THE UNIVERSITY OF
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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

by

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TABLE OF CONTENTS

		Page
Acknowledgements		ii
Table of Contents		iii
List of Tables		viii
List of Figures		x
List of Abbreviations		xiii
List of Symbols		xvi
List of Operational Definitions		xviii
Abstrak		xix
Abstract		xxi
CHAPTER 1	**INTRODUCTION**	
1.1	Background and Scope of the Research	1
1.2	Statement of the Problems	5
1.3	Aims of the Research	7
1.4	Significance of the Research	8
1.5	Thesis Structure	8
CHAPTER 2	**LITERATURE REVIEW**	
2.1	The Human Genetics	10
2.2	Influence of Genetics in Sports Performance	11
2.3	The Angiotensin I-Converting Enzyme (*ACE*) Gene and Human Performance	13
2.3.1	The *ACE* Gene	13
2.3.2	The *ACE I/D* Gene Polymorphism	17
2.3.3	The Distribution of *ACE I/D* Gene Polymorphism across Ethnicity	19
2.3.4	*I* allele and Endurance Performance	26
2.3.5	*D* allele and Strength or Power Performance	36
2.4	The Alpha-Actinin-3 (*ACTN3*) Gene and Human Performance	42
2.4.1	The *ACTN3* Gene	42
2.4.2	The *ACTN3 R/X* Gene Polymorphism	45
2.4.3	The Distribution of *ACTN3 R/X* Gene Polymorphism across Ethnicity	47
2.4.4	*R* allele and Strength or Power Performance	51
2.4.5	*X* allele and Endurance Performance	58
2.5	Exercise and Blood Pressure	62
2.5.1	Endurance Exercise Training and Blood Pressure	62
2.5.2	Resistance Exercise Training and Blood Pressure	63
2.5.3	Isometric Resistance Exercise Training and Blood Pressure	65
2.5.4	*ACE I/D* Gene Polymorphism, Isometric Exercise Training, and Blood Pressure	68
2.6	Research Gaps in the Field	70
CHAPTER 3	**RESEARCH OVERVIEW**	
3.1	Introduction	73
3.2	Research Objectives and Hypotheses	77
3.2.1	Study 1: Distributions of *ACE I/D* and *ACTN3 R/X* Gene	77

Polymorphisms in Malaysian and Australian Populations, and Their Association with Ethnicity
3.2.2 Study 2: The Effects of *ACE I/D* and *ACTN3 R/X* Gene Polymorphisms on Human Physical Performance in the Multi-Ethnic Malaysian Population 77
3.2.3 Study 3: The Influence of *ACE I/D* Gene Polymorphism on Cardiovascular and Muscular Adaptations to 8 Weeks of Isometric Handgrip Training in a Normotensive Population in Malaysia 78

CHAPTER 4 DISTRIBUTIONS OF *ACE I/D* AND *ACTN3 R/X* GENE POLYMORPHISMS IN MALAYSIAN AND AUSTRALIAN POPULATIONS, AND THEIR ASSOCIATION WITH ETHNICITY

4.1 Introduction 79
4.2 Aims 83
4.3 Methods and Participants 83
4.3.1 Study Design 83
4.3.2 Participants 84
4.3.2.1 Malaysian Population 84
4.3.2.2 Australian Population 85
4.3.3 Deoxyribonucleic Acid (DNA) Sample Collection 85
4.3.4 Genotype Determination 86
4.3.4.1 DNA Extraction 86
4.3.4.2 Molecular Analysis for *ACE I/D* Gene Polymorphism 87
4.3.4.3 Molecular Analysis for *ACTN3 R/X* Gene Polymorphism 88
4.4 Statistical Analysis 89
4.5 Results 90
4.5.1 Hardy-Weinberg Equilibrium Test 90
4.5.2 The Distribution of *ACE I/D* Gene Polymorphism 90
4.5.2.1 Multi-Ethnic Groups in Malaysia 90
4.5.2.2 Malaysian and Australian Populations 93
4.5.3 The Distribution of *ACTN R/X* Gene Polymorphism 95
4.5.3.1 Multi-Ethnic Groups in Malaysia 95
4.5.3.2 Malaysian and Australian Populations 97
4.6 Discussion 99
4.7 Conclusion 105

CHAPTER 5 THE EFFECTS OF *ACE I/D* AND *ACTN3 R/X* GENE POLYMORPHISMS ON HUMAN PHYSICAL PERFORMANCE IN THE MULTI-ETHNIC MALAYSIAN POPULATION

5.1 Introduction 106
5.2 Aims 110
5.3 Methods and Participants 111
5.3.1 Study Design 111
5.3.2 Participants 111
5.3.2.1 Malaysian Population 112
5.3.2.2 Australian Population 113

5.3.3 Anthropometric Measurements 113
5.3.4 Deoxyribonucleic Acid (DNA) Sample Collection 114
5.3.5 Genotype Determination 114
5.3.5.1 DNA Extraction 114
5.3.5.2 Molecular Analysis for *ACE I/D* Gene Polymorphism 115
5.3.5.3 Molecular Analysis for *ACTN3 R/X* Gene Polymorphism 116
5.3.6 Physical Tests 117
5.3.6.1 Yo-Yo intermittent Recovery Level 2 Test 118
5.3.6.2 Handgrip Strength Test 119
5.3.6.3 Leg Strength Test 119
5.4 Statistical Analysis 120
5.5 Results 122
5.5.1 Physical Characteristics of Participants 122
5.5.1.1 The Whole Cohort of Athletes and Controls in the Malaysian Population 122
5.5.1.2 Endurance, Strength/Power, and Intermittent Athletes in the Malaysian Population 123
5.5.2 The Distribution of *ACE I/D* Gene Polymorphism 125
5.5.2.1 Hardy-Weinberg Equilibrium 125
5.5.2.2 The Whole Cohort of Athletes and Controls in the Malaysian Population 125
5.5.2.3 Multi-Ethnic Groups of Athletes and Controls in the Malaysian Population 127
5.5.2.4 Endurance Athletes, Strength/power Athletes, Intermittent Athletes, and Controls in the Malaysian Population 129
5.5.2.5 Malay and Caucasian Intermittent Athletes 132
5.5.3 Interaction between Ethnicity and *ACE I/D* Gene Polymorphism on Endurance and Strength/Power Performances 132
5.5.4 Effects of *ACE I/D* Gene Polymorphism on Endurance and Strength/Power performances in the Malaysian Population 134
5.5.4.1 *ACE I/D* Gene Polymorphism and Endurance Performance in the Malaysian Population 134
5.5.4.2 *ACE I/D* Gene Polymorphism and Strength/Power Performance (Handgrip Strength) in the Malaysian Population 134
5.5.4.3 *ACE I/D* Gene Polymorphism with Strength/Power Performance (Leg Strength) in the Malaysian Population 135
5.5.5 The Distribution of *ACTN R/X* Gene Polymorphism 137
5.5.5.1 Hardy-Weinberg Equilibrium 137
5.5.5.2 The Whole Cohort of Athletes and Controls in the Malaysian Population 137
5.5.5.3 Multi-Ethnic Groups of Athletes and Controls in the Malaysian Population 139
5.5.5.4 Endurance Athletes, Strength/Power Athletes, Intermittent Athletes, and Controls in the Malaysian Population 141
5.5.5.5 Malay and Caucasian Intermittent Athletes 143

5.5.6 Interaction between Ethnicity and *ACTN3 R/X* Gene Polymorphism on Strength/Power and Endurance Performances 143
5.5.7 Effects of *ACTN3 R/X* Gene Polymorphism on Strength/Power and Endurance Performances in the Malaysian Population 145
5.5.7.1 *ACTN3 R/X* Gene Polymorphism and Strength/Power Performance (Handgrip Strength) in the Malaysian Population 145
5.5.7.2 *ACTN3 R/X* Gene Polymorphism and Strength/Power Performance (Leg Strength) in the Malaysian Population 147
5.5.7.3 *ACTN3 R/X* Gene Polymorphism and Endurance Performance in the Malaysian Population 147
5.6 Discussion 149
5.7 Conclusion 158

CHAPTER 6 THE INFLUENCE OF *ACE I/D* GENE POLYMORPHISM ON CARDIOVASCULAR AND MUSCULAR ADAPTATIONS TO 8 WEEKS OF ISOMETRIC HANDGRIP TRAINING IN A NORMOTENSIVE POPULATION IN MALAYSIA
6.1 Introduction 159
6.2 Aim 163
6.3 Methods and Participants 163
6.3.1 Study Design 163
6.3.2 Participants 164
6.3.3 Familiarization Session 167
6.3.4 Preliminary Assessments 167
6.3.5 *ACE I/D* Genotyping Determination 168
6.3.6 Cardiovascular and Muscular Assessments 169
6.3.7 Isometric Handgrip Training 170
6.4 Statistical analysis 171
6.5 Results 172
6.5.1 Characteristics of Participants 172
6.5.2 Cardiovascular and Muscular Responses 172
6.5.3 *ACE I/D* Gene Polymorphism and Cardiovascular Response 174
6.5.3.1 Systolic Blood Pressure (SBP) 174
6.5.3.2 Diastolic Blood Pressure (DBP) 177
6.5.3.3 Mean Arterial Pressure (MAP) 180
6.5.3.4 Pulse Pressure (PP) 183
6.5.3.5 Heart Rate (HR) 185
6.5.4 *ACE I/D* Gene Polymorphism and Muscular Response 187
6.5.4.1 Handgrip Strength (HGS) 187
6.6 Discussion 189
6.7 Conclusion 194

CHAPTER 7 GENERAL CONCLUSION AND FUTURE DIRECTIONS
7.1 General Conclusion 195
7.2 Strengths and Limitations 198
7.3 Future Directions 200

REFERENCES 202

APPENDICES
APPENDIX A
APPENDIX B
APPENDIX C
APPENDIX D
APPENDIX E

LIST OF THESIS RELEVANT PUBLICATIONS AND CONFERENCE PRESENTATIONS

LIST OF TABLES

		Page
Table 2.1	Distribution of *ACE I/D* gene polymorphism in different ethnic groups	22
Table 2.2	Studies that investigated the effect of *I* allele on endurance performance	29
Table 2.3	Studies that investigated the effect of *D* allele on strength/power performance	38
Table 2.4	Distribution of *ACTN3 R/X* gene polymorphism in different ethnic groups	49
Table 2.5	Studies that investigated the effect of *R* allele on strength/power performance	53
Table 2.6	Studies that investigated the effect of *X* allele on endurance performance	60
Table 5.1	Physical characteristics of athletes and controls in the Malaysian population	122
Table 5.2	Physical characteristics of endurance, strength/power and intermittent athletes in the Malaysian population	123
Table 5.3	Yo-Yo intermittent recovery level 2 performance and leg strength value in athletes from different sporting disciplines in the Malaysian population	124
Table 5.4	Yo-Yo intermittent recovery level 2 performance and handgrip strength value in athletes with different *ACE I/D* genotypes	134
Table 6.1	Physical characteristics of participants according to *ACE I/D* genotype	172
Table 6.2	Cardiovascular and muscular responses to 8 weeks of IHG training	173
Table 6.3	Cardiovascular and muscular responses to a session of IHG exercise	173
Table 6.4	Systolic blood pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups	174

