THE EFFECTS OF ANGIOTENSIN I-CONVERTING ENZYME (*ACE*) *I/D* AND ALPHA-ACTININ-3 (*ACTN3*) *R/X* GENE POLYMORPHISMS ON HUMAN PHYSICAL PERFORMANCE AND HEALTH WITHIN MALAYSIAN POPULATION

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by

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LIST OF ABBREVIATIONS

HERITAGE	*HE*alth, *RI*sk factors, exercise *T*raining *A*nd *GE*netics
ACE	angiotensin I-converting enzyme (italicized for gene)
ACTN3	alpha-actinin-3 (italicized for gene)
I	insertion
D	deletion
DNA	deoxyribonucleic acid
e.g.	for the sake of example
kb	kilo bases
RAS	renin-angiotensin system
ANG II	angiotensin II
ANG I	angiotensin I
ADH	antidiuretic hormone
bp	base pair
et al.	and others
VO_2max	maximal oxygen consumption
UK	United Kingdom
US	United States
R	arginine codon
X	premature stop codon
SMD	short middle distance
LD	long distance
SDH	succinate dehydrogenase
*bHA*D	beta hydroxyacyl-CoA dehydrogenase
*MCA*D	medium-chain acyl-CoA dehydrogenase

VT	ventilation threshold
MVC	maximal voluntary contraction
pH	power of hydrogen
NO	nitric oxide
pp	peak power
ST	strength training
PBS	phosphate buffered saline
rpm	revolutions per minute
Tris-HCl	tris hydrochloride
KCl	potassium chloride
EDTA	ethylenediaminetetraacetic acid
DTT	dithiothreitol
PMSF	phenylmethanesulfonylfluoride
dNTP	deoxynucleotide
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
dGTP	deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
SD	standard deviation
IHG	isometric handgrip
ANOVA	one-way analysis of variance
PCR	polymerase chain reaction
SBP	systolic blood pressure
DBP	diastolic blood pressure
MAP	mean arterial pressure

PP	pulse pressure
HR	heart rate
HGS	handgrip strength
ANG	angiotensin
BP	blood pressure
df	degrees of freedom
U	unit
NADH	nicotinamide adenine dinucleotide
CS	citrate synthase
USA	United States of America
min	minutes
sec	second
HWE	Hardy-Weinberg equilibrium

LIST OF SYMBOLS

%	percent
q	long arm
km	kilometre
m	metre
$ml \cdot kg^{-1} \cdot min^{-1}$	milliliter per kilogram per minute
g/m^2	grams per square meter
1-RM	one-repetition maximum
°/s	degrees / second
mmHg	millimeter of mercury
ml	milliliter
°C	celsius
µl	microliter
mg/ml	milligram/milliliter
g	grams
>	greater than
<	less than
1X	one times
mm	millimeter
Mg^{2+}	Magnesium ion
µm	micrometre
10X	ten times
=	equal to
≤	less than or equal to
≥	greater than or equal to

±	plus minus
X^2	chi-square
kg/m^2	kilogram per square meter
cm	centimeter
kg	kilogram
bpm	beats per minute
Δ	change
$p < 0.001$	all p values smaller than 0.001 (e.g., p = 0.000)

LIST OF OPERATIONAL DEFINITIONS

Other Bumiputra	The indigenous people of East Malaysia (Sabah and Sarawak).
Endurance athletes	Athletes who participate in an event that utilise predominant aerobic energy production (duration of exertion over 30 minutes, intensity of exertion moderate).
Strength/power athletes	Athletes who participate in an event that utilise mixed energy production (competitive exercise performance time (1–5 minutes); for combat sports, the duration of a single bout of competition was considered), but the intensity of exertion was higher and the balance between anaerobic/aerobic energy productions were shifted towards the anaerobic system.
Intermittent athletes	Athletes who participate in an event that utilise mixed anaerobic/aerobic energy production, with a duration of exertion ranging from 5 to 30 minutes and a moderate to high intensity of exertion.
Isometric exercise	Type of strength training that does not require any movement of the affected joint and the muscle length will remain unchanged during contraction.

KESAN POLIMORFISME GEN ANGIOTENSIN I-CONVERTING ENZYME *(ACE) I/D* DAN ALPHA-ACTININ-3 *(ACTN3) R/X* KE ATAS PRESTASI FIZIKAL DAN KESIHATAN MANUSIA DALAM POPULASI MALAYSIA

ABSTRAK

Perbezaan data set populasi dalam sorotan kajian semasa dengan laporan terhad di kalangan sampel Asia, di tambah pula dengan penemuan yang tidak konsisten di kalangan kumpulan etnik yang berbeza, serta kekurangan maklumat bagi penglibatan polimorfisme gen *ACE I/D* dan *ACTN3 R/X* dalam adaptasi latihan telah menghadkan keupayaan penyelidik untuk membuat kesimpulan yang bermakna yang berkaitan dengan kesan-kesan polimorfisme ini ke atas prestasi fizikal dan kesihatan manusia. Oleh itu, penyelidikan kedoktoran ini melaksanakan tiga siri kajian untuk mengkaji kesan polimorfisme gen *ACE I/D* dan *ACTN3 R/X* ke atas prestasi fizikal dan kesihatan manusia dalam populasi Malaysia. Dalam kajian pertama, sampel DNA telah diambil melalui sel bukal daripada 180 orang Asia dari Malaysia (70 lelaki, 110 perempuan) berumur 20.4 ± 1.6 tahun, dan 180 orang Kaukasia dari Australia (62 lelaki, 118 perempuan) berumur 23.3 ± 3.6 tahun. Dalam kajian kedua, sampel DNA telah diambil daripada 180 atlet terlatih Malaysia (148 lelaki, 32 perempuan) berumur 20.5 ± 1.9 tahun, 180 kawalan sedentari Malaysia, dan 33 atlet berkala Australia (semua lelaki) berumur 20.7 ± 4.0 tahun. Prestasi daya tahan dan muskular atlet Malaysia masing-masing telah dinilai dengan ujian 20 meter Yo-Yo berkala pemulihan tahap 2 dan ujian penguncupan sukarela maksimum. Dalam kajian yang ketiga, tiga puluh lelaki tidak terlatih normotensive, (ACE genotip: *II* = 10, *ID* = 10, dan *DD* = 10), menjalani latihan genggaman isometrik (IHG) (empat set 2

