ACE AND *ACTN3* POLYMORPHISMS, AEROBIC AND ANAEROBIC CAPACITIES, BONE AND MUSCULAR PERFORMANCE IN MALAY ATHLETES AND NON-ATHLETES

XIAO LI

UNIVERSITI SAINS MALAYSIA
2016

ACE AND *ACTN3* POLYMORPHISMS, AEROBIC AND ANAEROBIC CAPACITIES, BONE AND MUSCULAR PERFORMANCE IN MALAY ATHLETES AND NON-ATHLETES

by

XIAO LI

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

UNIVERSITI SAINS MALAYSIA
April 2016

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor, Assoc. Prof. Dr. Ooi Foong Kiew and co-supervisors, Prof. Dr. Zilfalil Bin Alwi for their continuous guidance throughout my PhD study, without them my PhD study would not have been possible. During my study, my supervisors shared with me their views and experiences, giving me encouragement and advices, which are indeed extremely valuable for my future research in life.

I am also indebted to Dr. Surini Yusoff for her expert advice and her assistance with genetic analysis, Dr. Juhara Haron for her help and guidance during the study period, Mr. Tan Shing Cheng for his assistance in genetic analysis, as well as the support and advice of Assoc. Prof. Dr. Chen Chee Keong.

I would also like to thank Ms. Jamaayah bt Meor Osman, Ms. Norlida Azalan, Mr. Muhamad Hanapi Muhamad Hussaini and Ms. Nur Hafizah Hamzah from Sports Science laboratory, Universiti Sains Malaysia for their assistance in data collection and also blood analysis throughout my study period. The assistance given by Ms. Nor Aini Sudin and Ms. Parimalah Velo are also very appreciated.

Furthermore, my sincere gratitude to all the lecturers and friends from Sports Science Unit and Human Genome Center in Universiti Sains Malaysia.

My gratitude also expends to Universiti Sains Malaysia for offering me graduate assistant scholarship and the employment as research assistant during my study period. Moreover, I also express my appreciation to Universiti Sains Malaysia Short Term Grant (No: 304/PPSP/61312051) provided by Universiti Sains Malaysia for the financial supporting of our research.

Great appreciation also goes to all my friends in Malaysia, as well as foreigner friends in Universiti Sains Malaysia for their help and valuable ideas during my study. To all participants involved in my study, I thank for the motivation and cooperation given by them during the process of data collection of my study.

Last but not the least, the invaluable and selfless supports from my family members and friends in China are highly appreciated. I will not be able to complete my PhD study without their endless love, kindness and encouragement, as well as confidence in me during my PhD study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

POLIMORFISME *ACE* AND *ACTN3*, KAPASITI AEROBIK DAN ANAEROBIK, TULANG DAN PRESTASI OTOT DALAM KALANGAN ATLET DAN BUKAN ATLET MELAYU

ABSTRAK

Kajian ini bertujuan untuk menyiasat hubungan antara polimorfisme gen *ACE* I/D dan polimorfisme gen *ACTN3* R577X, kapasiti aerobik dan anaerobik, tulang dan prestasi otot dalam kalangan atlet dan bukan atlet Melayu. Seramai 132 peserta telah menyertai kajian ini. Kesemua peserta atlet (atlet lelaki, n = 33; atlet wanita, n = 33) dan bukan atlet (lelaki bukan atlet, n = 33; perempuan bukan atlet, n = 33) Melayu telah dikenalpasti genotip polimorfisme *ACE* gen I/D dan polimorfisme *ACTN3* gen R577X mereka dengan menggunakan teknik PCR. Nisbah ekspiratori paksa "(FER)", pengambilan oksigen maksima (VO_{2max}) dan kuasa anaerobik 'Wingate' dan indeks keletihan peserta telah diukur. Sementara itu, pengukuran kuantitatif ultrabunyi iaitu kelajuan bunyi terhadap tulang "(SOS)" dan skor-T bagi kaki dan tangan dominan dan bukan dominan kesemua peserta telah diukur dengan menggunakan mesin sonometer tulang. Kebolehlenturan, kekuatan genggaman tangan, kekuatan kaki dan belakang badan, serta kuasa eksplosif lompatan kaki peserta juga telah diukur. Tork puncak otot (PT, penunjuk kekuatan otot), tork puncak per berat badan (PT/BW), dan purata kuasa (AVG.P) otot lutut dan bahu dominan dan bukan dominan dalam keadaan ekstensi dan fleksi pada $60^0.s^{-1}$, $180^0.s^{-1}$ serta $300^0.s^{-1}$ peserta diukur dengan menggunakan mesin dinamometer isokinetik BIODEX. Kajian ini menunjukkan genotip *ACE* II berkait dengan VO_{2max} yang lebih tinggi dalam kalangan atlet wanita