Page

Table 6.5	Diastolic blood pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups	177
Table 6.6	Mean arterial pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups	180
Table 6.7	Pulse pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups	183
Table 6.8	Heart rate at pre-, mid-, and post-training among *ACE I/D* genotype groups	185
Table 6.9	Handgrip strength at pre-, mid-, and post-training among *ACE I/D* genotype groups	187

LIST OF FIGURES

		Page
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).	15
Figure 2.2	The role of ACE in the circulating renin-angiotensin system (RAS). *Picture adapted from Athilingam et al. (2012).	16
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).	18
Figure 2.4	The genomic organization of the *ACTN3* gene on the long arm (q) of chromosome 11 on band 13.1. The *ACTN3* gene consists of 21 exons and 20 introns. *Picture adapted from Mayne (2006).	43
Figure 2.5	Skeletal muscle is composed of long cylindrical cells called muscle fibres. Each muscle fibre is composed of long tubes called myofibrils, which in turn, consist of filaments (actin and myosin). During muscle contraction, when these filaments are sliding past each other, the actin filament is anchored by the Z line, which is encompassed mainly by ACTN2 and ACTN3 proteins, for stabilization. *Picture adapted from and McMillan (2015).	44
Figure 2.6	A. DNA sequence and restriction endonuclease analysis of *ACTN3* gene exon 16 demonstrating the three possible *ACTN3 R/X* genotypes at position 577. Double peaks mean heterozygote. *Picture adapted from North et al. (1999). B. A visual detection of *ACTN3 R/X* genotypes. Lane 1: 50 bp DNA marker, lane 2: Homozygous *RR* (205 bp, and 86 bp), lane 3: heterozygous *RX* (205 bp, 108 bp, 97 bp, and 86 bp), and lane 4: homozygous *XX* (108 bp, 97 bp, and 86 bp). *Picture adapted from Mayne (2006).	46
Figure 3.1	The general research process	76
Figure 4.1	*ACE I/D* allele and genotype frequencies in the multi-ethnic groups in Malaysia	92
Figure 4.2	Frequencies of *ACE I/D* genotype in Malaysian and Australian populations	94

Page

Figure 4.3	*ACTN3 R/X* allele and genotype frequencies in the multi-ethnic groups in Malaysia	96
Figure 4.4	*ACTN3 R/X* genotype frequencies in Malaysian and Australian populations	98
Figure 5.1	*ACE I/D* allele and genotype frequencies in the whole cohort of athletes and controls in the Malaysian population	126
Figure 5.2	*ACE I/D* allele and genotype frequencies in multi-ethnic groups of athletes and controls in the Malaysian population	128
Figure 5.3	*ACE I/D* allele and genotype frequencies among endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population	131
Figure 5.4	*ACE I/D* genotype frequency in Malay and Caucasian intermittent athletes	133
Figure 5.5	The mean value of leg strength in athletes with different *ACE I/D* genotypes in the Malaysian population	136
Figure 5.6	*ACTN3 R/X* allele and genotype frequencies in the whole cohort of athletes and controls in the Malaysian population	138
Figure 5.7	*ACTN3 R/X* allele and genotype frequencies in multi-ethnic groups of athletes and controls in the Malaysian population	140
Figure 5.8	*ACTN3 R/X* allele and genotype frequencies among endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population	142
Figure 5.9	*ACTN3* R/X genotype frequencies in Malay and Caucasian intermittent athletes	144
Figure 5.10	The mean value of handgrip strength in athletes with different *ACTN3 R/X* genotypes in the Malaysian population	146
Figure 5.11	The mean value of leg strength in athletes with different *ACTN3 R/X* genotypes in the Malaysian population	148
Figure 6.1	Flow chart of the study design	165
Figure 6.2	Changes in systolic blood pressure value to IHG training in *ACE I/D* genotype groups	176
Figure 6.3	Changes in diastolic blood pressure value to IHG training in *ACE I/D* genotype groups	179

Page

Figure 6.4	Changes in mean arterial pressure value to IHG training in *ACE I/D* genotype groups	182
Figure 6.5	Changes in pulse pressure value to IHG training in *ACE I/D* genotype groups	184
Figure 6.6	Changes in heart rate value to IHG training in *ACE I/D* genotype groups	186
Figure 6.7	Changes in handgrip strength value to IHG training in *ACE I/D* genotype groups	188

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).

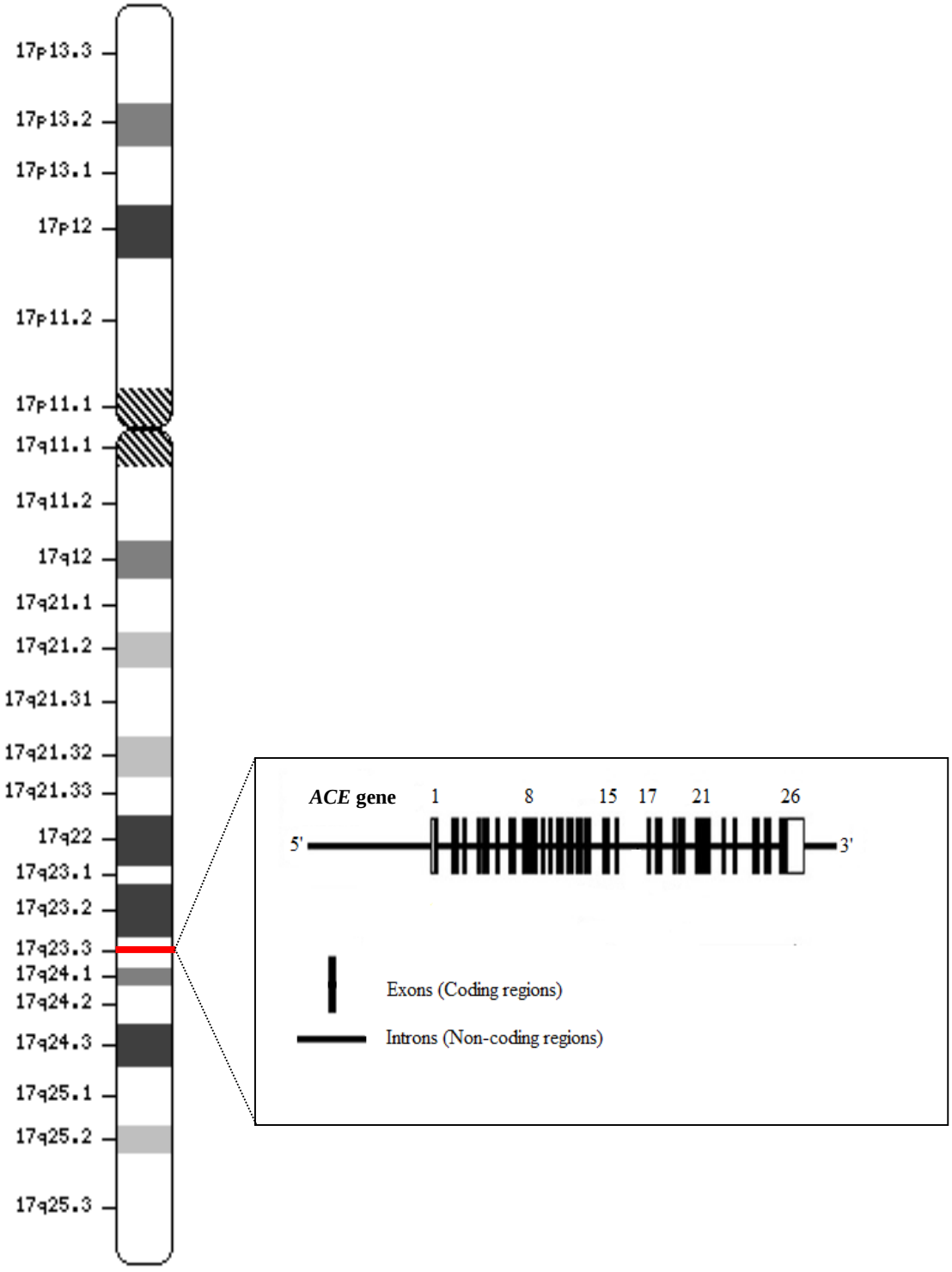


Figure 2.1 The genomic organization of the *ACE* gene on the long arm (q) of chromosome 17 on band 23.3. The *ACE* gene consists of 26 exons and 25 introns. *Picture adapted from Mayne (2006).