minit pengecutan isometrik pada 30% daripada penguncupan sukarela maksimum , dengan selang 1 minit rehat) 3 hari setiap minggu selama 8 minggu. Hasil kajian pertama menunjukkan bahawa pengagihan polimorfisme gen *ACE I/D* berubah di kalangan kumpulan etnik yang berbeza, tetapi tidak kepada polimorfisme gen *ACTN3 R/X*. Hasil yang diperolehi kajian kedua menunjukkan bahawa: a) Kesan polimorfisme ini pada prestasi daya tahan dan kekuatan/kuasa tidak berbeza mengikut kesukubangsaan. b) Alel *ACE D* dan alel *ACTN3 R* memberikan kelebihan dalam aktiviti-aktiviti yang memerlukan kekuatan/kuasa, dan c) Alel *ACE I* dan alel *ACTN3 R* tidak mempengaruhi prestasi daya tahan. Hasil daripada kajian terakhir menunjukkan bahawa polimorfisme gen *ACE I/D* mempunyai pengaruh positif dalam penyesuaian kardiovaskular dan otot berikut latihan genggaman isometrik di kalangan lelaki normotensive. Secara keseluruhan, kajian ini mengesahkan lagi tanggapan bahawa prestasi kekuatan/kuasa dipengaruhi oleh alel *ACE D* dan alel *ACTN3 R.* Sebagai tambahan, kajian ini menyimpulkan bahawa polimorfisme gen *ACE I/D* memodulatkan tindak balas kepada latihan genggaman isometrik dalam lelaki normotensive.

THE EFFECTS OF ANGIOTENSIN I-CONVERTING ENZYME (*ACE*) *I/D* AND ALPHA-ACTININ-3 (*ACTN3*) *R/X* GENE POLYMORPHISMS ON HUMAN PHYSICAL PERFORMANCE AND HEALTH WITHIN MALAYSIAN POPULATION

ABSTRACT

A disparity population data set in the current literature with limited reports among Asian samples, coupled with the inconsistent findings among different ethnic groups, and lack of information for the involvement of angiotensin I-converting enzyme (*ACE*) *I/D* and alpha-actinin-3 (*ACTN3*) *R/X* gene polymorphisms in training adaptation have limited the ability of researchers to draw meaningful conclusions pertaining to the effects of these polymorphisms on human physical performance and health. Therefore, this doctoral research implemented three series of studies to examine the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and health within the Malaysian population. In the first study, DNA samples were retrieved via buccal cell from 180 Asians from Malaysia (70 males, 110 females) aged 20.4 ± 1.6 years, and 180 Caucasians from Australia (62 males, 118 females) aged 23.3 ± 3.6 years. In the second study, DNA samples were retrieved from 180 well-trained Malaysian athletes (148 males, 32 females) aged 20.5 ± 1.9 years, 180 Malaysian sedentary controls, and 33 intermittent Australian athletes (all males) aged 20.7 ± 4.0 years. Endurance and muscular performances of Malaysian athletes were evaluated with 20 meters Yo-Yo intermittent recovery level 2 and maximal voluntary contraction tests, respectively. In the third study, thirty normotensive, untrained males (*ACE* genotype: *II* = 10, *ID* = 10, and *DD* = 10),

undergone isometric handgrip training (four sets of 2 minutes isometric contractions at 30% of maximal voluntary contraction, with 1 minute resting interval) 3 days per week for 8 weeks. The result from the first study indicated that the distribution of *ACE I/D* gene polymorphism varied among different ethnic groups, but not to *ACTN3 R/X* gene polymorphism. The findings obtained from the second study demonstrated that: a) The effects of these polymorphisms on endurance and strength/power performances did not vary by ethnicity, b) The *ACE D* allele and *ACTN3 R* allele conferred an advantage in activities that require strength/power, and c) The *ACE I* allele and *ACTN3 X* allele did not influence endurance performance. Finding from the final study demonstrated that *ACE I/D* gene polymorphism had a positive influence in cardiovascular and muscular adaptations following isometric handgrip training among normotensive men. Overall, this research reaffirms the notion that strength/power performance is influenced by the *ACE D* allele and *ACTN3 R* allele. In addition, this research concludes that the *ACE I/D g*ene polymorphism modulates response to isometric handgrip training in normotensive men.

CHAPTER 1

INTRODUCTION

1.1 Background and Scope of the Research

Genetics play a key role in almost every aspect of human physical performance and health. The influence of the genetic factor on human physical performance and health has been extensively studied over the past several decades (Bouchard et al., 1997, MacArthur and North, 2005, Bouchard and Hoffman, 2011). The first strong evidence for genetic involvement in human physical performance came from a family study known as the *HE*alth, *RI*sk factors, exercise *T*raining *A*nd *Ge*netics (HERITAGE) Family Study (Bouchard et al., 1995). Since then, efforts have been made to identify candidate genes to human physical performance. Through the first annual version of human gene map for performance and health-related fitness, several genes or markers related to physical performance and health-related phenotypes have been identified (Rankinen et al., 2001, Rankinen et al., 2002, Rankinen et al., 2004, Wolfarth et al., 2005, Rankinen et al., 2006, Bray et al., 2009). The most updated version of this yearly publication revealed that there are 239 genes associated with human physical performance (Bray et al., 2009).

Two of the most extensively investigated genes associated with human physical performance are angiotensin I-converting enzyme (*ACE*) and alpha-actinin-3 (*ACTN3*) genes (Ma et al., 2013). It has been suggested that possession of the *I* allele of the *ACE I/D* gene polymorphism may influence endurance performance as the

presence of *I* allele of the *ACE I/D* gene polymorphism has been reported to be more pronounced among endurance athletes, such as elite distance runners (Myerson et al., 1999, Alvarez et al., 2000, Hruskovicova et al., 2006, Min et al., 2009), rowers (Gayagay et al., 1998, Ahmetov et al., 2008b), triathletes (Collins et al., 2004, Shenoy et al., 2010), and long-distance swimmers (Tsianos et al., 2004b). Also, individuals with *I* allele have also been reported to have a higher capacity of maximal oxygen consumption (VO_2max) (Hagberg et al., 1998, Goh et al., 2009), higher percentage of slow twitch muscle fibres (Zhang et al., 2003), greater cardiac output (Hagberg et al., 2002), and higher heat tolerance (Heled, 2004). Meanwhile, possession of the *R* allele of the *ACTN3 R/X* gene polymorphism may offer additive effects on strength/power performance as the *RR* genotype (two copies of *R* allele) was observed more frequently in strength/power-oriented athletes, such as Russian power athletes (Druzhevskaya et al., 2008), elite-level bodybuilders and power lifters (Roth et al., 2008), gymnasts (Massidda et al., 2009), Indian power athletes (Kothari et al., 2011), and Polish power athletes (Cieszczyk et al., 2011), when compared with endurance athletes and controls. Individuals with *R* allele have also been reported to have greater strength/power capacity (Clarkson et al., 2005a, Moran et al., 2006b, Vincent et al., 2007, Norman et al., 2009, Shang et al., 2012, Erskine et al., 2014).