dan lelaki, dan juga berkait dengan PT otot yang lebih tinggi dalam kalangan atlet wanita. Sementara itu, genotip *ACE* ID berkait dengan kuasa eksplosif lompatan kaki yang lebih tinggi dan indeks keletihan yang lebih rendah dalam kalangan atlet wanita, dan juga berkait dengan PT otot iaitu kekuatan dan kuasa yang lebih tinggi dalam kalangan atlet lelaki. Dalam kalangan wanita bukan atlet, genotip DD berkait dengan status kesihatan tulang yang lebih baik. Kajian ini juga mendapati bahawa atlet wanita bergenotip *ACTN3* RR dan RX berkait dengan kebolehlenturan yang lebih tinggi. Sementara itu, atlet wanita yang bergenotip RR berkait dengan kuasa eksplosif lompatan kaki yang lebih tinggi. Dalam kalangan atlet lelaki, genotip RR berkait dengan purata kuasa yang lebih tinggi. Atlet lelaki dan wanita yang bergenotip RR berkait dengan PT otot dan AVG.P, iaitu kekuatan dan kuasa otot yang lebih tinggi. Wanita bukan atlet yang bergenotip RR pula berkait dengan status kesihatan tulang yang lebih baik. Secara kesimpulan, penemuan semasa yang didapati dari kajian ini boleh digunakan sebagai panduan kepada badan-badan sukan dan jurulatih dalam pengenalpastian dan pemilihan atlet elit di Malaysia, khususnya kumpulan etnik Melayu.

ACE AND *ACTN3* POLYMORPHISMS, AEROBIC AND ANAEROBIC CAPACITIES, BONE AND MUSCULAR PERFORMANCE IN MALAY ATHLETES AND NON-ATHLETES

ABSTRACT

This study investigated the association between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism, aerobic and anaerobic capacities, bone and muscular performance in Malay athletes and non-athletes. A total of 132 participants were recruited in this study. Malay athletes (male athletes, n=33; female athletes, n=33) and non-athletes (male non-athletes, n=33; female non-athletes, n=33) participants were genotyped for *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism by using PCR technique. Forced expiratory ratio (FER), maximal oxygen uptake (VO_{2max}) and Wingate anaerobic power were measured. Meanwhile, the quantitative ultrasound measurements of bone speed of sound (SOS) and T-score of the participants' dominant and non-dominant legs and arms were measured using a bone sonometer. Participants' flexibility, handgrip strength, back and leg strength, leg explosive jump power were also measured. Muscular peak torque (PT, an indicator of muscular strength), peak torque per body weight (PT/BW) and average power (AVG.P) of the participants' dominant and non-dominant knee and shoulder extension and flexion at $60^0.s^{-1}$, $180^0.s^{-1}$ and $300^0.s^{-1}$ were measured using BIODEX isokinetic dynamometer. The present study found that *ACE* II genotype was associated with higher VO_{2max} in female and male athletes, and was associated higher muscular PT in female athletes. Meanwhile, ID genotype was associated with higher leg explosive jump power and lower fatigue index in female

athletes, and was associated with higher muscular PT i.e. strength and power in male athletes. In female non-athletes, DD genotype was associated with better bone health status. This study also found that *ACTN3* RR and RX genotypes were associated with higher flexibility in female athletes. Meanwhile, RR genotype was associated with leg explosive jump power in female athletes. In male athletes, RR genotype was associated with higher mean power. In both female and male athletes, RR genotype was associated with higher muscular PT and AVG.P, i.e. muscular strength and power. In female non-athletes, RR genotype was associated with better bone health status. In conclusion, the present findings obtained from this study can be used to guide the decisions of sports bodies and coaches in talent identification and selection of elite athletes in Malaysia, especially Malay ethnic group.

CHAPTER 1
INTRODUCTION

1.1 Study background

Efficient human movement is influenced by environmental and behavioral factors including training, diet and genetic endowment, and it is believed that genetic endowment is one of the factors that can increase the possibility of an individual to become an elite athlete (Paparini *et al.*, 2007). Lucia *et al.* (2010) also mentioned that athletic champion status is a complex polygenic trait in which numerous candidate genes, complex gene-gene interactions and environment-gene interactions are involved.

Genetic factors determine 20-80% of the variations in a wide variety of traits that is relevant to athletic performances, such as oxygen uptake, cardiac output and relative proportion of fast and slow fibers in skeletal muscle (MacArthur and North 2007). Several previous studies have identified a large number of individual genes underlying the influence of these traits towards athletic performance, and it was found that more than 200 genes and quantitative trait loci have been associated with athletic performance and physical fitness traits (Ginevičienė *et al.*, 2011a; Ahmetov *et al.*, 2009).

The talent of a sports person can be defined by the complement of genes that he or she inherited from his both parents. Over the course of evolution, families pass on their genetic coding from one generation to the next and over time certain characteristics of genes are added, subtracted and altered (Schoenfelder, 2010). The development of technology for rapid deoxyribonucleic acid (DNA) sequencing and genotyping has allowed the identification of some of the individual genetic variations that contribute to athletic performance (Patel and Greydanus, 2002). The process of talent identification by the sports associations and coaches can be revolutionized by the discovery and characterization of genetic variants that strongly influence athletic performance, with genetic analysis being added to the existing battery of physiological, biochemical and psychological tests that form the current basis for selecting talented athletes for further training (Patel and Greydanus, 2002).

Genetic predisposition has great implications in the characterization of an individual as a great athlete despite the specific training and nutritional follow-up factors. Studies of genes that influence human physical performance show a strong heritability of key endurance and strength phenotypes. Endurance phenotype includes maximal oxygen uptake while strength phenotype include muscular strength (Ginevičienė *et al.*, 2011a; Ahmetov *et al.*, 2009).