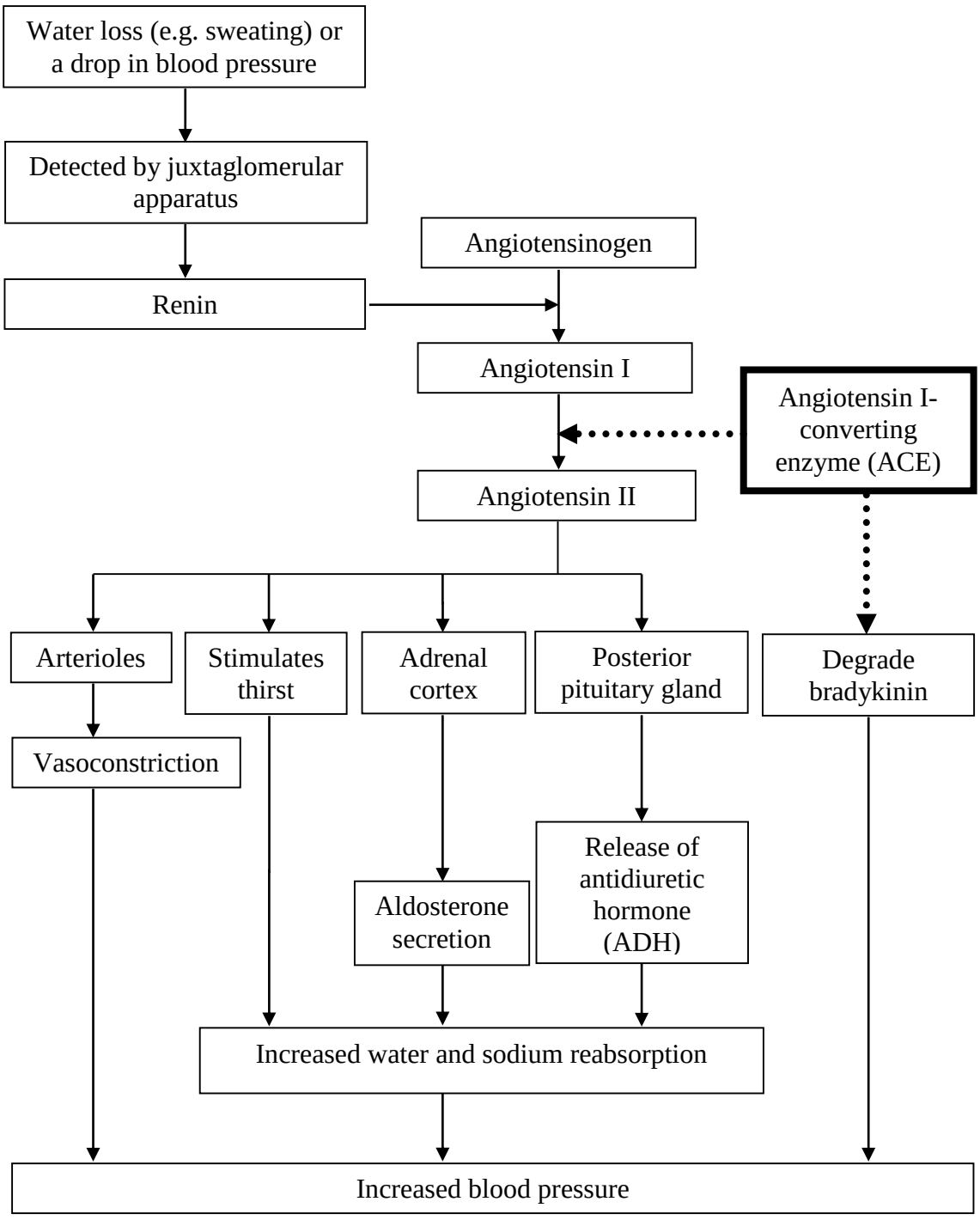


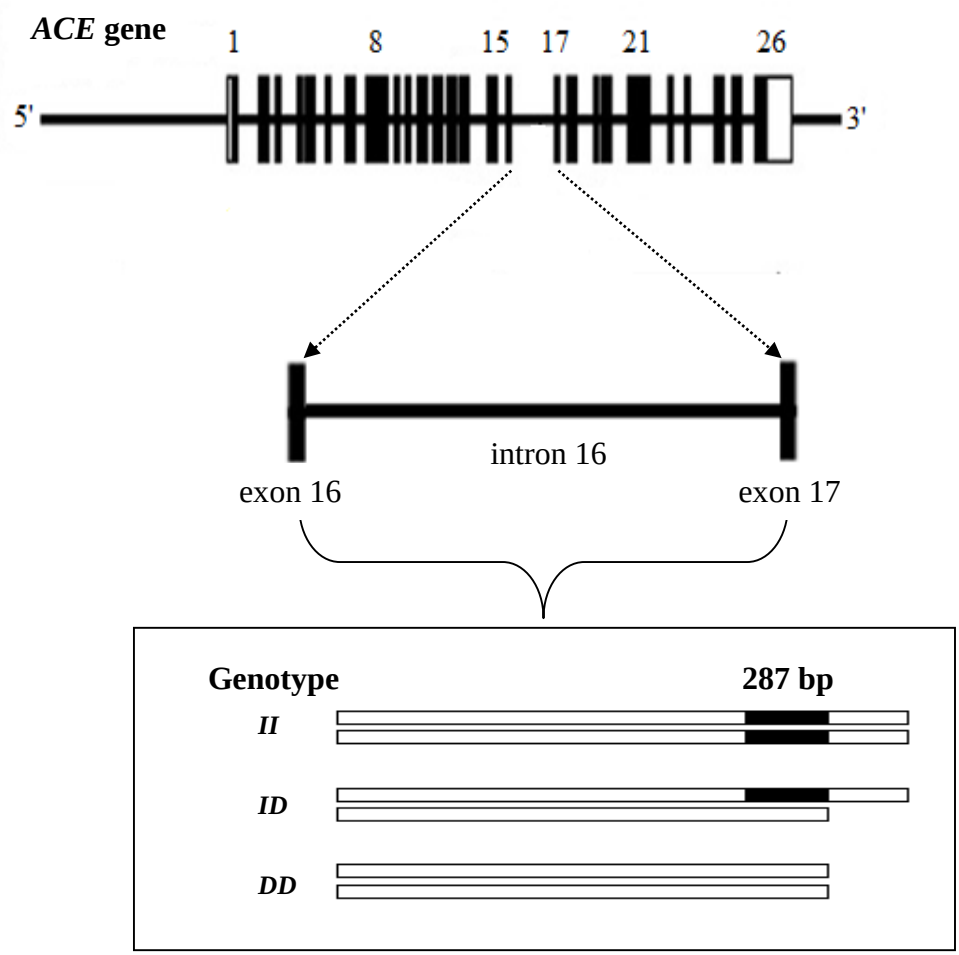
Figure 2.2 The role of ACE in the circulating renin-angiotensin system (RAS). *Picture adapted from Athilingam et al. (2012).

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.

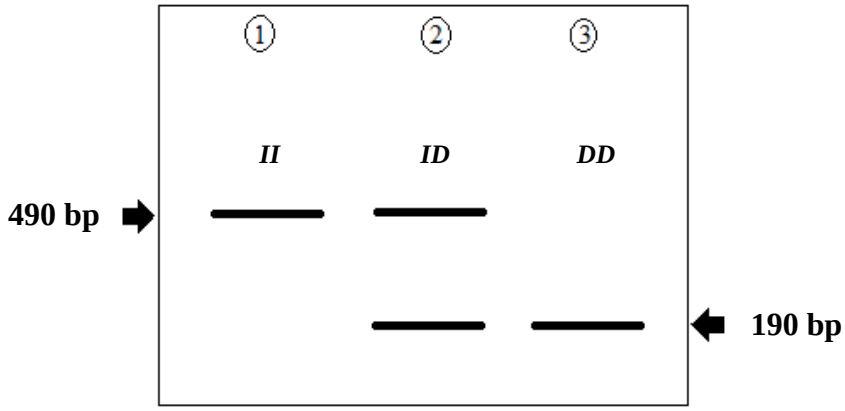


Figure 2.3 A. The *ACE I/D* gene polymorphism is characterized by an insertion or deletion of a 287-bp alu sequence at intron 16. B. A visual detection of *ACE I/D* genotypes. Lane 1: Homozygous *II* genotype (490 bp), Lane 2: Heterozygous *ID* genotype (490 bp and 190 bp), and Lane 3: Homozygous *DD*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

Table 2.1 Continued

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		I	*D*		
	Greek Cypriot	0.39	0.61	48	Batzer et al. (1994)
	Greenland Native	0.55	0.45	41	Batzer et al. (1996)
	Nguni	0.40	0.60	43	Soodyall et al. (1996)
	Nigerian	0.27	0.73	11	Batzer et al. (1994)
		0.37	0.63	80	Barley et al. (1994)
	Coastal Papua New Guinean	0.66	0.34	48	Perna et al. (1992)
	(PNG)	0.74	0.26	68	Perna et al. (1992)
	Highland Papua New Guinean	0.38	0.62	48	Soodyall et al. (1996)
	(PNG)	0.48	0.52	73	Foy et al. (1996)
	Sotho	0.73	0.27	111	Rupert et al. (2003)
	Alaska Native	0.74	0.26	51	Rupert et al. (2003)
	Kenyan	0.38	0.62	85	Scott et al. (2005)
	Black (Brazil)	0.57	0.43	92	Pereira et al. (2001)
	Mulatto (Brazil)	0.65	0.35	40	Pereira et al. (2001)
	Jamaican	0.41	0.59	311	Scott et al. (2010)
	Southwest African American	0.33	0.67	44	Scott et al. (2010)
	Northeast African American	0.44	0.56	72	Scott et al. (2010)
	Southeast African American	0.47	0.53	74	Scott et al. (2010)

2.3.4 *I* allele and Endurance Performance

A study by Gayagay et al. (1998) among sixty-four Australian national rowers had been the first report to display the additive effect of possession of the *I* allele of the *ACE* gene on endurance performance as a relative excess of both *I* allele and *II* genotype, which was found in the rowers, when compared to the controls. A similar finding was reported by Montgomery et al. (1998) for thirty-three elite male high-altitude mountaineers and 1,906 British males. Montgomery et al. (1998) also demonstrated that the UK army military recruits, who possessed *II* genotype, showed an 11-fold greater improvement in duration of exercise after a 10-week general physical training program when compared to those with *DD* genotype. Since then, *ACE* gene has attracted much attention worldwide as a candidate gene related to endurance performance (Bouchard and Hoffman, 2011).

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). Besides, 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).

Apart from that, some studies have attempted to identify if possession of the *I* allele would enhance the effect of exercise training on training adaptation. In comparison with *D* allele carrier, the *I* allele carrier was shown to enhance mechanical efficiency in trained muscle following an 11-week program of aerobic training (Williams et al., 2000), increased aortic distensibility due to chronic prolonged training (Tanriverdi et al., 2005a), greater exercise adherence to 6-month exercise training at 60% and 85% of maximal exercise capacity (Thompson et al., 2006), as well as higher improvement in 30 minutes' (at 70% of heart rate reserve) running speed performance after a 6-week anaerobic and aerobic training program (Cam et al., 2007).

While these findings are convincing, the effect of possession of the *I* allele on endurance performance has remained inconclusive at present as several studies failed to replicate the association between *I* allele and endurance athlete status (Taylor et al., 1999, Rankinen et al., 2000c, Scott et al., 2005, Oh, 2007, Amir et al., 2007, Tobina et al., 2010, Gineviciene et al., 2011, Ash et al., 2011). In addition, some cross-sectional studies revealed that individuals with *D* allele have better endurance performance (Tobina et al., 2010) and VO_2max values (Zhaoa et al., 2003) compared to those individuals with *I* allele. Moreover, individuals with the *DD* genotype had also been reported with a 14 to 38% greater increase in VO_2max with training than those subjects with *II* genotype (Rankinen et al., 2000b).

Reasons for these inconsistent findings are unknown, but ethnicity could be one of the factors associated with these findings based on the previous reports that the distribution of *ACE I/D* gene polymorphism varies across ethnic groups. Hence, to control this potential bias, population-specific research is warranted to confirm the

effect of possession of the *I* allele on endurance performance. Moreover, to date, there are limited studies have examined possession of the *I* allele on endurance performance among Asian population, such as Malaysian population, compared to Caucasian population (Ma et al., 2013). This limited data set raises the question of whether the effect of possession of the *I* allele on endurance performance that previously reported for Caucasians population would also appear in Asian population such as in Malaysian population. A summary of studies that investigated the effect of possession of the *I* allele on endurance performance is presented in Table 2.2.