Hence, there is a growing body of evidence amplifying the significance of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in human physical performance (Yang et al., 2003, Cam et al., 2007, Voroshin and Astratenkova, 2008, Druzhevskaya et al., 2008, Goh et al., 2009, Kothari et al., 2011, Ma et al., 2013). On the contrary, some studies have failed to demonstrate the influences of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance (Sonna et al., 2001, Lucia et al., 2006,

Moran et al., 2006a, Amir et al., 2007, Ahmetov et al., 2008a, Döring et al., 2010). Therefore, it has remained uncertain if human physical performance is indeed influenced by *ACE I/D* and *ACTN3 R/X* gene polymorphisms. Moreover, it has been speculated that the inconsistencies observed in the present findings may be due to small sample size and ethnicity differences (Zilberman-Schapira et al., 2012).

The distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms have been reported to vary across ethnic groups in general populations worldwide (Batzer et al., 1994, Batzer et al., 1996, Mills et al., 2001, Clarkson et al., 2005a, Jayapalan et al., 2008). As for *ACE I/D* gene polymorphism, the frequency of *I* allele in the Caucasian population ranged from 0.78 to 0.23. However, in the Asian population, this frequency ranged from about 0.76 to 0.42. The distribution of *ACE I/D* gene polymorphism among the three ethnic groups in Malaysia; Malay (*I* allele: 0.71, *D* allele: 0.29), Chinese (*I* allele: 0.63, *D* allele: 0.37), and Indian (*I* allele: 0.58, *D* allele: 0.42), were reported to be different to each other with *I* and *D* alleles found to be more prevalent among the Malays (0.71) and Indians (0.42), respectively (Jayapalan et al., 2008). On the other hand, as for *ACTN3 R/X* gene polymorphism, the frequency of *R* allele in the Caucasian population ranged from 0.61 to 0.50, while in the Asian population, the frequency of *R* allele varied from 0.53 to 0.39.

The different pattern in the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms across different ethnicities were apparently consistent with the research findings on the effect of *ACE I/D* gene polymorphism in the susceptibility to certain diseases (Ishigami et al., 1995, Barley et al., 1996, Staessen et al., 1997, Kunz et al., 1998, Fujisawa et al., 1998, Sagnella et al., 1999, Ng et al., 2005). For instance,

a study by Ng et al. (2005) showed that the association between *ACE I/D* gene polymorphism and diabetic nephropathy was more common in the Asian population than those from the Caucasian population. Based on the findings in the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in general populations worldwide and the research findings on the effect of *ACE I/D* gene polymorphism in disease susceptibility, there is a possibility that the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance may vary depending on the ethnic origin, which indicates that such findings previously reported for Caucasian population may not be relevant and could be different in Asian population. Nevertheless, whether the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance vary between different ethnic groups has remained unclear at present due to insufficient comparative analyses across ethnicities in the current literature (Zilberman-Schapira et al., 2012, Ma et al., 2013). A recent meta-analysis showed that the effects of the *ACE I/D* and the *ACTN3 R/X* gene polymorphisms on human physical performance have been mostly reported among Caucasian population and less reported in the Asian population (Ma et al., 2013).

Therefore, more research in the Asian population is needed to understand ethnic differences of the *ACE I/D* gene polymorphism, especially where the preliminary data suggest individual variation in response to exercise training could be influenced by this variant (Hagberg et al., 1999, Folland et al., 2000, Williams et al., 2000, Zhang et al., 2002, Giaccaglia et al., 2008). For instance, the *ACE I/D* gene polymorphism has been reported to influence adaptation to light weight lifting and walking training (Giaccaglia et al., 2008), isometric and dynamic leg training (Folland et al., 2000), as well as aerobic training (Hagberg et al., 1999, Williams et al., 2000,

Zhang et al., 2002). The results of these studies demonstrate that individuals with the same genotype of *ACE I/D* gene polymorphism exhibited similar adaptations to the training. While the deficient of the ACTN3 protein due to *ACTN3 R/X* gene polymorphism had been reported does not have any harmful health effects (North et al., 1999), the *ACE I/D* gene polymorphism had been reported to be associated with several disease such as hypertension (Barley et al., 1996, Sagnella et al., 1999). Moreover, several studies showed that blood pressure response to exercise training for health management also vary among individuals with different genotypes of *ACE I/D* gene polymorphism (Hagberg et al., 1999, Zhang et al., 2002, Kim, 2009). For instance, a study by Hagberg et al. (1999) found that after 9 months of endurance exercise training at 75 to 85 % of VO_2max, *I* allele carriers had reduced systolic and diastolic blood pressure more than *D* allele carriers. Despite these findings, it has remained unknown if the *ACE I/D* gene polymorphism can also influence cardiovascular and muscular responses to isometric handgrip training that had been found to be superior to the dynamic resistance exercise training in controlling and preventing high blood pressure in a normotensive population.

1.2 Statement of the Problems

The current literature pertaining to the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance has appeared to be inconsistent, which is speculated to be due to ethnicity factor. Furthermore, compelling evidence indicates that the influences of the *ACE I/D* and the *ACTN3 R/X* gene polymorphisms on human physical performance may vary across ethnicity. However, whether the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical

performance vary across ethnicity have remained unclear due to the disparity discovered in the data set for population-based studies in the current literature. Moreover, despite available evidence supporting the effect of *ACE I/D* gene polymorphism on adaptation to certain training programs, there is no evidence at present that the *ACE I/D* gene polymorphism may influence adaptation to isometric handgrip training in controlling and preventing high blood pressure in normotensive individuals.

Therefore, more comprehensive studies on *ACE I/D* and *ACTN3 R/X* gene polymorphisms across different ethnicities, particularly among the Asian population, are needed to determine if the effects of these variants vary across ethnicity. To the author's knowledge, there are limited studies examining the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within multi-ethnic Malaysian population. Based on the report by Jayapalan et al. (2008) on the distribution of *ACE I/D* gene polymorphism among multi-ethnic Malaysian populations, there is a possibility that the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance may vary between ethnic groups in Malaysia. Hence, such genetic information could be useful to identify potential elite athletes in Malaysia and to assist coaches in optimizing their athlete's training program. Besides that, a training study that implements the isometric handgrip exercise is also warranted to examine the training responses among normotensive individuals with different genotypes of *ACE I/D* gene polymorphism that may identify individuals who will lower resting blood pressure the most with this training program for health management.