One popular gene that has been associated with the tendency of individual towards sports is Angiotension I-Convertion Enzyme (ACE) gene. ACE is encoded by the *ACE* gene located on chromosome 17 at position q23.3. The size of the gene is 44,778 bases, with 21 kb contains 26 exons and 25 introns. There are two forms of ACE in human, the production of which depends on whether it is encoded by

somatic *ACE* (*sACE*) or germinal or testicular *ACE* (*gACE*) (Riordan, 2003; Brown *et al.*, 2006). Somatic ACE is the longer form of ACE in human which is transcribed from exons 1-12 and 14-26, while germinal ACE (shorter form) is transcribed from exons 13-26 (Tsianos *et al.*, 2004; Eynon *et al.*, 2009a). The D allele has been shown to be associated with increased sprinter performance and muscle powers based on a research conducted by Woods *et al.* (2000) on short distance swimmers. This allele was also found to be related to an increase in the strength of the quadricipital thigh muscle in response to nine-week isometric strength training (Folland *et al.*, 2000; Cięszczyk *et al.*, 2011). Amir *et al.* (2007) found the overrepresentation of the *ACE* gene D allele and DD genotype among elite Israeli marathon athletes. Similar finding was also reported by Tobina *et al.* (2010). They noted that the DD genotype was significantly higher than the II genotype amongst the Japanese athletes, and the average running speed was significantly higher for athletes with the combined DD and ID genotypes than those with the combined II genotype.

Another popular candidate gene that has shown association with athlete performance is α-actinin-3 (*ACTN3*) gene due to the replacement of arginine (R) to stop codon Ter (X), at position 577 of amino acid (MacArthur and North, 2007). It results in the deficiency of α-actinin-3 protein. The presence of α-actinin-3 is required for optimal fast fiber performance in power athletes (MacArthur and North, 2007) which majority of the power athletes has the RR and RX genotypes. The absence of α-actinin-3 provides some advantage to endurance athlete where the majority of the athletes has the XX genotype.

It is very intricate and complex to become an elite athlete. Many gene variants that influence physical performance in one population might not have the same effect in another. The genotype and phenotypic variance exists in different ethnicities and populations (Peng *et al.*, 2008). For example, discernable deviations can be observed in the genetic profiles of individuals within a less genetically heterogeneous ancestry, e.g., among Europeans and Han Chinese (Zilberman-Schapira *et al.*, 2012). Most countries are mixture of different races, caused by history of migration centuries ago. Any genetic analysis with different ethnic groups might lead to misleading results.

1.2 Research gap of the study

Based on prior studies on association between human sports performance and these two genes, to our knowledge, to date there are limited studies focusing on the athletic performance and genetic factors among Malay population. Additionally, limited study has been performed to investigate *ACE* I/D and *ACTN3* R577X polymorphisms in Malay male and female athletes in Malaysia, and limited study has investigated the association between the *ACE* I/D genotypes, *ACTN3* R577X genotypes, muscular strength and explosive power, aerobic and anaerobic capacities, bone and the other sports ability related parameters in this population. Hence, the present study was designed to address the paucity of this information. The present study aimed to examine the association between *ACE* I/D and *ACTN3* R577X polymorphisms, aerobic- and anaerobic-orientated phenotypes, bone health status and muscular strength and power among Malay male and female athletes and non-athletes.

1.3 Research questions

1) What are the genetic similarities (relatedness) between Malay athletes and Malay non-athletes in Malaysia?

2) Is there any association between *ACE* and *ACTN3* polymorphisms, aerobic capacitiy, i.e. VO_{2max}, Wingate anaerobic capacity, i.e. anaerobic power, bone health status, i.e. bone speed of sound, and muscular performance, i.e. isokinetic muscular strength and power in Malay male and female athletes and non-athletes?

3) Are *ACE* gene I/D and *ACTN3* gene R577X polymorphisms important in determining an individual athletic potential in Malaysia, especially in Malay population?

1.4 Objectives of the study

1.4.1 General objective:

To examine the association of *ACE* gene and *ACTN3* gene polymorphisms with aerobic and anaerobic capacities, bone, muscular performance and other sports ability related parameters in Malay male and female athletes and non-athletes in Malaysia.

1.4.2 Specific objectives:

1) To examine the presence and frequency distributions of *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism in Malay male and female athletes and non-athletes.

2) To examine the association between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with physical and physiological characteristics i.e. percent body fat, resting heart rate and blood pressure, aerobic capacity i.e. maximal oxygen uptake (VO_{2max}) and forced expiratory ratio (FER), anaerobic capacities i.e. mean power, peak power, anaerobic capacity, anaerobic power and fatigue index (FI) in Malay male and female athletes and Malay male and female non-athletes.

3) To examine the association between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with quantitative ultrasound measurements of bone speed of sound i.e. SOS and also T-score in Malay male and female athletes and Malay male and female non-athletes.

4) To examine the association between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with flexibility, hand grip strength, back and leg strength and leg explosive power in Malay male and female athletes and Malay male and female non-athletes.

5) To examine the association between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with muscular strength and power measured using isokinetic dynamometer, i.e. peak torque, peak torque per body weight and average power in Malay male and female athletes and Malay male and female non-athletes.

6) To examine the correlation between quantitative ultrasound measurement of the bone of lower limbs, muscular performance and anaerobic capacities in Malay athletes and Malay male and female non-athletes.

1.5 Hypotheses of the study

H_{A1}: There are associations between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with physical and physiological characteristics i.e. percent body fat, resting heart rate, blood pressure, aerobic capacity i.e. maximal oxygen uptake (VO_{2max}) and forced expiratory ratio (FER), anaerobic capacities i.e. mean power, peak power, anaerobic capacity, anaerobic power and fatigue index (FI) in Malay male and female athletes and Malay male and female non-athletes.