Table 2.2 Studies that investigated the effect of possession of the *I* allele on endurance performance

Reference	Population	Study group (sample size)	p value	Results
Studies that reported the effect of possession of the *I* allele on endurance performance				
Shenoy et al. (2010)	Asian (Indian)	National level army triathletes (n=29) Controls (n=101)	0.02	*I* allele frequency in triathletes (0.85), controls (0.52)
Cieszcyzk et al. (2010)	Caucasian (Polish and Lithuanian)	Elite judo players (n=28) Controls (n=115)	0.02	*I* allele frequency in judo players (0.61), controls (0.44)
Goh et al. (2009)	Asian (Singaporean)	National rugby union players (n=17)	0.03	$V0_2$max was higher for the subjects with *II* genotype
Min et al. (2009)	Asian (Japanese)	Runners:- • Short distance (n=107) • Middle distance (n=62) • Long distance (n=108)	0.001	*I* allele frequency in runners:- • Short distance (0.44) • Middle distance (0.48) • Long distance (0.66)
Ahmetov et al. (2008b)	Caucasian (Russian)	Rowers (n=230) Controls (n=855)	< 0.05	*I* allele frequency in rowers (0.35), controls (0.29)
Voroshin and Astratenkov (2008)	Caucasian (Russian)	Elite 400 m distance runners (n=33)	< 0.01	*II* genotype favoured endurance performance and heart rate recovery
Cam et al. (2007)	Caucasian	Non-elite athletes (n=55)	< 0.05	Better improvement in aerobic endurance performance in *II* genotype carriers
Mayne (2006)	Caucasian	Athletes:- • Endurance (n=27) • Strength/power (n=63) Controls (n=48)	< 0.01	*I* allele frequency in endurance athletes (0.24), strength/power athletes (0.07), controls (0.13)

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Thompson et al. (2006)	Caucasian	A six-month program with the subjects (n=110)	< 0.01	*I* allele might increase adherence to exercise training regimen
Hruskovicová et al. (2006)	Caucasian	Marathon runners (n=104) • 1^{st} to 50^{th} places (n=20) • 51^{st} to 100^{th} places (n=28) • 101^{st} to 150^{th} places (n=26) • 151^{st} to 200^{th} places (n=30) Half-marathon runners (n=222) Inline skaters (n=18) Controls (n=252)	< 0.01	*I* allele frequency in marathon runners; • 1^{st} to 50^{th} places (0.65) • 51^{st} to 100^{th} places (0.52) • 101^{st} to 150^{th} places (0.56) • 151^{st} to 200^{th} places (0.55), half-marathon runners (0.48), inline skaters (0.61), controls (0.47)
Tanriverdi et al. (2005a)	Caucasian (Turkey)	• Endurance athletes (n=56) • Controls (n=46)	< 0.05	Aortic distensibility was increased by prolonged training in endurance athletes with the *II* genotype
Tanriverdi et al. (2005b)	Caucasian (Turkey)	• Endurance athletes (n=56) • Controls (n=46)	< 0.0001	Regular isotonic exercise improved endothelium-dependent vasodilatation, especially in those with the *II* genotype
Collins et al. (2004)	Caucasian	Triathletes:- • Fastest finishers (n=100) • Slowest finishers (n=100) Controls (n=199)	0.036	*I* allele frequency in triathletes:- • Fastest finishers (0.52) • Slowest finishers (0.48), controls (0.42)
Tsianos et al. (2004a)	Caucasian	Climbers (n=195)	0.01	*I* allele frequency for those who reached the summit was 0.47 than 0.21 for those who did not reach the summit

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Tsianos et al. (2004b)	Caucasian	Swimmers:- • 10 km distances (n=19) • 25 km races (n=16)	0.01	*I* allele frequency in swimmers:- • 10 km distances (0.29) • 25 km races (0.59)
Kasikcioglu et al. (2004)	Caucasian	•Elite wrestlers (n=29) •Controls (n=51)	< 0.001	*I* allele carriers had higher VO_2max than D allele carriers
Heled (2004)	Caucasian	Healthy male (n=58)	< 0.05	*I* allele increased heat tolerance
Zhang et al. (2003)	Asian (Japanese)	Untrained healthy young (n=41)	< 0.01	*I* allele carriers had higher percentage of slow twitch muscle fibres than *D* allele carriers
Hagberg et al. (2002)	Caucasian	Postmenopausal • Sedentary (n=20) • Physically active (n=20) • Endurance athletes (n=22)	< 0.05	*I* allele carriers had 25% greater cardiac output than *D* allele carriers
Nazarov et al. (2001)	Caucasian (Russian)	Swimmers:- • Long distance (n=12) • Middle distance (n=24) • Short distance (n=16) Controls (n=449)	0.042	*I* allele frequency in swimmers:- • Long distance (0.54) • Middle distance (0.65) • Short distance (0.31), controls (0.50)
Williams et al. (2000)	Caucasian	Army recruits (n=116)	< 0.025	*I* allele conferred an enhanced mechanical efficiency in trained muscle
Alvarez et al. (2000)	Caucasian (Spain)	Cyclists (n=25) Long-distance runners (n=25) Handball players (n=15) Controls (n=400)	0.0009	*I* allele frequency in cyclists (0.28), long-distance runners (0.25), handball players (0.20), controls (0.16)

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Myerson et al. (1999)	Caucasian	Runners:- • ≤ 200 m (n=20) • 400-3000 m (n=37) • ≥ 5000 m (n=34) Controls (n=1906)	0.009	*I* allele frequency in runners:- • ≤ 200 m (0.35) • 400-3000 m (0.53) • ≥ 5000 m (0.62), controls (0.49)
Hagberg et al. (1998)	Caucasian	Postmenopausal • Sedentary (n=19) • Physically active (n=19) Athletes (n=20)	< 0.05	The *II* genotype group had a 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$ higher VO_2max than the *ID* genotype group after accounting for the effect of habitual physical activity level
Montgomery et al. (1998)	Caucasian	Elite male British mountaineers (n=25) Controls (n=1906)	0.02	*I* allele frequency in mountaineers (0.70), controls (0.45)
Montgomery et al. (1998)	Caucasian	Army recruits (n=66)	0.001	*I* allele improved endurance performance
Gayagay et al. (1998)	Caucasian (Australian)	National rowers (n=64) Controls (n=114)	0.03	*I* allele frequency in rowers (0.57), controls (0.43)
Studies that reported possession of the *I* allele did not influence endurance performance				
Ash et al. (2011)	Caucasian (Ethiopian)	Athletes (n=114) • Endurance runners (n=76) • Sprint and power event athletes (n=38) Controls:- • Ethiopian population (n=317) • Endurance athlete-matched (n=410)	> 0.05	Elite endurance athlete status in Ethiopians was not influenced by *ACE I/D* gene polymorphism

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Holdys et al. (2011)	Caucasian (Polish)	Athletes (n=166) • Speed-strength (n=35) • Endurance-speed-strength (n=71) • Endurance (n=50) Controls (n=83)	> 0.05	No difference in mean values for the VO_2max between athletes with different genotypes
Gineviciene et al. (2011)	Caucasian (Lithuanian)	Athletes:- • Endurance (n=64) • Speed/ power (n=47) • Mix (n=33) • Team sports (n=49) Controls (n=250)	0.025	*II* genotype carriers had greater grip strength and vertical jump than *ID* and *DD* genotype carriers
Tobina et al. (2010)	Asian (Japanese)	Elite long distance (over 5000 m) runners (n=37) Control (n=335)	> 0.05	• The frequency of the *II* genotype in athletes was not significantly higher compared to non-athletes • Endurance performance was better in *DD* and *ID* genotype individuals than in *II* genotype individuals
Oh (2007)	Asian (Korean)	Elite mixed athletes (n=139) Controls (n=163)	> 0.05	The excess of *I* allele in long distance runners was not significant
Amir et al. (2007)	Caucasian (Israeli)	Athletes:- • Endurance (n=79) • Power (n=42) Controls (n=247)	< 0.05	*D* allele and *DD* genotype seemed to be higher in Israeli elite marathon runners than in sprinters

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Scott et al. (2005)	Kenyan	Athletes:- • International endurance (n=70) • National endurance (n=221) Controls (n=85)	0.39	Elite Kenyans' endurance athlete status was not influenced by *ACE I/D* gene polymorphism
Zhaoa et al. (2003)	Asian (Singaporean)	Students with prior exercise/ military training (n=67)	< 0.05	Subjects with the *DD* genotype had significantly higher level of VO_2max than other genotypes
Nazarov et al. (2001)	Caucasian	Swimmers:- • Long distance (n=12) • Middle distance (n=24) • Short distance (n=16) Controls (n=449)	0.656	No difference was found in frequencies between elite long distance swimmers and controls
Nazarov et al. (2001)	Caucasian	Track and field athletes:- • Long distance (n=10) • Middle distance (n=7) • Short distance (n=14) Controls (n=449)	0.687	No difference was found in frequencies between elite long distance track, field athletes, and controls
Sonna et al. (2001)	Caucasian	Army recruits (n=147)	> 0.05	*II* genotype did not have a strong effect on aerobic power or endurance in healthy young American adults
Rankinen et al. (2000b)	Caucasian and Black	Sedentary:- • Caucasian (n=476) • Black (n=248)	0.042	Individuals with the *DD* genotype had 14 to 38% of greater increase in VO_2max with training than *II* genotype carriers

Table 2.2 Continued

Reference	Population	Study group (sample size)	p value	Results
Rankinen et al. (2000c)	Caucasian	Endurance athletes (n=192) Controls (n=189)	> 0.05	*ACE I/D* gene polymorphism was not associated with the higher cardiorespiratory endurance performance level
Taylor et al. (1999)	Caucasian (Australian)	National athletes aerobic sports(n=120) Controls (n=685)	> 0.05	*II* genotype did not confer elite athletic ability

2.3.5 *D* allele and Strength or Power Performance

As the elevation level of the ACE activity in individuals with *D* allele of the *ACE I/D* gene polymorphism increased the production of ANG II (a potent growth factor in cardiac and vascular tissues) in the skeletal muscle renin-angiotensin system, which is the potential mechanism where muscle cell growth and hypertrophy may be activated (Geisterfer et al., 1998, Kai et al., 1998), this allele has been thought to influence strength or power performance (Jones and Woods, 2003, Sayed-Tabatabaei et al., 2006). Therefore, several studies have attempted to examine the effect of possession of the *D* allele on strength or power performance. The *D* allele carriers were reported to possess the greatest muscle strength (Hopkinson et al., 2004, Williams et al., 2005, Costa et al., 2009b). In several case-control studies, the frequency of *D* allele was found to be higher among those athletes involved in strength or power-oriented events (Woods et al., 2001, Nazarov et al., 2001, Paulauskas et al., 2009, Costa et al., 2009a, Kikuchi et al., 2012, Wang et al., 2013, Eidera et al., 2013).

Montgomery et al. (1997) reported that the left ventricular mass was increased by 18% in male Caucasian military recruits after a 10-week physical training period, with the highest left ventricular growth observed in subjects with the *DD* genotype compared to other subjects with *II* and *ID* genotypes. Possession of the *D* allele may have protective effects to muscle damage as the subjects with *DD* genotype had recorded the lowest blood creatine kinase values in response to eccentric contractions than the other genotype carriers (Yamin et al., 2007). Meanwhile, in a study carried out by Folland et al. (2000), young adult men with *D* allele had been reported to have greater strength gains after following a 9-week strength training program compared to

those with *I* allele. Similarly, greater gain in strength was observed in older individuals with *DD* genotype after 18 months of walking and light weight training (Giaccaglia et al., 2008).

Nevertheless, the inconsistent findings in other studies have caused the effect of possession of the *D* allele on strength performance to remain inconclusive (Thomis et al., 2004, Kasikcioglu et al., 2004, Moran et al., 2006a, Charbonneau, 2007, Eynon et al., 2009a, Rodríguez-Romo et al., 2010, Scott et al., 2010, Gineviciene et al., 2011, Wang et al., 2013). Reasons for these inconsistent findings are unknown, but it could be due to ethnicity factor, and limited reports from Asian population such as from Malaysian population (Ma et al., 2013) as previously explained for the effect of possession of the *I* allele on endurance performance. Therefore, future research involving Asian population, particularly Malaysian population is warranted to confirm the effect of possession of the *D* allele on strength/power performance. The list of studies that investigated the effect of possession of the *D* allele on strength/power performance is summarized in Table 2.3.