1.3 Aims of the Research

The main objective of this research had been to examine the influences of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance, as well as health, within the Malaysian population. The specific objectives of this research are as the following:

(i) To investigate ethnic variation on *ACE I/D* and *ACTN3 R/X* gene polymorphisms by comparing the distribution of the data between Malaysian and Australian populations, as well as between four ethnic groups (Malay, Chinese, Indian, and Other Bumiputra) in Malaysia.

(ii) To examine the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on athletic status and human physical performance in the Malaysian population and to determine if the effects of these polymorphisms on human physical performance differ by ethnicity.

(iii) To examine the effect of *ACE I/D* gene polymorphism on cardiovascular and muscular adaptations following an 8-week isometric handgrip training on cardiovascular and muscular adaptations among normotensive men.

1.4 Significance of the Research

The findings retrieved from the series of experiments in this doctoral research project provide better comprehension on the involvement of the genetic factor on human physical performance among different ethnic groups. Furthermore, this research provides more information concerning *ACE I/D* gene polymorphism and training adaptation. In fact, besides establishing the influences of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within multi-ethnic Malaysian population, this study could be able to assist sports coaches in developing talent of athletes based on their genetic traits. In addition, the findings obtained from this doctoral research project also provide valuable information on training adaptation for health management and its association to genetic traits.

1.5 Thesis Structure

This thesis includes three separate studies relating to the influences of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and training intervention for health. A detailed review of the topic is discussed in Chapter 2 of the literature review. Meanwhile, Chapter 3 gives an overview of the research plan and the objectives of each study undertaken in this research project. The first study that was designed to examine the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms within the Malaysian population and its association with ethnicity are presented in Chapter 4. This is followed by the second study that investigated the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within the Malaysian population and determined if those effects differ by

ethnicity in Chapter 5. The final study, which is presented in Chapter 6, examined the influence of *ACE I/D* gene polymorphism on training adaptations for health. Lastly, the overall conclusion of this doctoral research project is presented in Chapter 7.

CHAPTER 2

LITERATURE REVIEW

2.1 The Human Genetics

Genetics is a branch of science that studies heredity and how an organism inherits, as well as transfers characteristics from one generation to the next (Winter et al., 2002). Human genetics, then, emphasizes on the variation that occurs in human beings (Winter et al., 2002). The fundamental component in genetics is known as a gene, which is a region of deoxyribonucleic acid (DNA) that contains particular codes for making a specific protein that is required for building tissues for the formation of organs (Mulvihill et al., 2011).

Generally, genes that control human physical traits occur in pairs called alleles, which are alternative forms of genes located on loci (positions) on the same chromosome (Pitman, 1993). For example, high or low, round or wrinkled, red or white. Each human inherits two alleles for each gene from the mother and the father (Walker, 2009). Alleles can exist in the form of dominant and recessive, and if a gene is composed of a pair of dominant alleles or only one dominant allele is present, the characteristic from the dominant allele will appear over the characteristic carried by the recessive allele (Pitman, 1993). However, the recessive allele is able to show its characteristic if paired with another recessive allele (Pitman, 1993). Thus, this natural selection creates a scenario, whereby different phenotype (characteristic) outcomes can result from one gene (Winter et al., 2002). Moreover, the differences in allele may

be significant to explain the variation in human physiology (e.g. muscle strength) (Mulvihill et al., 2011).

2.2 Influence of Genetics in Sports Performance

The ability of an individual to maximize personal potential is enormously complex. Undoubtedly, some factors, such as the volume of training, motivation, and environment, attribute to the success of an athlete (Baker and Davids, 2006). Nevertheless, if training is a crucial determinant for an athlete to increase the levels of body strength, agility, speed, and endurance, it does not seem to be a comprehensive factor of human physical performance as the genetic factor is more responsible for determining human innate potential (Baker and Davids, 2006). The genetic factor is likely to give a major impact towards physical trait, as a study among more than one million Swedish men reported that 81% of their body height was attributable to a genetic factor, while the left 19% were influenced by environment (Silventoinen et al., 2008).

The data obtained from twin studies are the best evidence to estimate precisely the contribution of genetic factors on physical performance and limit the environmental factor (Chatterjee and Das, 1995, Calvo et al., 2002, Maridaki, 2006, Alonso et al., 2014). For instance, a study by Chatterjee and Das (1995) among 30 pairs of monozygotic and 20 pairs of dizygotic twins showed that vital capacity, vertical jump, and heart rate were influenced more by genetic factors than environmental factors. Meanwhile, a study among 32 Caucasian male twins, who had similar environmental backgrounds, showed that the heritability of anaerobic capacity

was estimated between 20 and 70%, as measured with jumping tasks and the Wingate test (Calvo et al., 2002). A similar result was also successfully replicated in a study among 15 pairs of preadolescents and 15 pairs of adolescent female twins, which then supported a strong influence of genetic factors on muscular strength and power performance (Maridaki, 2006). The aerobic performance was also reported to be under genetic control through a twin study conducted in northeast Brazil that observed the rate of heritability of aerobic power to be 77% (Alonso et al., 2014).

Nonetheless, Bouchard and colleagues (1995) were the first to examine the association between the genetic factor and the human physical performance via the HERITAGE family study which involved 484 Whites from 99 families and 260 Blacks from 105 families that were exercise trained for 20 weeks and were tested for maximal oxygen consumption (VO_2max) on a cycle ergometer twice before and twice after the training program. Results from the HERITAGE family study found that there was 2.5 times more variance in changes in aerobic fitness between families than within families in responses to exercise interventions (Bouchard et al., 1995). Since then, this association has continued to be extensively investigated, as reported in the first (Rankinen et al., 2001, Rankinen et al., 2002, Rankinen et al., 2004, Wolfarth et al., 2005, Rankinen et al., 2006, Bray et al., 2009) and the second annual versions of the human gene map for performance and health-related fitness (Rankinen et al., 2010, Hagberg et al., 2011, Roth et al., 2012, Pérusse et al., 2013, Loos et al., 2015). In the initial publication of the human gene map for performance and health-related fitness by Rankinen and colleagues (2001), several genes or markers identified had been related to physical performance phenotypes. Subsequently, the number of genes identified had begun to increase and the last article of this yearly publication revealed

that 239 genes were associated with physical performance, which included cardio-respiratory endurance, elite endurance, athlete status, muscle strength, muscle performance traits, and exercise intolerance of variable degrees (Bray et al., 2009). From this large number of genes, two genes that have been extensively examined are the angiotensin I-converting enzyme (*ACE*) and the alpha-actinin-3 (*ACTN3*) genes (Ma et al., 2013). The *ACE* and *ACTN3* genes were suggested as the strongest candidate genes with the highest number of positive findings related to endurance and strength/ power performances, respectively (Ma et al., 2013).