H_{A2}: There are associations between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with quantitative ultrasound measurements of bone speed of sound i.e. SOS and also T-score in Malay male and female athletes and Malay male and female non-athletes.

H_{A3}: There are associations between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with flexibility, hands grip strength, back and leg strength, leg explosive power in Malay male and female athletes and Malay male and female non-athletes.

H_{A4}: There are associations between *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with muscular strength and power i.e. peak torque, peak torque per body weight and average power in Malay male and female athletes and Malay male and female non-athletes.

H_{A5}: There are correlations between quantitative ultrasound measurement of the bone of lower limbs, muscular performance and anaerobic capacities in Malay athletes and Malay male and female non-athletes.

1.6 Significance of the study

If the present study can find that there are association of *ACE* gene I/D polymorphism and *ACTN3* gene R577X polymorphism with aerobic and anaerobic capacities, bone, muscular performance and other parameters related to sports ability in Malay male and female athletes and non-athletes, the results of the present study can then be used in talent identification of elite athletes and champions athletes in Malaysia. At the same time the efficiency of elite athlete selection can be improved manpower and material resources can also be saved. It is also hoped that results obtained from this study can guide the decisions of sports bodies, coaches and athletes in the formulation of talent identification that can benefit Malaysian athletes.

CHAPTER 2
LITERATURE REVIEW

2.1 Sports disciplines

The two major sports disciplines, i.e. endurance and sprint or power performance involve different types of muscle metabolism. Endurance sports require high level of aerobic or cardiorespiratory fitness which is represented by maximal oxygen uptake (VO_{2max}) of an individual (Plowman and Smith, 2013). Endurance discipline depends on aerobic energy metabolism, and the examples are long distance swimming, triathlon, skiing, medium and long distance running, race walking, mountaineering and cycling. Meanwhile, sprint or power discipline requires predominantly anaerobic energy metabolism (Brown *et al.*, 2006) or power-generating muscle metabolism (Plowman and Smith, 2013). Examples of sprint or power discipline are short distance running, weightlifting and track and field events such as high jump and long jump. Besides the two major sports disciplines, the third sports discipline is the endurance-speed-strength discipline where athletes are involved with an intermediate character of energy metabolism. Examples of endurance-speed-strength discipline are hockey, tennis, football, volleyball, basketball, handball, rugby and boxing.

2.2 Aerobic capacity

Cardio-respiratory fitness includes the function of both the heart and the lungs, it reflects the efficiency of the circulatory system to deliver oxygen that is taken into the blood through the lungs and the heart to circulate the blood through the arteries and veins (Bouchard *et al*., 1999). Cardio-respiratory of individual indicates how fit an individual is aerobically. The maximal ability of an individual to consume oxygen is dependent on his/her cardiorespiratory function and the capacity of skeletal muscle mitochondria to consume oxygen. Maximal oxygen consumption (VO_{2max}) is one of the important predictors of cardiorespiratory fitness, and it is used for assessing one's aerobic capacity (Bassett and Howley, 2000). It is also an indicator of the cardiovascular system to deliver oxygenated blood to working muscles and utilization of oxygen by the muscles during exercise (Heyward, 2014). Treadmill running VO_{2max} test is a common test used for direct assessing VO_{2max} of an individual (Joyner and Coyle, 2008), which is accepted as a standard cardio-respiratory fitness measurement. While running on treadmill with increasing the speed and grade of treadmill gradually, participant's oxygen and carbon dioxide concentrations are assessed by metabolic cart, and the volume of expired air is also recorded. VO_{2max} is the volume of oxygen consumption at the exhausting level of running during approximately 10-15 minutes (Bouchard *et al.*, 1999; Heyward and Gibson, 2014).

2.3 Anaerobic capacity

Anaerobic activity is defined as activity involving energy expenditure that uses anaerobic metabolism, i.e. without the usage of oxygen, lasting less than 90 seconds and utilising an exhaustive effort (Heyward and Gibson, 2014). Wingate anaerobic test is the most common test which can assess the anaerobic fitness of an individual. Two major energy sources are required during the Wingate anaerobic test (MacDougall and Wenger, 1991). The first energy source is the adenosine triphosphate-phosphocreatine (ATP-PCr) system, which lasts for 3 to 15 seconds during maximum effort. The second system is anaerobic glycolysis, which can be sustained for the remainder of the all-out effort. Therefore, the Wingate anaerobic test can measure muscles' ability to work using both the ATP-PCr and glycolytic systems. Sports persons involving in sports events such as football, sprinting, soccer, baseball and gymnastics require anaerobic metabolism during competition. The Wingate anaerobic test was designed to measure an individual's peak power, mean power and percent fatigue (Inbar *et al.*, 1996). Besides Wingate anaerobic test, the tests which can assess an individual's power and/or anaerobic capacity are vertical jump test and standing long jump test (Bar-Or, 1987).

2.4 Bone health

Bone health is influenced by age, gender, race, nutrition, life style, exercise, and hormonal factors as well as muscle strength (Hochberg, 2007; Blain *et al.*, 2001; Taaffe et al., 2001; Burr, 1997). Regarding association between muscular strength and bone health, Ahedi *et al.*, (2014) investigated the relationship between muscle

strength and bone mineral density (BMD) of the hip and spine in 321 Tasmanian older adults and reported that hip BMD was positively related to the muscular strength, and the authors concluded that higher muscular strength may maintain bone health and prevent bone fragility and fractures (Ahedi *et al.*, 2014). Similarly, Lee *et al.* (2014) also reported that muscular strength is associated with BMD of hip in healthy elderly women. Regarding the associations between bone mineral density and muscle anaerobic capacities, such as explosive power, Nasri *et al.* (2013) found that hand grip strength and explosive leg power were significantly correlated with BMD of both spine and legs among fifty adolescent combat sports athletes aged 17 years.