Table 2.3 Studies that investigated the effect of possession of the *D* allele on strength/power performance

Reference	Population	Study group (sample size)	p value	Results
Studies that reported the effect of possession of the *D* allele on strength/power performance				
Eidera et al. (2013)	Caucasian (Polish)	Polish power athletes (n=100) Controls (n=354)	0.014	*D* allele frequency in power athletes (0.63), controls (0.53)
Wang et al. (2013)	Caucasian (European, Commonwealth, Russian, and American cohorts)	Swimmers;- • Short middle distance (SMD) (≤ 400 m) (n=125) • Long distance (LD) (> 400 m) (n=68) Controls (n=1694)	0.003 0.005	*D* allele was overrepresented in short-and-middle-distance swimmers
Ahmetov et al. (2013)	Caucasian	Healthy physically active pupils (n=457)	0.037	High results for standing long-jump test in boys with *D* allele
Kikuchi et al. (2012)	Asian (Japanese)	International wrestlers (n=52) National wrestlers (n=83) Controls (n=333)	0.000 0.002	*D* allele frequency in international wrestlers (0.59), national wrestlers (0.44), controls (0.26)
Costa et al. (2009a)	Portuguese	Swimmers:- •Elite short distance (n=25) •Elite middle distance (n=14) •Average short distance (n=23) •Average middle distance (n=9) Controls (n=100)	< 0.05	*D* allele frequency in swimmers:- •Elite short distance (0.78) •Elite middle distance (0.54) •Average short distance (0.61) •Average middle distance (0.72) controls (0.62)
Costa et al. (2009b)	Portuguese	Swimmer:- •Short distance (n=22) •Middle distance (n=13) Triathletes (n=23)	< 0.05	Higher right grip strength in *D* allele carriers compared to those with *I* allele carriers

Table 2.3 Continued

Reference	Population	Study group (sample size)	p value	Results
Paulauskas et al. (2009)	Caucasian (Lithuanian)	Wrestlers (n=16) Controls (n=116)	< 0.05	*D* allele frequency in wrestlers (0.66), controls (0.55)
Giaccaglia et al. (2008)	Caucasian	Older sedentary men and women (n=213)	0.04	*DD* genotype individuals showed greater gains in knee extensor strength compared to *II* genotype individuals
Yamin et al. (2007)	Caucasian (Israeli)	Healthy physical education students (n=70)	0.02	*II* genotype imposed an increased risk of developing muscle damage, whereas the *DD* genotype may have protective effects
Charbonneau (2007)	Caucasian and African American	Older sedentary adults	0.02	Caucasian males who carried at least one *D* allele exhibited more hypertrophy than *II* genotype carriers
Cerit et al. (2006)	Caucasian	Non-elite Turkish army recruits (n=186)	0.001	*DD* genotype seemed to have an advantage in development in short-duration aerobic performance
Williams et al. (2005)	Caucasian	Untrained men (n=81)	0.026	*DD* genotype carriers had greater muscle strength than other genotype carriers
Cam et al. (2005)	Caucasian (Turkish)	Running performance:- •Superior (n=30) •Mediocre (n=29) •Poor (n=29)	0.019	Better performance in short duration aerobic endurance was influenced by the *D* allele

Table 2.3 Continued

Reference	Population	Study group (sample size)	p value	Results
Kasikcioglu et al. (2004)	Caucasian	Elite wrestlers (n=29) Controls (n=51)	< 0.001	Left ventricular mass was found to be higher in *DD* genotype carriers (126.2 ± 2.9 g/m^2) than *II* (85.5 ± 4.0 g/m^2) or *ID* (110.1 ± 2.3 g/m^2) genotype carriers
Hopkinson et al. (2004)	Caucasian	Chronic Obstructive Pulmonary Disease Patients (n=103) Controls (n=101)	0.04	The *D* allele was associated with greater isometric quadriceps strength
Hernández et al. (2003)	Caucasian	Endurance athletes (n=61)	0.031	The extent of exercise-induced left ventricular hypertrophy in endurance athletes was influenced by the *D* allele
Graf et al. (2001)	Caucasian	Endurance trained elite athletes (n=83)	0.039	The highest ANG II plasma resting concentration was found in athletes with *DD* genotype
Myerson et al. (2001)	Caucasian	British Army homozygous for the *DD* (n=79) and *II* (n=62) genotypes	0.0009	Left ventricular growth was greater in the *DD* genotype carriers than other genotype carriers
Nazarov et al. (2001)	Caucasian (Russian)	Swimmers long distance (n=12) Swimmers middle distance (n=24) Swimmers short distance (n=16) Controls (n=449)	0.001	*D* allele frequency in swimmers:-long distance (0.46), middle distance (n=35), short distance (n=69), controls (0.50)
Woods et al. (2001)	Caucasian	Swimmers (n=56) Military recruits (n=1248)	0.005	*D* allele frequency in swimmers (0.59), military recruits (0.41)
Folland et al. (2000)	Caucasian	33 healthy males	< 0.05	The *ID* and *DD* genotypes carriers had greater strength gains than the *II* genotype carriers

Table 2.3 Continued

Reference	Population	Study group (sample size)	p value	Results
Montgomery et al. (1997)	Caucasian	Military recruits (n=460)	< 0.01	Left ventricular growth rose significantly only among the *DD* genotype carriers
Studies that reported possession of the *D* allele did not influence strength/power performance				
Wang et al. (2013)	Asian (Japanese and Taiwanese)	Swimmers SMD (≥ 100 m) (n=166) Swimmers LD (200-400 m) (n=160) Controls (n=1252)	< 0.05	The *I* allele was overrepresented in the short-distance swimmer group
Gineviciene et al. (2011)	Caucasian (Lithuanian)	Elite athletes (n=193) Controls (n=250)	< 0.05	Speed and power in Lithuanian athletes were determined by *I* allele
Rodríguez-Romo et al. (2010)	Caucasian (European)	Non-athletic young adults (n=281)	> 0.05	*ACE I/D* gene polymorphism did not seem to exert a major influence on muscle 'explosive' power
Scott et al. (2010)	Jamaican and Caucasian (US)	Jamaican sprinters (n=116) US sprinters (n=114) Jamaican controls (n=311) US controls (n=191)	0.37	No excess in *DD* genotype in elite sprint athletes relative to controls
Eynon et al. (2009a)	Caucasian (Israeli)	Sprinters (n=81) Controls (n=240)	0.00007	*D* allele frequency in sprinters (0.49), controls (0.66)
Charbonneau (2007)	Caucasian and African American	Older sedentary adults (n=243)	> 0.05	Skeletal muscle strength was not influenced by the *D* allele
Moran et al. (2006a)	Caucasian (Greeks)	Teenagers (n=1027)	< 0.05	Strength phenotypes were influenced by the *I* allele
Thomis et al. (2004)	Caucasian	Twins, who participated in the Leuven Twin & Training Study (n=57)	> 0.05	No evidence for the effect of *D* allele on skeletal muscle
Kasikcioglu et al. (2004)	Caucasian	Elite wrestlers (n=29) Controls (n=51)	> 0.05	The frequency in athletes did not differ significantly from controls

2.4 The Alpha-Actinin-3 (*ACTN3*) Gene and Human Performance

2.4.1 The *ACTN3* Gene

As illustrated in Figure 2.4, the *ACTN3* gene is located on the long arm of chromosome 11 (11q13.1) and it spans approximately 16,407 kb of genomic DNA, which embraces 21 exons and 20 introns. The *ACTN3* gene codes the ACTN3 protein, which is a member of the alpha-actinin-binding proteins (North et al., 1999). In humans, the alpha-actinin-binding proteins play an important role for structuring and for performing regulatory functions in cytoskeleton organization, as well as muscle contraction (Mills et al., 2001). In fact, four specific genes are responsible for the expression of different types of alpha-actinin-binding proteins; *ACTN1* and *ACTN4* genes code for non-muscle proteins (Kaplan et al., 2000), whereas *ACTN2* and *ACTN3* gene codes for myofibrillar proteins that are localized at the boundaries of sarcomeres in the contractile apparatus of muscle Z disk (Mills et al., 2001).

As portrayed in Figure 2.5, the Z line, which is composed of ACTN2 and ACTN3 proteins, acts to stabilize and to integrate the muscle contractile apparatus by cross-linking actin filaments and other structural components (Mills et al., 2001). The ACTN2 protein is expressed in all skeletal muscle fibres, while the ACTN3 protein is expressed only in the fast-twitch skeletal muscle fibre (Mills et al., 2001).

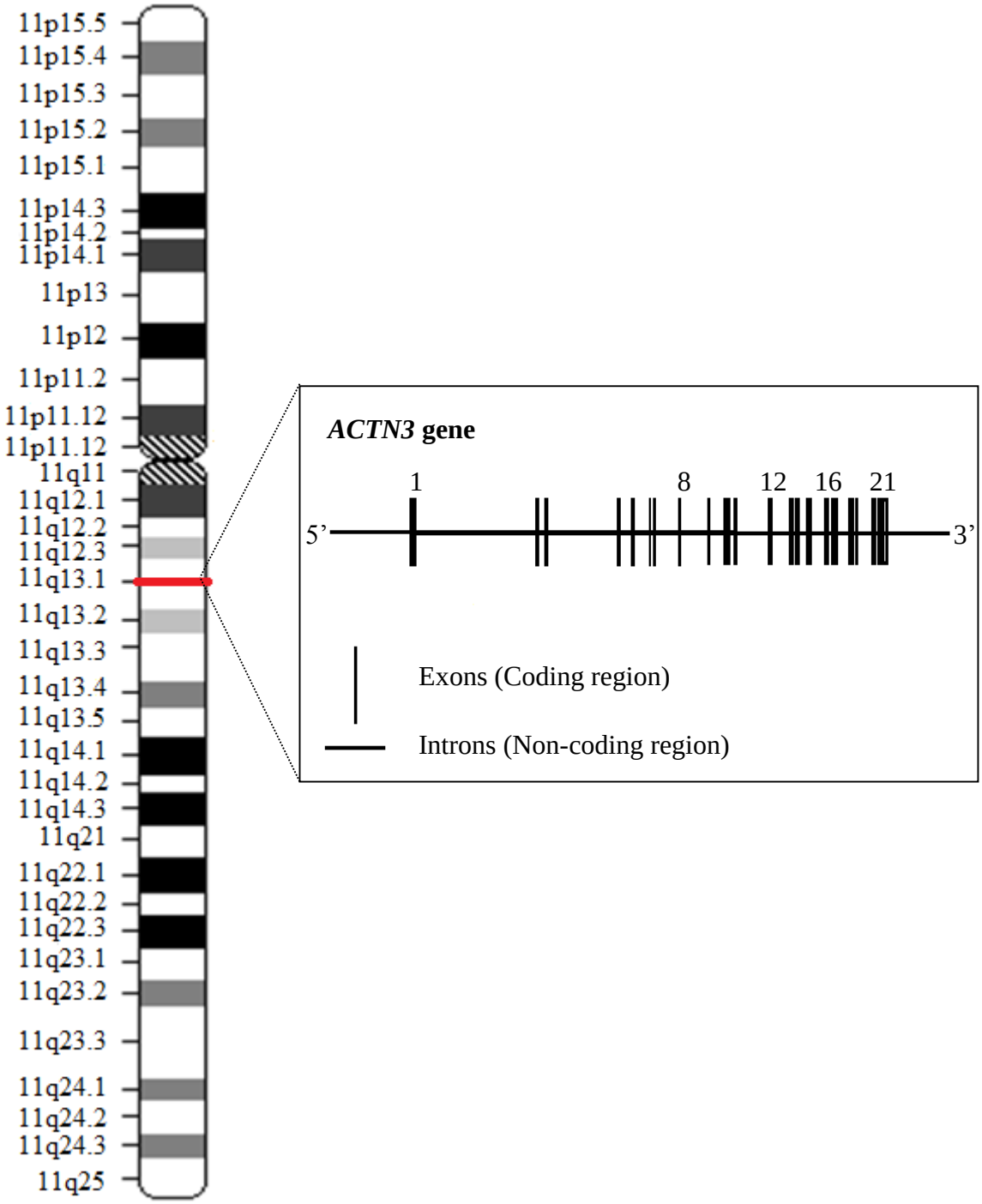


Figure 2.4 The genomic organization of the *ACTN3* gene on the long arm (q) of chromosome 11 on band 13.1. The *ACTN3* gene consists of 21 exons and 20 introns. *Picture adapted from Mayne (2006).