2.3 The Angiotensin I-Converting Enzyme (*ACE*) Gene and Human Performance

2.3.1 The *ACE* Gene

In humans, the *ACE* gene is located on the long arm (q) of chromosome 17 (17q23.3), spans 21 kilo bases (kb) in length, and comprises of 26 exons and 25 introns, as illustrated in Figure 2.1 (Sayed-Tabatabaei et al., 2006). The *ACE* gene is responsible for producing ACE (Sayed-Tabatabaei et al., 2006), and it has been identified as a key component in the renin-angiotensin system (RAS); which is a hormone system that regulates blood pressure, water fluid balance, and tissue growth (Silverthorn, 2007). In addition, as illustrated in Figure 2.2, the main role of ACE in circulating RAS is to produce angiotensin II (ANG II), which is a potent vasopressor and aldosterone-stimulating peptide from angiotensin I (ANG I) (Coates, 2003), and to degrade bradykinin, a potent vasodilator that lowers blood pressure (Coates, 2003). Other than that, the plasma ACE level has been shown to differ between individuals, but identical

between family members, which indicates that the interindividual variation in the plasma ACE level is determined by genetic factors (Cambien et al., 1988). Among several polymorphisms in the *ACE* gene, the *ACE I/D* gene polymorphism (rs4646994) was found to have a strong linkage with the level of plasma ACE as it accounted for 47% of the total phenotypic variance of ACE activity (Rigat et al., 1990).

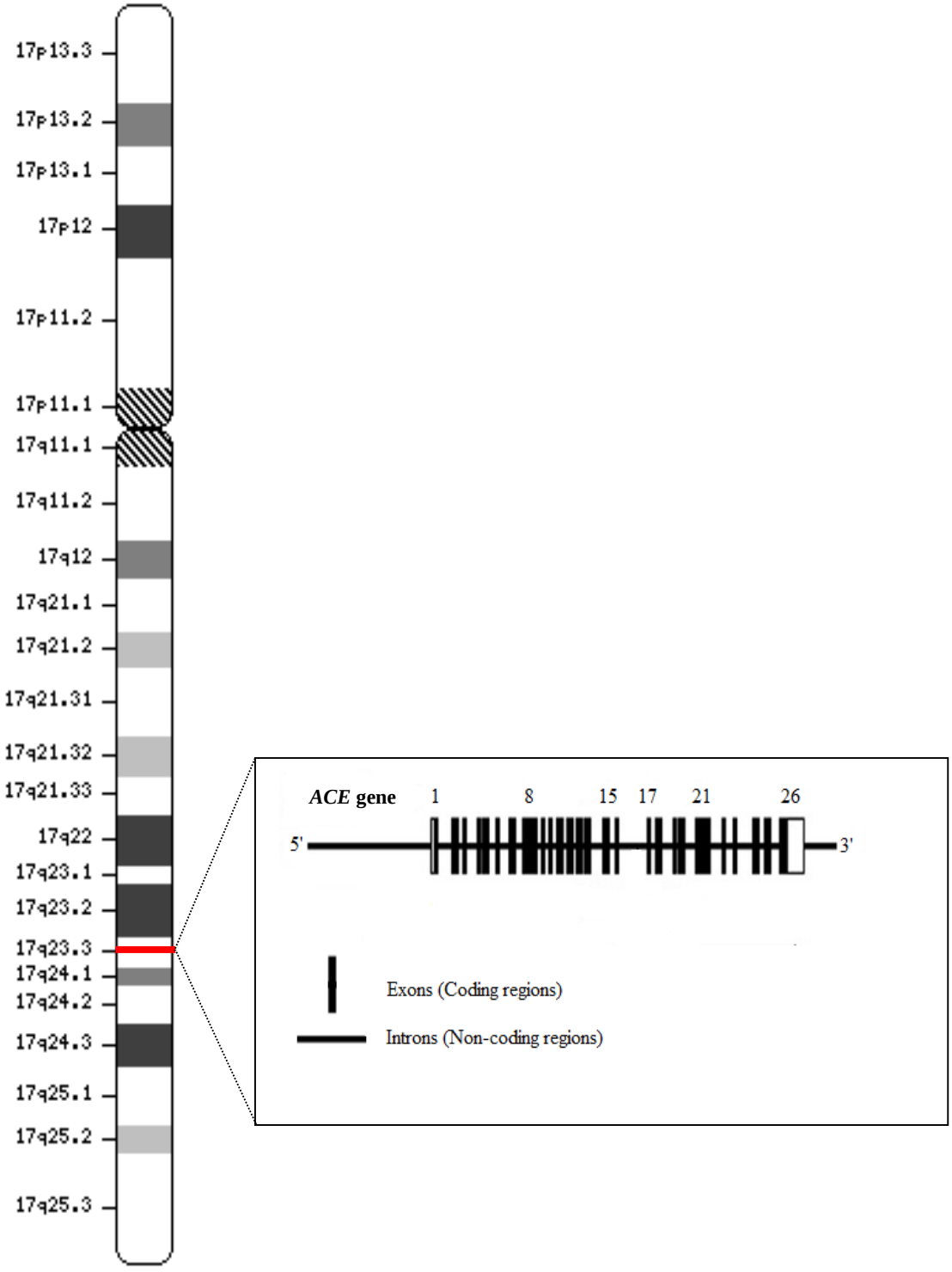


Figure 2.1 The genomic organization of the *ACE* gene on the long arm (q) of chromosome 17 on band 23.3. The *ACE* gene consists of 26 exons and 25 introns. *Picture adapted from Mayne (2006).