It is well known that osteoporosis is a systematic bone disease characterized by loss of bone contents and progressive deterioration of microarchitecture of the bone, which would lead to bone fragility and fractures eventually (Wass and Owen, 2014; Tung and Iqbal, 2007). In 1994, the World Health Organization recommended that dual energy X-ray absorptiometry (DXA) is the gold standard method for the diagnosis of osteoporosis and measurement of bone mineral density (BMD) (W.H.O., 1994). Nevertheless, many other techniques are available to evaluate bone health in recent years (W.H.O., 2004). One of the popular techniques is quantitative ultrasound (QUS), which uses sound waves to diagnose osteoporosis and assess bone health of an individual (Miura *et al.*, 2008; Mészáros *et al.*, 2007; Baroncelli, 2008; Mimura *et al.*, 2008). Today, more and more researchers use devices based on quantitative ultrasound to evaluate osteoporosis due to its portability, proper practicality and cheaper cost for the public to access. Ng and Sundram (1998) reported that quantitative ultrasound provides bone speed of sound (SOS) results which can

contribute additional information on bone contents and microarchitectures as well as BMD. The speed of sound of bone, which is an alternative to DXA for osteoporosis screening, can be measured by quantitative ultrasound through bone at the phalanx, radius, tibia and metatarsal (Njeh *et al.*, 2001; Giangregorio and Webber, 2004).

2.5 Muscular strength and power

Muscular strength and power not only impacts the quality of personal daily life, but also can reflect a person's sports ability. Muscular strength is the maximal force that a muscle can exert. Human skeletal muscle consists of slow-twitch (ST) and fast-twitch (FT) which is determined by different protein types or myosin isoform to control the speed of contraction of muscle cells. ST cells can elevate blood supply and aerobic enzyme content in order to create higher muscular aerobic capacity and power, and FT cells have greater muscular anaerobic capacity and power through storing higher concentration of glycogen and anaerobic enzymes (Heyward and Gibson, 2014). In sports science fields, one of the most common methods to test muscular strength and power is manual muscle testing, i.e. hand grip strength testing, back and leg strength testing, standing long jump and vertical jump testing. Although those manual muscular strength and power testing are less objective when an individual has ability to generate high force, these testings are easier and straighter to use and assess an individual’s strength and power (Keasays *et al.*, 2000).

Another measurement of muscular strength and power usually examines the isometric strength and power, i.e. the maximal force exerted when the limb is not moving. Muscular strength and power is the product of force and velocity. By

definition, therefore, strength and power can be measured only when the limb is in motion. BIODEX isokinetic dynamometer can be used to measure subjects' muscular strength and power. Perhaps the most important reason for isokinetic testing is that it provides an effective way to attain objective measures. It is demonstrated that this instrument provided mechanically valid, reliable and reproducible measures of strength and power. Many studies have been performed to document this validity and reliability, but there is controversy about which is the most clinically significant testing speed. A specific muscular power and strength measured by BIODEX isokinetic dynamometer can be assessed as peak torque, peak torque per body weight and average power etc.. Peak torque is highest muscular force output, which is similar to a one repetition maximum effort in isotonics, and average power is the mean value of how effectively the muscle can perform work over time (Plowman and Smith, 2013). Isokinetic muscular extension and flexion power and strength were normally assessed at 3 angular velocities of movement (with a rest period of 10 seconds between the trials): $60^0.s^{-1}$, $180^0.s^{-1}$and $300^0.s^{-1}$ (Pincivero *et al.*, 1997). Slowest speed tests are generally conducted with 5 repetitions i.e. $60^0.s^{-1}$, and faster speed tests are usually performed at 10 to 15 repetitions, i.e. $180^0.s^{-1}$and $300^0.s^{-1}$ respectively. Testing at each velocity should be consisted of 5 sub-maximal followed by 2-3 maximal repetitions for warm-up purposes. During the testing procedure, each participant was given verbal encouragement as well as visual feedback from an investigator in an attempt to achieve a maximal effort level (Hald and Bottjen, 1987).

2.6 *Angiotensin converting enzyme* gene ID polymorphism and human physical performance in different populations and races

Physical fitness is a complex phenotype influenced by environmental and genetic factors. Meanwhile variations in human physical performance and athlete ability have been recognized as a strong heritable component. The talent of a sports person can be defined by the complement of genes that he/she inherited from his/her both parents. Over the course of evolution, families pass on their genetic coding from one generation to the next and certain characteristics of genes are added, subtracted and altered over time. It was estimated that the heritability of athlete status is at approximately 66% in a twin pair study by Schoenfelder (2010), but the author did not report whether it was influenced by single or multiple genes. In the last two decades, many sports science studies have been conducted to investigate the relationship of genetics and elite athletic performance, and the association of genetic characteristics and their impact on training and exercise. It was expected that, with the rapid development of gene-based technologies, more and more researches will be carried out in the future to identify genetic predispositions as a contributing factor to athletic abilities and performance (Patel and Greydanus, 2002).