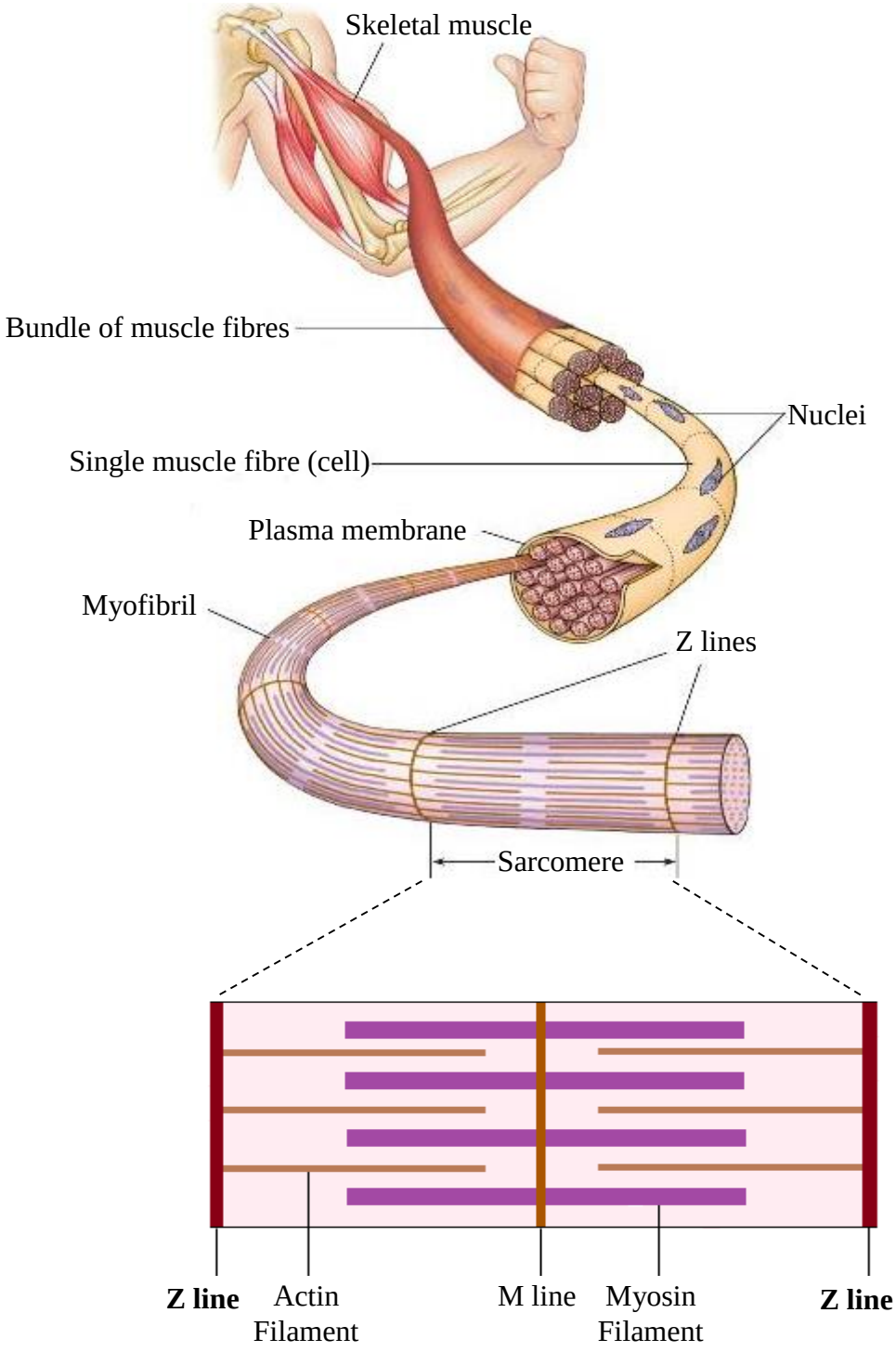


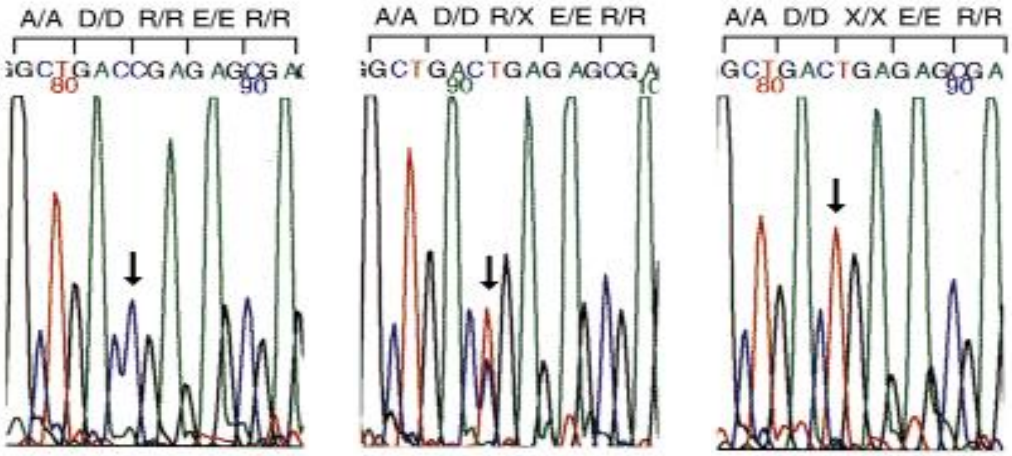
Figure 2.5 Skeletal muscle is composed of long cylindrical cells called muscle fibres. Each muscle fibre is composed of long tubes called myofibrils, which in turn, consist of filaments (actin and myosin). During muscle contraction, when these filaments are sliding past each other, the actin filament is anchored by the Z line, which is encompassed mainly by ACTN2 and ACTN3 proteins, for stabilization. *Picture adapted from Starr and Mcmillan (2015).

Due to the location of the ACTN3 protein, this protein is likely to give static and stable function in maintaining the ordered myofibrillar array with greater coordination to generate high muscle power and velocity during movements (Mills et al., 2001). The expression of ACTN3 protein was reported to depend on *ACTN3 R/X* gene polymorphism (North et al., 1999).

2.4.2 The *ACTN3 R/X* Gene Polymorphism

As shown in Figure 2.6A, the *ACTN3 R/X* gene polymorphism (rs1815739) is identified as a nonsense mutation, which specifically occurred in codon 577 of exon 16, where the translation (C to T) at nucleotide position 1,747 alters the production of arginine codon (*R*) to a premature stop codon (*X*) (North et al., 1999). The wild-type and the null mutations for *ACTN3 R/X* gene polymorphism are characterized by *R* and *X* alleles, respectively (North et al., 1999). The *R* allele codes for functioning *ACTN3* gene that results in the production of ACTN3 protein, whilst incomplete sequence in the *X* allele, preventing the production of that protein (North et al., 1999). MacArthur et al. (2007) suggested that the loss of ACTN3 protein leads to alterations in skeletal muscle metabolism towards more efficient aerobic metabolism. Given these different physiological functions, possession of the *R* and *X* alleles may grant beneficial effects for strength/power and endurance activities, respectively. Figure 2.6B displays the identification of the *ACTN3 R/X* genotype through gel visualization, where the PCR product is fragmented by enzyme *Dde*I and the presence of *R* allele is characterized by 205 bp and 86 bp, whereas for *X* allele are 108 bp, 97 bp, and 86 bp (North et al., 1999).

(A)



Homozygous wild-type (*RR*) | **Heterozygote (*RX*)** | **Homozygous null mutation (*XX*)**

(B)

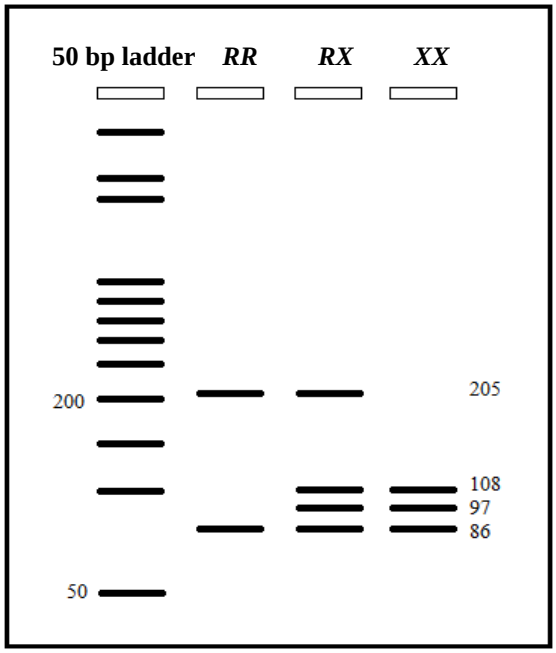


Figure 2.6 A. DNA sequence and restriction endonuclease analysis of *ACTN3* gene exon 16 demonstrating the three possible *ACTN3 R/X* genotypes at position 577. Double peaks mean heterozygote. *Picture adapted from North et al. (1999). B. A visual detection of *ACTN3 R/X* genotypes. Lane 1: 50 bp DNA marker, lane 2: Homozygous *RR* (205 bp, and 86 bp), lane 3: heterozygous *RX* (205 bp, 108 bp, 97 bp, and 86 bp), and lane 4: homozygous *XX* (108 bp, 97 bp, and 86 bp). *Picture adapted from Mayne (2006).

More than one billion people worldwide have reported an absence of ACTN3 protein (North et al., 1999). Moreover, it has been suggested that under certain environmental conditions, this gene variation could evolve to accommodate the energy expenditure requirements of people (North, 2008). Nevertheless, the distribution of *ACTN3 R/X* gene polymorphism is different across several populations.

2.4.3 The Distribution of *ACTN3 R/X* Gene Polymorphism across Ethnicity

There is evidence that the distribution of the *ACTN3 R/X* gene polymorphism varies between ethnic groups. The highest frequencies of *R* and *X* alleles were observed in the Black population. The prevalence of *R* and *X* alleles in the Black population ranged from 0.92 to 0.42 and 0.58 to 0.08, respectively. Among the different ethnic groups in the Black population, the lowest rate of ACTN3 deficiency was reported in the Nigerian group (Yang et al., 2007). The trend observed in Nigerians was similar to the findings obtained for Kenyans (Yang et al., 2007) and African Bantu (Mills et al., 2001). Meanwhile, the trend reported in Jamaican (Scott et al., 2010) and Highland Papua New Guinean (Mills et al., 2001) populations was remarkably comparable with the Northeast and the Southeast African Americans (Scott et al., 2010) and Ethiopians (Yang et al., 2007), respectively.