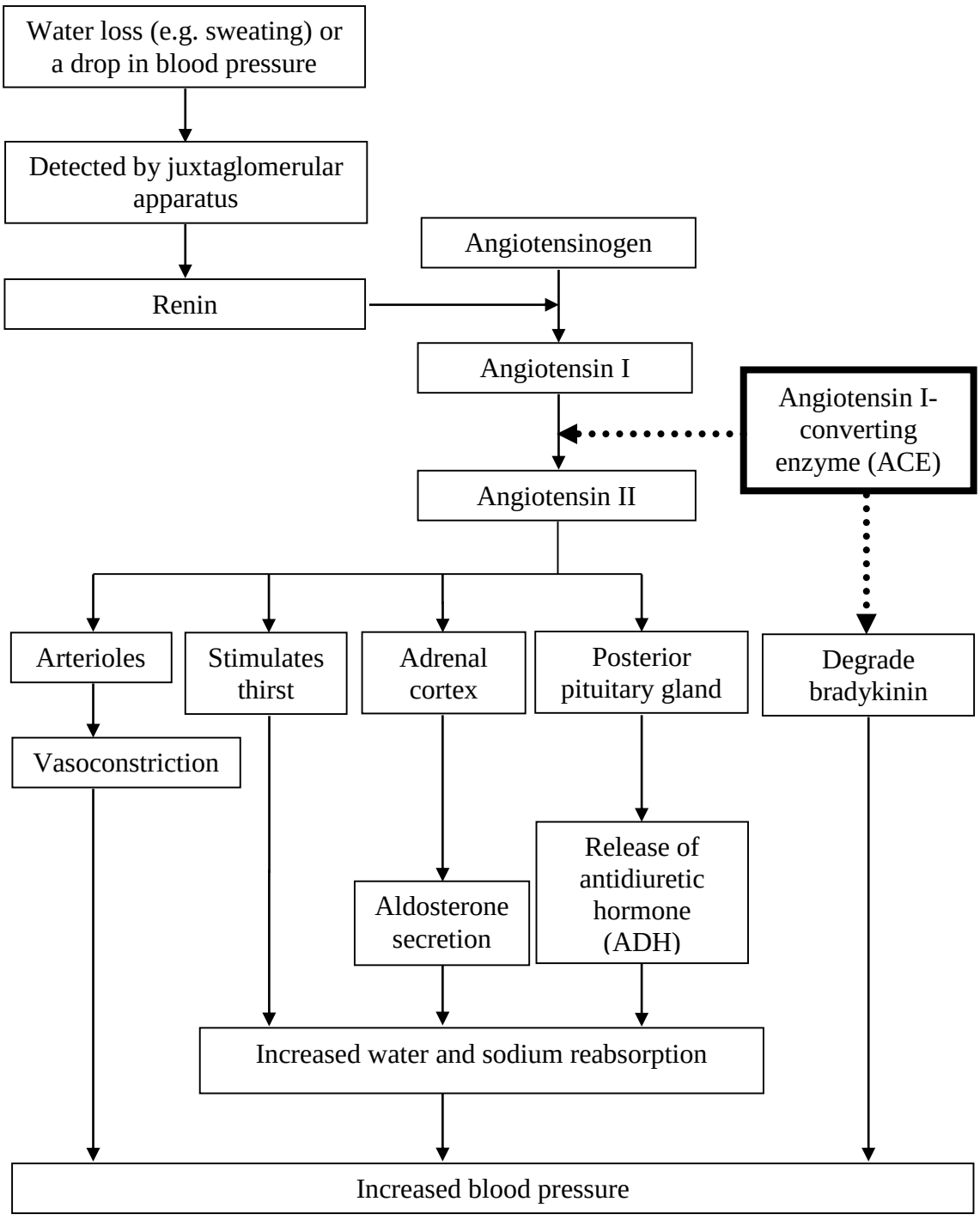


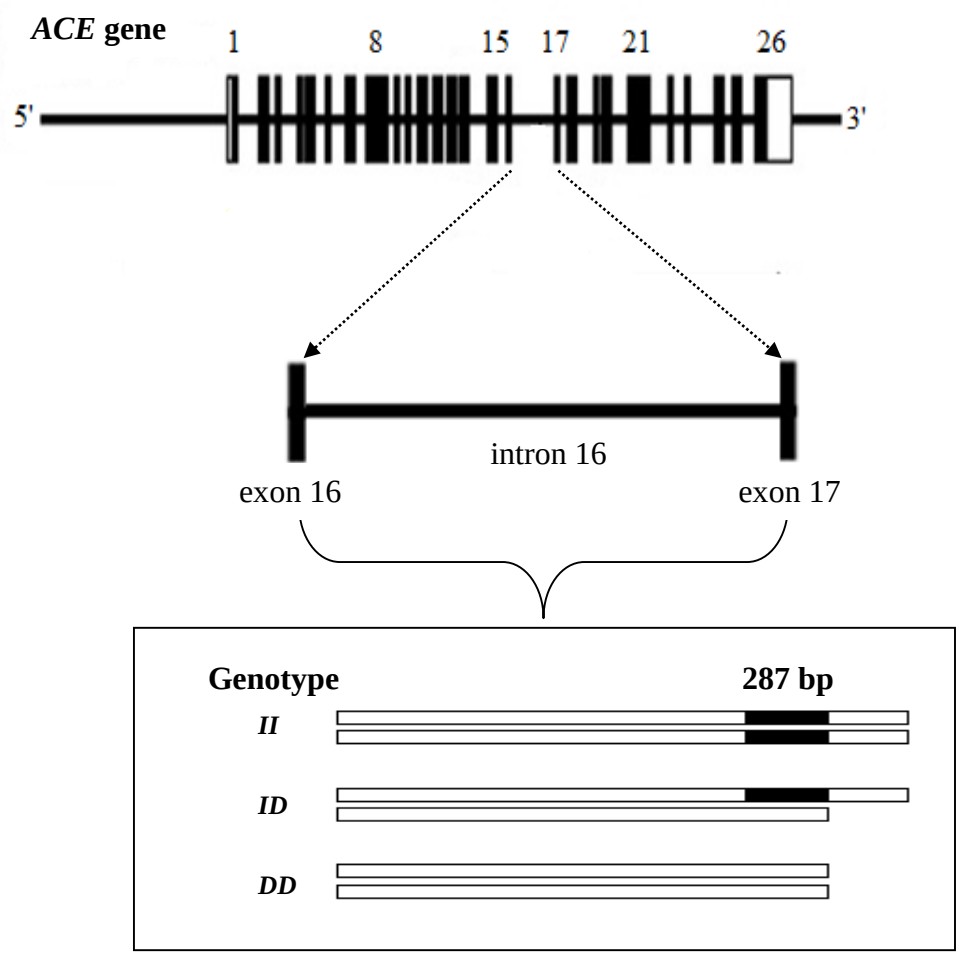
Figure 2.2 The role of ACE in the circulating renin-angiotensin system (RAS). *Picture adapted from Athilingam et al. (2012).