Angiotensin converting enzyme (ACE) is a component of circulating renin-angiotensin system (RAS) which influences circulatory homeostasis through the degradation of vasodilator kinins and generation of vasopressor angiotensin II (Ang II). Genetic polymorphisms within the *ACE* gene could be associated with various phenotypic characteristics such as diseases and human performances. To date, one of the most popular genetic polymorphisms that has been shown to be associated

with athlete performance is *ACE* gene, which contains a restriction fragment length polymorphism consisting of the insertion, I (presence of Alu repeat) and deletion, D (absence of Alu repeat) of 287 bp of Alu repeat located in intron 16 (Tsianos *et al.*, 2004; Maffulli *et al.*, 2013; Guth and Roth, 2013). Previous studies investigating the influence of the polymorphism and various phenotypic characteristics have produced inconsistent findings due to the inter-ethnic variations of the *ACE* allele distribution. For example, some previous studies showed that I allele was associated with fatigue resistance in skeletal muscle and endurance performance, while the D allele has been associated with power or sprint performances. Nevertheless, controversy still exists in the above conclusion, in which some studies have reported that I allele was associated with a better power or sprint performance rather than with endurance athletic abilities. This section discusses the ethnic variations of *ACE* allele distribution in different populations and races, including African, American, European, Asian populations etc.. Additionally, association between *ACE* ID polymorphism and human fitness among various populations and races were discussed.

2.6.1 Renin-angiotensin system (RAS)

According to Basso and Terragno (2001), Tigerstedt and Bergman discovered the rate-limiting enzyme renin, and reported the effect of renal extracts about one hundred years ago. Then the renin-angiotensin system (RAS) continues to be an estimable subject for subsequent research. It is well known that the endocrine renin-angiotensin system (RAS) is a key regulator of circulatory homeostasis. In other words, it is important for regulating blood pressure and fluid homeostasis

(Wang *et al.*, 2008; Puthucheary *et al.*, 2011a; Paul *et al.*, 2006). Renin is a 37 kDa aspartyl protease that converts angiotensinogen to decapeptide angiotensin I (Ang I). Ang I is in turn acted upon by the peptidyl dipeptidase ACE to generate octapeptide angiotensin II (Ang II).

Agonist action of Ang II on angiotensin type-1 receptor (AT1R) causes vasoconstriction in arterial blood pressure. Ang II also affects renal sodium reabsorption and adrenal aldosterone production, leading to salt and water retention, which further influences blood volume and pressure (Wang *et al.*, 2008; Myerson *et al.*, 1999). Previous studies have shown that the vasoconstrictor peptide angiotensin II also plays an important role in vascular smooth muscle growth (Geisterfer *et al.*, 1988; Naftilan, 1992).

2.6.2 Angiotensin converting enzyme (ACE)

Angiotensin converting enzyme (ACE) is a component of circulating renin-angiotensin system (RAS) which influences circulatory homeostasis through the degradation of vasodilator kinins and generation of vasopressor angiotensin II (Ang II). ACE is a monomeric, membrane bound, zinc and chloride dependent peptidyl dipeptidase that catalyzes the conversion of decapeptide angiotensin I to octapeptide angiotensin II, by removing carboxy terminal dipeptide (Brown *et al.*, 2006).

ACE is encoded by the *ACE* gene located on chromosome 17 at position q23.3. The size of the gene is 44,778 bases, with 21 kb contains 26 exons and 25 introns.

There are two forms of ACE in human, the production of which depends on whether it is encoded by somatic ACE (sACE) or germinal or testicular ACE (gACE) (Riordan, 2003). Somatic ACE is the longer form of ACE in human which is transcribed from exons 1-12 and 14-26, while germinal ACE (shorter form) is transcribed from exons 13-26 (de Souza *et al.*, 2013; Eynon *et al.*, 2009a).

According to Jasinska and Krzyzosiak (2004), Alu sequences and repeats are the most frequent and simple sequence repeats, which are short segments of DNA interspersed throughout the genome and come in many varieties. In human *ACE* gene, the Alu insertion/deletion polymorphism can be found in intron 16, which involves either the presence or the absence of a 287 bp fragment. Most studies on the ancestral human genome in the recent history of evolutionary found that the frequency of each polymorphic genotype of the Alu insertion/deletion polymorphism in *ACE* gene varies across different ethnic populations. For example, one of those studies showed that the frequency of insertion/insertion (II), insertion/deletion (ID) and deletion/deletion (DD) genotypes of *ACE* polymorphism was 44.1%, 43.4% and 12.5% respectively among Caucasian Italian population (Scanavini *et al.*, 2002).

2.6.3 Ethnic variations of *ACE* allele distribution in difference populations and races

It has been reported that there are inconsistent findings on the influence of *ACE* gene polymorphisms on phenotypic characteristics across different populations, due to the inter-ethnic variations of the *ACE* allele distribution (Barley *et al.*, 1994; Barley *et al.*, 1996; Mathew *et al.*, 2001; Harrap *et al.*, 2003; Sagnella *et al.*, 1999).

Previous studies with the related data demonstrating the ethnic distribution of ACE ID polymorphism in different populations and races are tabulated in Table 2.1.