On the other hand, the distribution of *R* and *X* alleles in the Caucasian populations ranged from 0.61 to 0.50 and 0.50 to 0.39, respectively. Among the Caucasians, the highest frequency of *R* allele was noted for the Russian population (Druzhevskaya et al., 2008). Other than that, the distribution patterns of *R* and *X* alleles

in the Australian population (Yang et al., 2003) had been similar to the pattern discovered in the Iranian population (Fattahi and Najmabadi, 2012).

Meanwhile, in the Asian populations, the frequencies of *R* and *X* alleles vary from 0.53 to 0.39 and 0.61 to 0.47, respectively. The lowest prevalence of *R* allele was observed among Indians (Kothari et al., 2011). Besides, the distribution patterns of *R* and *X* alleles in Javanese population (Mills et al., 2001) were closely similar to those of Japanese population (Yang et al., 2007).

Although these data suggest variation in the distribution of the *ACTN3 R/X* gene polymorphism across different populations, the data lacks a comprehensive understanding for ethnic variation in the distribution of the *ACTN3 R/X* gene polymorphism as most of the findings involved the Black and the Caucasian populations with limited evidence within Asian populations. Therefore, more studies involving Asian populations are required to bridge the gap in the current literature. A list of population-based studies conducted across different ethnic groups is summarized in Table 2.4.

Table 2.4 Distribution of *ACTN3 R/X* gene polymorphism in different ethnic groups

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		R	***X***		
Asian	Asian pooled	0.53	0.47	55	Clarkson et al. (2005a)
		0.50	0.50	28	Mills et al. (2001)
	Javanese	0.46	0.54	48	Mills et al. (2001)
	Indian	0.39	0.61	150	Kothari et al. (2011)
		0.52	0.48	125	Goel and Mittal (2007)
	Japanese	0.47	0.53	97	Yang et al. (2007)
Caucasian	Caucasian Pooled	0.50	0.50	469	Clarkson et al. (2005a)
	European	0.58	0.42	107	Mills et al. (2001)
	Iranian	0.56	0.44	210	Fattahi and Najmabadi (2012)
	Australian	0.56	0.44	436	Yang et al. (2003)
	Spanish	0.52	0.48	123	Lucia et al. (2006)
	Italian	0.58	0.42	31	Massidda et al. (2012)
		0.57	0.43	53	Massidda et al. (2009)
	Polish	0.60	0.40	254	Cieszczyk et al. (2011)
	Israeli	0.51	0.49	240	Eynon et al. (2009b)
	Russian	0.61	0.39	1197	Druzhevskaya et al. (2008)
Black	Aboriginal Australian	0.71	0.29	87	Mills et al. (2001)
	African American	0.62	0.38	28	Clarkson et al. (2005a)
		0.73	0.27	45	Mills et al. (2001)
	Southwest African American	0.76	0.24	44	Scott et al. (2010)
	Northeast African American	0.83	0.17	72	Scott et al. (2010)
	Southeast African American	0.82	0.18	74	Scott et al. (2010)

Table 2.4 Continued

Racial group	Ethnic group	Allele frequency		Sample size (n)	References
		R	***X***		
	African Bantu	0.90	0.10	78	Mills et al. (2001)
	South African Bantu	0.89	0.11	88	Mills et al. (2001)
	Native American	0.57	0.43	7	Mills et al. (2001)
	Highland Papua New Guinean	0.64	0.36	39	Mills et al. (2001)
	Hispanic	0.42	0.58	25	Clarkson et al. (2005a)
		0.59	0.41	32	Mills et al. (2001)
	Jamaican	0.86	0.14	311	Scott et al. (2010)
	Ethiopian	0.66	0.34	105	Yang et al. (2007)
	Kenyan	0.91	0.09	158	Yang et al. (2007)
	Nigerian	0.92	0.08	60	Yang et al. (2007)

2.4.4 *R* allele and Strength or Power Performance

A study by Yang et al. (2003) had been the first to link the *ACTN3* gene to human physical performance. This study suggested that the *R* allele of *ACTN3 R/X* gene polymorphism could enhance sprint ability, as at least one copy of *R* allele was found in all male Olympian power and female elite sprint athletes (Yang et al., 2003). Moreover, Niemi and Majamaa (2005) reported similar findings with a higher frequency of the *R* allele in Finnish sprinters and a lower frequency of *R* allele in endurance athletes. They also reported that none of the top Finnish sprinters were predisposed for two copies of *X* allele (Niemi and Majamaa, 2005). Similar findings were also reported by Eynon et al. (2009b) with the frequency of *R* allele significantly higher in sprinters than endurance athletes and controls of Israeli population. Furthermore, 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.

Besides the positive finding on the effect of possession of the *R* allele on strength/power athletic status, 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). Possession of the *R* allele was also found to influence training adaptation (Clarkson et al., 2005b, Delmonico et al., 2007, Ichinoseki-Sekine et al., 2010, Gentil et al., 2011). After a 2-month home-based resistance training program, *R* allele carrier demonstrated greater

improvement in muscle strength than those with *X* allele (Ichinoseki-Sekine et al., 2010). Likewise, absolute peak power value change with strength training was found to be higher in the *RR* group than in the *XX* group (Delmonico et al., 2007). Possession of the functional *R* allele had also been reported to be associated with susceptible muscle damage (Clarkson et al., 2005b) and muscle thickness (Gentil et al., 2011) in response to training.

Contrary to the positive findings, several studies failed to identify the effect of possession of the *R* allele on strength/power performance (Clarkson et al., 2005a, Lucia et al., 2006, Santiago et al., 2010, McCauley et al., 2010, Scott et al., 2010, Djarova et al., 2011, Gineviciene et al., 2011, Wang et al., 2013, Ahmetov et al., 2013). For example, possession of the *R* allele had been reported to have no influence on knee extensor isometric strength (McCauley et al., 2010) and ventilatory thresholds (Lucia et al., 2006). In another study, individuals with *XX* genotype exhibited a greater absolute and relative one-repetition maximum (1-RM) gain after following resistance training (Clarkson et al., 2005a), as well as better grip strength and vertical jump (Gineviciene et al., 2011), compared to *RR* carriers. As previously explained in the effects of *ACE I/D* gene polymorphism on physical performance, these inconsistent findings could be due to ethnicity factor, and limited reports from Asian population (Ma et al., 2013). Hence, future study among Asian population, particularly Malaysian population is warranted to confirm the effect of possession of the *R* allele on strength/power performance. The list of studies that investigated the effect of possession of the *R* allele on strength/power performance is summarized in Table 2.5.

Table 2.5 Studies that investigated the effect of possession of the *R* allele on strength/power performance

Reference	Population	Study group (sample size)	p value	Results
Studies that reported the effect of possession of the *R* allele on strength/power performance				
Erskine et al. (2014)	Caucasian	Untrained healthy male (n=51)	< 0.05	*R* allele carriers had greater quadriceps femoris muscle volume, maximum isoinertial strength (1-RM), and maximum power than *X* allele carriers
Shang et al. (2012)	Asian (Chinese)	Young male soldiers (n=452)	0.021	*R* allele carriers displayed significantly higher handgrip strength than individuals with *X* allele
Kothari et al. (2011)	Asian (Indian)	Power athletes (n=72) Endurance athletes (n=83) Controls (n=150)	< 0.01	*R* allele frequency in power athletes (0.71), endurance athletes (0.31), controls (0.39)
Cieszczyk et al. (2011)	Caucasian (Polish)	Power athletes (n=158) Controls (n=254)	0.008	*R* allele frequency in power athletes (0.69), controls (0.60)
Pimenta et al. (2011)	Caucasian (Brazilian)	Soccer professional athletes (n=37)	< 0.05	*R* allele carriers were less susceptible to eccentric damage
Gentil et al. (2011)	Caucasian (Brazilian)	Men (n=141)	< 0.05	Only carriers of *R* allele showed an increase in muscle thickness in response to training
Djarova et al. (2011)	Zulu South African	Cricketers (n=14) Controls (n=17)	< 0.05	A high *R* allele frequency was found among cricketers with lower uric acid and lactic acid levels
Ichinoseki-Sekine et al. (2010)	Asian (Japanese)	Untrained elderly women (n=35)	< 0.05	Sit-ups and one-leg standing with open eyes were improved in the *R* allele group, but not in the *X* allele group

Table 2.5 Continued

Reference	Population	Study group (sample size)	p value	Results
Norman et al. (2009)	Caucasian	Moderate to well-trained men and women (n=120)	< 0.05	Repeated exercise bouts prompted an increase in peak torque in the *RR* genotype group
Massidda et al. (2009)	Caucasian (Italian)	Elite gymnasts (n=35) Controls (n=53)	< 0.04	*R* allele frequency in gymnasts (0.73), controls (0.57)
Eynon et al. (2009b)	Caucasian (Israeli)	Sprinters (n=74) Endurance runners (n=81) Controls (n=240)	< 0.05	*R* allele frequency in sprinters (0.69), endurance runners (0.43), controls (0.51)
Roth et al. (2008)	Caucasian and Black	Strength athletes Caucasian (n=52) Strength athletes Black (n=23) Controls Caucasian (n=668) Controls Black (n=208)	< 0.05	The *R* allele was overrepresented in elite strength (bodybuilder and power lifter) athletes
Druzhevskaya et al. (2008)	Caucasian (Russian)	Strength/sprint athletes (n=486) Controls (n=1197)	< 0.05	*R* allele frequency in strength/sprint (0.67), controls (0.61)
Vincent et al. (2007)	Caucasian	Healthy young males (n=90)	0.03	*R* allele carriers showed significantly higher relative dynamic quadriceps torques at 300°/s, compared to *XX* genotype carriers
Delmonico et al. (2007)	Caucasian	Older men (n=71) Older women (n=86)	< 0.05	• In men, absolute peak power (PP) changed with strength training (ST) in the *RR* genotype group, which was higher than in other groups • In women, relative PP changed with ST in the *RR* genotype group, which was higher than in other groups

Table 2.5 Continued

Reference	Population	Study group (sample size)	p value	Results
Mayne (2006)	Caucasian	Strength/power athletes (n=44) Endurance athletes (n=27) Controls (n=48)	< 0.01	*R* allele frequency in strength/power athletes (0.61), endurance athletes (0.56), controls (0.37)
Moran et al. (2006b)	Caucasian (Greek)	Adolescent (n=922)	0.003	Subjects with *RR* and *RX* genotypes had faster 40 m sprint time than *XX* genotype subjects
Niemi and Majamaa (2005)	Caucasian (Finnish)	Top endurance athletes (n=20) Endurance athletes (n=40) Top sprinters (n=23) Sprinters (n=68) Controls (n=1060)	0.03	The frequencies of *XX* and *RR* genotypes were higher and lower among Finnish endurance athletes, respectively, and none of the top Finnish sprinters had *XX* genotype
Clarkson et al. (2005b)	• Caucasian • African-American • Hispanic • Asian • Other	Sedentary:- • Caucasians (n=115) • African-Americans (n=4) • Hispanics (n=6) • Asians (n=20) • Other (n=11)	0.03	Subjects with *XX* genotype had the lowest muscle strength and the lowest resting creatine kinase activity than other genotype carriers
Clarkson et al. (2005a)	• Caucasian	Women (n=286)	< 0.05	*XX* genotype carriers had lower baseline strength than *RX* genotype carriers
Yang et al. (2003)	Caucasian (Australian)	Sprinters (n=107) Endurance athletes (n=194) Controls (n=436)	0.001	*R* allele frequency in sprinters (0.72), endurance athletes (0.54), controls (0.56)