2.3.2 The *ACE I/D* Gene Polymorphism

As shown in Figure 2.3A, the *ACE I/D* gene polymorphism refers to the presence (Insertion "*I*") or absence (Deletion "*D*") of a-287 base pair (bp) alu repetitive sequence in intron 16 of *I* or *D* alleles on chromosome 17, respectively (Rigat et al., 1992). The ACE level was reported lower in individuals with two copies of *I* allele (Rigat et al., 1990). This led to a decrease in the conversion of ANG I to ANG II, which resulted in less vasoconstriction in skeletal muscle, and thus, an increased delivery of oxygenated blood to the working muscles (Sayed-Tabatabaei et al., 2006). Conversely, individuals with two copies of *D* allele had been reported to have a higher level of ACE (Rigat et al., 1990), which resulted in higher level of ANG II and led to a greater vasoconstriction, as well as reduced oxygenated blood flow to the working muscle (Jones and Woods, 2003, Sayed-Tabatabaei et al., 2006). Given these opposing physiological characteristics, *I* and *D* alleles may confer advantageous for endurance and strength/power events, respectively.

The *ACE I/D* gene polymorphism may result in three possible genotypes of *II* (with low ACE serum levels), *ID* (with intermediate ACE serum levels), and *DD* (with high ACE serum levels) (Rigat et al., 1990). Figure 2.3B shows a visual detection of *ACE I/D* genotypes with the presence of *I* and *D* alleles, which are presented by 490 bp and 190 bp, respectively (Rigat et al., 1992). Moreover, the distribution of *ACE I/D* gene polymorphism has been widely studied across many populations with allele and genotype frequencies being reported to vary across different racial groups (Barley et al., 1994, Batzer et al., 1994, Batzer et al., 1996, Jayapalan et al., 2008).

A.



B.

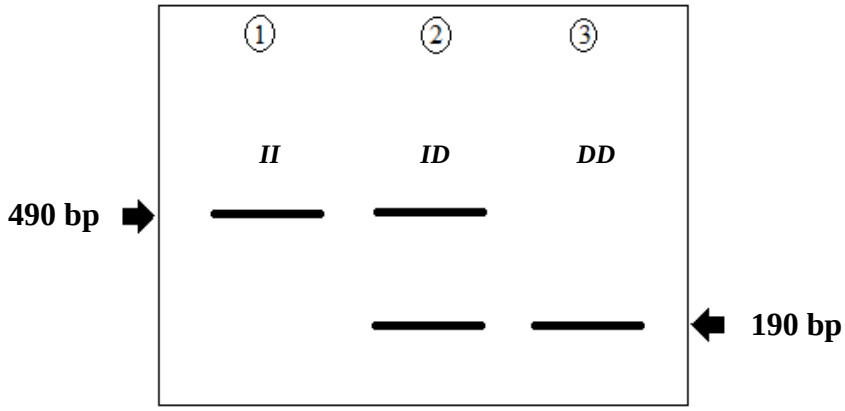


Figure 2.3 A. The *ACE I/D* gene polymorphism is characterized by an insertion or deletion of a 287-bp alu sequence at intron 16. B. A visual detection of *ACE I/D* genotypes. Lane 1: Homozygous *II* genotype (490 bp), Lane 2: Heterozygous *ID* genotype (490 bp and 190 bp), and Lane 3: Homozygous *DD* genotype (190 bp). *Pictures adapted from Mayne (2006).

2.3.3 The Distribution of *ACE I/D* Gene Polymorphism across Ethnicity

The current literature has observed variation in the distribution of the *ACE I/D* gene polymorphism in different racial and ethnic groups. Among the racial groups, the highest frequency of *I* allele was reported in the Black (Australian Aboriginal) population (0.97) (Lester et al., 1999), while the *D* allele was reported highest among the Caucasian population (0.77) (Tiret et al., 1992). In addition, the distribution patterns of *I* and *D* alleles in the Black population were about 0.97 to 0.27 and 0.73 to 0.03, respectively. In Black population, the Australian Aboriginal population was reported to have the highest frequency of *I* allele as compared to other ethnic groups of Black population (Lester et al., 1999). Other than that, the *D* allele was reported to be the most prevalent among Nigerians (Batzer et al., 1994) and Somalis (Bayoumi, 2006). Meanwhile, the trend observed among Amerindians (Vargas-Alarcon et al., 2003) was closely similar to those reported for Pima Indians (Foy et al., 1996), Coastal Papua New Guineans (Perna et al., 1992), Sothos (Rupert et al., 2003), Mulatto (Pereira et al., 2001), and Alaska Natives (Rupert et al., 2003).

On the other hand, in the Caucasian populations, the frequencies of *I* and *D* alleles ranged from 0.78 to 0.23 and 0.77 to 0.22, respectively. Among the Caucasians, the highest frequencies of *I* and *D* alleles were noted for Mexican (Vargas-Alarcon et al., 2003) and European (Tiret et al., 1992) populations, respectively. Nonetheless, *I* allele was observed to be less prominent among European (Tiret et al., 1992) and Caucasian populations from the Middle East, such as Egyptians (Ulu et al., 2006) and Omanis (Wang and Staessen, 2000). Moreover, the occurrence of *I* allele among Mexicans (Vargas-Alarcon et al., 2003) was observed to have close similarities with

the studies conducted by (Cambien et al., 1992) in European population. Furthermore, the high frequency of *D* allele observed among Europeans (Tiret et al., 1992) was fairly similar with those reported for Egyptians (Ulu et al., 2006) and Omanis (Wang and Staessen, 2000). The distribution trend of *ACE I/D* gene polymorphism in Australian samples (Lester et al., 1999, Lea et al., 2005) was reportedly identical with studies among the Brazilian (Pereira et al., 2001) and European (Renner et al., 2002) populations. In addition, results retrieved from several studies conducted in the same ethnic group, such as Turkish, were markedly similar to each other (Erdoğan et al., 2004, Cam et al., 2005, Sipahi et al., 2006, Berdeli and Cam, 2009). Nevertheless, varying results have been reported from studies in European populations. While Cambien et al. (1992) reported that the frequency of *I* allele was 0.73 in their cohort sample, *I* allele was found to be less frequent in other studies; 0.23 (Tiret et al., 1992), 0.43 (Vassilikioti et al., 1996), and 0.51 (Batzer et al., 1996).