Sagnella *et al.* (1999) studied the frequencies of *ACE* ID polymorphism among 1577 men and women living in the South London belonging to three main ethnic groups: whites, people of African descent i.e. Caribbeans and West Africans and people of South Asian Indian origin. The study found that the distribution of the II, ID and DD genotypes was 18.4%, 49.6% and 32.0% respectively in whites, 18.4%, 50.5% and 30.9% in African descent and 18.3%, 41.8% and 39.8% in those of South Asian origin. Among people of African descent, it was found that there were no statistically significant difference in the II, ID and DD genotype frequencies between West Africans (18.1%, 49.6% and 32.2%, respectively) and Caribbeans (20.6%, 53.7% and 25.7%, respectively). In another study, Mathew *et al.* (2001) investigated the distribution of the II, ID and DD genotypes among African Americans, Indians and whites. They reported that the II, ID and DD genotypes distribution was 11%, 60% and 29% in African Americans, 31%, 50% and 19% in Indians and 31%, 40% and 29% in whites. They also reported that there was a significant difference on the frequency of the deletion allele among African Americans (59%), Indians (49%) and whites (44%).

Table 2.1: Previous studies on the ethnic distribution of *ACE* insertion (I)/deletion (D) polymorphism in different populations and races

Study	Subjects	Population	Race	Sample size	The distribution of *ACE* I/D polymorphism				
					II (%)	ID (%)	DD (%)	I allele	D allele
Schachter *et al.* (1994)	Centenarian	French	Caucasian	310	16.6	43.8	39.6	0.385	0.615
Miller *et al.* (1996)	Normal	British	Caucasian	1906	24.0	50.0	26.0	0.490	0.510
Montgomery *et al.* (1998)	Army	UK	Caucasian	78	25.6	59.0	15.4	0.551	0.449
Montgomery *et al.* (1999)	Army	UK	Caucasian	123	24.0	57.0	19.0	0.525	0.475
Sagnella *et al*. (1999)	Normal	Londoner	Caucasian	462	18.4	49.6	32.0	0.432	0.568
			African descent	462	18.4	50.5	30.9	0.437	0.563
			West African	176	18.1	49.6	32.2	0.429	0.571
			Caribbean	286	20.6	53.7	25.7	0.474	0.526
			South Asian	442	18.3	41.8	39.8	0.392	0.608
Alvarez *et al.* (2000)	Athlete	Spanish	Caucasian	60	25.0	58.0	17.0	0.540	0.460
	Normal		Caucasian	400	16.0	45.0	39.0	0.380	0.620
Mathew *et al.* (2001)	Normal	American	Caucasian	82	31.0	40.0	29.0	0.510	0.490
			African	142	11.0	60.0	29.0	0.410	0.590
			Indian	136	31.0	50.0	19.0	0.560	0.440
Nazarov *et al.* (2001)	Athlete	Russian	Caucasian	217	19.0	51.0	29.0	0.445	0.555
	Normal		Caucasian	449	23.0	52.0	24.0	0.490	0.510
Collins *et al.* (2004)	Athlete	African	South African	447	21.7	51.7	26.6	0.475	0.525
	Normal		South African	199	17.6	49.8	32.7	0.425	0.575
Scott *et al.* (2005b)	Athlete	African	Kenyan	291	15.1	51.2	33.7	0.407	0.593
	Normal		Kenyan	85	14.1	48.2	37.7	0.382	0.618
Yang *et al.* (2006)	Normal	Chinese	Asian	221	41.0	49.4	9.6	0.654	0.346
	Patient		Asian	-	38.8	42.1	19.1	0.598	0.402

Table 2.1: Previous studies on the ethnic distribution of *ACE* insertion (I)/deletion (D) polymorphism in different populations and races (continued)

Study	Subjects	Population	Race	Sample size	The distribution of *ACE* I/D polymorphism				
					II (%)	ID (%)	DD (%)	I allele	D allele
Amir *et al.* (2007)	Athlete	Israelite	Asian	121	12.0	36.0	52.0	0.300	0.700
	Normal		Caucasian	247	10.0	46.0	43.0	0.340	0.660
Cam *et al.* (2007)	Normal	Turkish	Caucasian	55	21.8	41.8	36.4	0.427	0.573
Jayapalan et al. (2008)	Normal	Malaysian	Malay	274	51.1	39.4	9.5	0.708	0.292
			Chinese	150	40.0	46.7	13.3	0.634	0.366
			Indian	213	35.7	45.2	19.2	0.583	0.417
Berdeli and Cam (2009)	Normal	Turkish	Caucasian	1063	16.1	47.7	36.2	0.399	0.601
Deeba *et al.* (2009)	Patient	South Indian	Asian	185	23.0	45.0	32.0	0.460	0.540
	Normal			201	37.0	42.0	21.0	0.580	0.420
Min *et al*. (2009)	Athlete	Japanese	Asian	227	32.5	42.6	24.9	0.538	0.462
Cieszczyk *et al.* (2010)	Athlete	Polish	Caucasian	28	28.6	64.3	7.1	0.607	0.393
	Normal			115	19.2	50.4	30.4	0.443	0.557
Jayapalan *et al.* (2010)	Patient	Malaysian	South East Asian	62	30.6	56.4	13.0	0.588	0.412
				-	36.4	47.3	16.3	0.601	0.399
Shaikh *et al*. (2012)	Patient	Pakistani	Asian	464	26.1	54.2	19.7	0.535	0.465
	Normal			150	28.0	32.0	40.0	0.440	0.560
Wang *et al.* (2013)	Athlete	European	Caucasian	191	21.9	43.4	34.7	0.436	0.564
	Athlete	Japanese	East Asian	362	42.3	42.2	15.5	0.634	0.366
	Normal	Taiwanese USA	Caucasian	1248	24.1	49.3	26.6	0.488	0.512
	Normal	Japanese Taiwanese	East Asian	1244	45.0	43.7	11.3	0.669	0.331
Zhou *et al.* (2013)	Normal	Chinese	Asian	260	11.9	23.1	65.0	0.235	0.765
	Patient		Asian	343	30.9	37.5	31.6	0.497	0.504
Jastrzębski *et al.* (2014)	Athlete	Polish	Caucasian	121	30.6	53.7	15.7	0.574	0.426
	Normal		Caucasian	115	19.2	50.4	30.4	0.443	0.557

In French centenarians, Schachter *et al.* (1994) observed that the frequency of II, ID and DD genotypes was 16.6%, 43.8% and 39.6% among 310 French centenarians. Besides, 1063 healthy white Western Turkish Caucasians were examined for the prevalence of the *ACE* ID polymorphism by Berdeli and Cam (2009). Their results showed that the *ACE* gene I allele frequency was 39.9% and D allele frequency was 60.1%, and the frequency of the *ACE* gene II, ID and DD genotype was 16.1%, 47.7% and 36.2% respectively. In another study involving Turkish athletes (Caucasian), it was found that the frequency of II, ID and DD genotypes was 21.8%, 41.8% and 36.4% amongst female non-elite Caucasian Turkish athletes (Cam *et al.*, 2007). The distribution of II, ID and DD genotypes was 12.5%, 50% and 37.5% among 80 white male gymnasts from the Italian population (Morucci *et al.*, 2014).

In a study comparing Asian and Caucasian populations (Li *et al.*, 2012), the authors found that the II, ID and DD genotypes distribution were 36.4%, 47.3% and 16.3% in Asians versus 24.9%, 46.5% and 28.6% in Caucasians, nevertheless no significant difference in the distribution of the genotypes between Asians and Caucasians was observed. Regarding study on distribution of *ACE* ID polymorphism in the Malaysian population, a previous study carried out by Jayapalan *et al.* (2008) has recruited 274 Malays, 150 Chinese and 213 Indians ethnic subgroups in Malaysia. They found that the distribution of the II, ID and DD genotypes in the Malay subgroup was 51.1%, 39.4% and 9.5%, respectively. In Chinese, the distribution was 40.0%, 46.7% and 13.3% respectively, and the distribution was 35.7%, 45.2% and 19.2% respectively in Indian subgroup.

2.6.4 Association between *ACE* gene I/D polymorphism and human physical and fitness performance

Genetic predisposition has great implications in the characterisation of an individual as a great athlete apart from the specific training and nutritional follow-up factors. Studies of genes that influence human physical performance show a strong heritability of key endurance and strength phenotypes. Endurance phenotypes include maximal oxygen uptake, lactate threshold and economy movement, while strength phenotypes consist of muscle strength and sprint performance (Ginevičienė *et al.*, 2011a).

It is known that the presence of the extra fragment has been found to be associated with lower circulating and tissue ACE activity, while the absence of the 287 bp fragment was associated with relatively higher ACE activity (Myerson *et al.*, 1999). The three genotype variants exist are II, ID and DD. Even though this marker lies in an intronic region, it was shown to be functional and gave strong and consistent marker for ACE activity (Jones *et al.*, 2002). Based on previous studies, the I allele has been proven to be associated with fatigue resistance in skeletal muscle and endurance performance, while the D allele has been associated with power or sprint performances (Tsianos *et al.*, 2004; Montgomery *et al*., 1999). Min *et al.* (2009) found that the effects of *ACE* ID polymorphism on endurance- and power-oriented performance are more prominent in male athletes. *ACE* ID genotype was highly distributed in both short-distance and long-distance runners. Several previous studies with the related data demonstrating the association between *ACE* insertion (I) /

deletion (D) polymorphism and human endurance, muscular strength and power status in different population and races are summarized in Table 2.2.

Table 2.2: Previous studies on the association between *ACE* insertion (I)/deletion (D) polymorphism and human endurance, muscular strength and power status in different populations and races

Study	Discipline	Level of athletes	Ethnicity & Population	Sample size	Frequency of allele/genotype and association of allele/genotype with human fitness performance
Ash *et al.* (2011)	Endurance runners, sprint and power event athletes	National or international	Ethiopian East African	524 **A** 317 **C**	There was no association between *ACE* gene variation and elite endurance status amongst Ethiopians population.
Cieszczyk *et al.* (2009)	Rowers	Olympic and World champion	Polish Caucasian	55 **MA** 115 **C**	The frequency of I allele in rower's group differed significantly from which in controls.
Collins *et al.* (2004)	Triathlons	Mixed	South African born	100 fastest finishers 100 slowest finishers 166 **C**	Excess of I allele in 'fast finisher' group ($p = 0.036$) and a linear trend for increasing I allele frequency from the controls through the slow finishers to the fast finishers ($p = 0.033$)
Gayagay *et al.* (1998)	Rowers	National	Australian Caucasian	43 **MA** 21 **FA** 75 **MN** 39 **FN**	An excess of the *ACE* I allele ($p < 0.02$) and II genotype ($p = 0.03$) were observed in Australian national rowers compared to the normal population.

A = athletes; C = control; FA = female athletes; MA = male athletes; FN = female non-athletes; MN = male non-athletes. "p" indicates statistical significant level.