Meanwhile, in the Asian population, the frequencies of *I* and *D* alleles ranged from 0.76 to 0.42 and 0.58 to 0.24, respectively. *I* allele was reported to be most prominent in the Javanese population (Sasongko et al., 2005), whilst the highest frequency of *D* allele was observed in the Kazakh sample (Aĭtkhozhina and Liudvikova, 2003). Besides, a study by Jayapalan et al. (2008) that looked into different ethnic groups in Malaysia observed higher frequencies of *I* and *D* alleles among Malays and Indians, respectively. Moreover, the frequency of *I* allele among the Malays in this study was markedly similar with Thai (Nitiyanant et al., 1997), *S*ingaporean Chinese (Lee, 1994), and Javanese (Sasongko et al., 2005) populations, while the higher *D* allele frequency detected among Indians was closely identical to previous finding of other Indian groups in Asia (Saha et al., 1996, Movva et al., 2007).

Additionally, the trend observed in the Chinese population in Malaysia was noticeably close to those reported for Hong Kong Chinese (Young et al., 1995), Taiwanese (Chuang et al., 1997), and Japanese (Tamaki et al., 2002) populations.

It is quite clear from these observations that ethnic variation has been demonstrated to be existed in the distribution of *ACE I/D* gene polymorphism. Nevertheless, to the best of author's knowledge, there is no report available for the distribution of *ACE I/D* gene polymorphism in certain ethnic groups particularly those from Asian population such as indigenous people of East Malaysia (will be referred as 'Other Bumiputra'). Hence, further studies are warranted to obtain the prevalence data of *ACE I/D* gene polymorphism in different ethnic groups to observe ethnic specificity in the distribution of *ACE I/D* gene polymorphism. The list of studies pertaining to the distribution of *ACE I/D* gene polymorphism across different ethnic groups is summarized in Table 2.1.

Table 2.1 Distribution of *ACE I/D* gene polymorphism in different ethnic groups

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		I	***D***		
Asian	Malaysian pooled	0.65	0.35	637	Jayapalan et al. (2008)
	Malaysian Malay	0.71	0.29	274	Jayapalan et al. (2008)
	Indian	0.55	0.45	460	Movva et al. (2007)
		0.55	0.45	166	Saha et al. (1996)
	Malaysian Indian	0.58	0.42	213	Jayapalan et al. (2008)
	Chinese	0.60	0.40	102	Huang et al. (2004)
		0.59	0.41	147	Saha et al. (1996)
	Hong Kong Chinese	0.63	0.37	183	Young et al. (1995)
	Malaysian Chinese	0.63	0.37	150	Jayapalan et al. (2008)
	Singaporean Chinese	0.69	0.31	671	Koh et al. (2003)
		0.70	0.30	189	Lee (1994)
	Taiwanese	0.64	0.36	189	Chuang et al. (1997)
	Japanese	0.67	0.33	1245	Matsubara et al. (2002)
		0.60	0.40	2168	Tamaki et al. (2002)
		0.69	0.31	90	Lau et al. (2002)
		0.67	0.33	113	Kario et al. (1997)
		0.67	0.33	46	Yoshida et al. (1995)
	Thai	0.70	0.30	298	Nitiyanant et al. (1997)
	Javanese	0.76	0.24	136	Sasongko et al. (2005)
	Kazakh	0.42	0.58	145	Aĭtkhozhina and Liudvikova (2003)
	Korean	0.61	0.39	13914	Yoo (2005)

Table 2.1 Continued

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		I	*D*		
Caucasian	European	0.48	0.52	2413	Stephens et al. (2005)
		0.48	0.52	3001	Mattace-Raso et al. (2004)
		0.46	0.54	522	Renner et al. (2002)
		0.41	0.59	357	Ferrieres et al. (1999)
		0.43	0.57	84	Vassilikioti et al. (1996)
		0.51	0.49	57	Batzer et al. (1996)
		0.49	0.51	186	Barley et al. (1994)
		0.73	0.27	733	Cambien et al. (1992)
		0.23	0.77	98	Tiret et al. (1992)
	Brazilian	0.42	0.58	65	Sprovieri and Sens (2005)
		0.46	0.54	150	Pereira et al. (2001)
	Australian	0.46	0.54	244	Lea et al. (2005)
		0.56	0.44	634	van Bockxmeer et al. (2000)
		0.46	0.54	100	Lester et al. (1999)
	Breton	0.58	0.42	41	Batzer et al. (1994)
	French	0.47	0.53	346	Marre et al. (1997)
		0.48	0.52	54	Batzer et al. (1996)
	French Acadian	0.48	0.52	53	Batzer et al. (1996)
	Greek Cypriot	0.51	0.49	46	Batzer et al. (1996)
	Egyptian	0.33	0.67	188	Salem (2008)
		0.28	0.72	188	Ulu et al. (2006)
	Emirate	0.39	0.61	164	Bayoumi et al. (2006)
	Omanis	0.29	0.71	159	Wang and Staessen (2000)
	Syrian	0.40	0.60	127	Salem (2008)

Table 2.1 Continued

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		I	*D*		
	Sudanese	0.36	0.64	70	Bayoumi et al. (2006)
	Mexican	0.78	0.22	300	Vargas-Alarcon et al. (2003)
	Swiss	0.37	0.63	43	Batzer et al. (1996)
	Turkish Cypriot	0.33	0.67	33	Batzer et al. (1994)
	Turkish	0.40	0.60	1063	Berdeli and Cam (2009)
		0.51	0.49	38	Sipahi et al. (2006)
		0.41	0.59	88	Cam et al. (2005)
		0.47	0.53	103	Erdoğan et al. (2004)
	Iranian	0.60	0.40	167	Abdi Rad and Bagheri (2011)
	Greek	0.38	0.62	352	Eleni et al. (2008)
	Slovenian	0.49	0.51	218	Zorc-Pleskovic et al. (2005)
	German	0.49	0.51	719	Mondry et al. (2005)
		0.51	0.49	163	Hohenfellner et al. (2001)
	Croatian	0.51	0.49	172	Barbalic et al. (2004)
	Polish	0.57	0.43	111	Zak et al. (2003)
	Italian	0.69	0.31	31	Massidda et al. (2012)
		0.52	0.48	92	Rigoli et al. (2004)
		0.43	0.57	684	Di Pasquale et al. (2004)
	Colombian	0.54	0.46	69	Camelo et al. (2004)
	Chilean	0.57	0.43	117	Jalil et al. (1999)
Black	Amerindian (Teenek and Nahuas)	0.61-0.78	0.22-0.39	68	Vargas-Alarcon et al. (2003)
	Pima Indian	0.71	0.29	184	Foy et al. (1996)
	Australian Aboriginal	0.97	0.03	53	Lester et al. (1999)
	Somalis	0.27	0.73	53	Bayoumi et al. (2006)