Table 2.5 Continued

Reference	Population	Study group (sample size)	p value	Results
Studies that reported possession of the *R* allele did not influence strength/power performance				
Wang et al. (2013)	Caucasian and Asian	Caucasian swimmers;- • Short middle distance (SMD) (≥ 400 m) (n=125) • Long distance (LD) (≥ 400 m) (n=68) Caucasian Controls (n=1694) Asian swimmers;- • SMD (≥ 100 m) (n=166) • LD (200-400 m) (n=160) Asian Controls (n=1252)	> 0.05	Swimmer status in either Caucasians or East Asians was not influenced by *R* allele
Ahmetov et al. (2013)	Caucasian (Russian)	Middle school-age children (n=457)	< 0.05	High strength/power performance was not influenced by *R* allele
Djarova et al. (2011)	Zulu South African	Cricket players (n=14) Controls (n=17)	> 0.05	No significant difference was identified between two studied groups
Gineviciene et al. (2011)	Caucasian (Lithuanian)	Power athletes (n=51) Endurance athletes (n=77) Mixed athletes (n=65) Controls (n=250)	> 0.05	There was no significant difference in allele and genotype frequencies between the sports groups
Gineviciene et al. (2011)	Caucasian (Lithuanian)	Power athletes (n=51) Endurance athletes (n=77) Mixed athletes (n=65) Controls (n=250)	< 0.05	The grip strength and the vertical jump were better among athletes with *XX* genotype compared to athletes with *RR* genotype

Table 2.5 Continued

Reference	Population	Study group (sample size)	p value	Results
Scott et al. (2010)	Jamaican and US African American	Sprinter:- • Jamaican (n=116) • US (n=114) Controls:- • Jamaican (n=311) • US (n=191)	0. 67	Elite sprint athlete was not influenced by *R* allele
McCauley et al. (2010)	Caucasian	Older males aged 60-70 years old (n=100)	> 0.05	*R* allele did not affect the absolute and the relative high velocity strength
Santiago et al. (2010)	Caucasian (Spanish)	Healthy young adults (n=284)	> 0.05	*R* allele did not seem to influence the ability to produce peak (explosive) power
Lucia et al. (2006)	Caucasian (European)	Endurance runners (n=52) Cyclists (n=50) Controls (n=123)	> 0.05	• No significant difference existed between the genotype groups • No difference was found in indices of endurance performance (VO_2peak or ventilator thresholds) between athlete carriers of each *ACTN3 R/X* genotype
Clarkson et al. (2005a)	Caucasian and Asian	Caucasian women (n=286) Asian women (n=23)	< 0.05	Women with *XX* genotype displayed the greatest 1-RM response to training compared with those women with *RR* genotype after resistance training

2.4.5 *X* allele and Endurance Performance

A study using an ACTN3 knockout mouse model demonstrated that the lack of ACTN3 protein in an *X* allele carrier has resulted in alterations of skeletal muscle metabolism, explicit to aerobic metabolism, nicotinamide adenine dinucleotide (NADH), and succinate dehydrogenase (SDH) (MacArthur et al., 2007). The activity of the enzyme citrate synthase (CS), which is commonly found in slow- twitch fibre and also a marker for mitochondrial oxidative capacity, was found to be 22% significantly higher in mice with null mutation (*XX*) compared to wild-type mice (*RR*) (MacArthur et al., 2007). Moreover, the higher activity of beta hydroxyacyl-CoA dehydrogenase (*bHA*D) and medium-chain acyl-CoA dehydrogenase (*MCA*D), which are two mitochondrial enzymes involved in fatty acid oxidation in mice with deficiency of ACTN3 protein, suggested an increased reliance on aerobic instead of anaerobic metabolism (North, 2008).

Meanwhile, in another study involving an ACTN3 knockout mouse model, muscles with deficit in ACTN3 protein showed an increment in the twitch half relaxation time (MacArthur et al., 2008), which would significantly prolong the time taken for muscles to relax, and consequently, become detrimental to activities that require a repeated rapid contraction, such as sprinting and weight lifting, but providing another possible explanation that possession of the *X* allele may confer an advantage for endurance performance (Allen et al., 2008). The *X* allele appears to enhance endurance performance due to the higher frequency of the *XX* genotype in endurance athletes compared to controls, although this result is only reflected in female athletes (Yang et al., 2003). In addition, the frequency of *X* allele was also markedly over-

represented in Israeli endurance athletes (Eynon et al., 2009b) and Chinese female endurance athletes (Shang et al., 2010) compared to strength/power athletes and controls. Thus, the *X* allele has been suggested to give beneficial effects on endurance performance due to the higher VO_2max in the *X* allele carrier than those with *R* allele (Lucia et al., 2007).

Nonetheless, despite these positive findings, some studies have shown that *X* allele failed to influence endurance performance (Moran et al., 2006b, San Juan et al., 2006, Ahmetov et al., 2008a, Gomez-Gallego et al., 2009, Döring et al., 2010, Kothari et al., 2011). As previously explained in the effects of possession of the *R* allele on strength/power performance, these conflicting findings could be due to ethnicity factor, and limited reports from Asian population (Ma et al., 2013). Therefore, to further investigate the role of *X* allele on endurance performance, future research must involves Asian population, especially Malaysian population. The list of studies that investigated the effect of possession of the *X* allele on endurance performance is summarized in Table 2.6.

Table 2.6 Studies that investigated the effect of possession of the *X* allele on endurance performance

Reference	Population	Study group (sample size)	p value	Results
Studies that reported the effect of possession of the *X* allele on endurance performance				
Ahmetov et al. (2011)	Caucasian (Russian)	Sub elite Russian speed skaters (n=34) Controls (n=60)	< 0.05	*XX* genotype carriers exhibited a higher proportion of slow twitch fibres
Ahmetov et al. (2011)	Caucasian (Russian)	Speed skaters:- • Sprinters (n=39) • Middle distance (n=53) • Endurance-oriented (n=23) Controls (n=1301)	0.046	The frequency of *XX* genotype was significantly higher in endurance-oriented speed skaters than other skaters
Shang et al. (2010)	Asian (Chinese)	Endurance athletes:- • Male (n=132) • Female (n=118) Controls:- • Male (n=298) • Female (n=152)	0.02	• *X* allele frequency in female endurance athletes (0.51), controls (0.41) • No significant difference was observed in male endurance athletes vs. controls
Eynon et al. (2009b)	Caucasian (Israeli)	Endurance runners (n=81) Sprinters (n=74) Controls (n=240)	< 0.05	*X* allele frequency in endurance runners (0.47), sprinters (0.30), controls (0.45)
Lucia et al. (2007)	Caucasian (European)	Adult McArdle's disease patients (n=40) Sedentary controls (n=40)	< 0.05	Carriers of the *X* allele had a higher VO_2max at the ventilation threshold (VT) than those with *R* allele
Yang et al. (2003)	Caucasian (Australian)	Female Sprinters (n=35) Female Endurance athletes (n=72) Female Controls (n=292)	< 0.001	*X* allele frequency in sprinters (0.29), endurance athletes (0.47), controls (0.56)

Table 2.6 Continued

Reference	Population	Study group (sample size)	p value	Results
Studies that reported possession of the *X* allele did not influence endurance performance				
Kothari et al. (2011)	Asian (Indian)	Athletes:- Endurance (n=83) Power (n=72) Controls (n=150)	0.07	The *X* allele or the *XX* genotype was present at higher frequency in endurance athletes, but displayed insignificant association
Döring et al. (2010)	Caucasian (North America, Finland and Germany)	Male elite endurance athletes (n=316) Controls (n=304)	> 0.01	The prevalence of the *XX* genotype was similar in endurance athletes and controls
Gomez-Gallego et al. (2009)	Caucasian (Spanish)	• Elite professional road cyclists (n=46) • Healthy men (n=46)	< 0.05	Endurance phenotype trait was influenced by *R* allele
Ahmetov et al. (2008a)	Caucasian (Russian)	Rowers (n=54)	0.016	Male rowers with *RR* genotype showed better results in long-distance rowing than carriers of *RX* or *XX* genotypes
Ahmetov et al. (2008a)	Caucasian (Russian)	Endurance-oriented athletes (n=456) Controls (n=1211)	< 0.05	*X* allele was underrepresented in Russian endurance athletes
Moran et al. (2006b)	Caucasian (Greeks)	Adolescent (n=922)	> 0.05	Endurance phenotype was not influenced by *X* allele
San Juan et al. (2006)	Caucasian (Spanish)	Healthy, non-athletic elderly women (age 61–80 years) (n=23)	> 0.05	Complete deficiency of ACTN3 did not affect aerobic capacity

2.5 Exercise and Blood Pressure

At present, hypertension has become one of the significant risk factors for chronic diseases, such as heart attack and stroke (Chobanian et al., 2003). Moreover, the National Health and Morbidity Survey 2011 reported that 32.7% of adults aged 18 years old and above in Malaysia suffered from hypertension (Survey, 2011). Thus, it has been suggested that high blood pressure can be controlled and prevented with lifestyle modifications, such as dieting and exercising (Pescatello et al., 2004). In general, exercise helps to strengthen the heart where the heart will pump more blood with less effort, thus decreasing the force on arteries, which in turn lowers blood pressure (Dicker, 2010). Nevertheless, the optimal exercise training program in preventing and controlling high blood pressure has remained unclear as the magnitude of the exercise training effect may vary across different training prescriptions (Williams et al., 2007).

2.5.1 Endurance Exercise Training and Blood Pressure

Endurance exercise, which involves large muscle groups in dynamic activities such as walking and swimming, has shown to lower resting blood pressure in both hypertensive and normotensive individuals (Pescatello and Kulikowich, 2001, Cornelissen and Fagard, 2005b, Baynard et al., 2008, Goldberg et al., 2012). In fact, a recent meta-analysis study reported that endurance exercise training at a moderate to high intensity less than 210 minutes per week for less than 24 weeks reduced the resting systolic and diastolic blood pressure by 3.5 and 2.5 mmHg, respectively, with the largest reduction in blood pressure more pronounced in hypertensive than in

normotensive individuals (Cornelissen and Smart, 2013). Meanwhile, several studies have demonstrated that a similar magnitude of blood pressure reduction was observed for up to 22 hours after cessation of a single set of moderate-intensity endurance exercise training (Brownley et al., 1996, MacDonald et al., 2001, Cornelissen and Fagard, 2005b). It also showed that moderate-intensity endurance exercise training increases exercise capacity (Tsai et al., 2004) and decreases heart rate during exercise by 11.3 mmHg in hypertensive patients (Wilmore et al., 2001). Taken together, these findings suggest that endurance exercise training does not only help lower resting blood pressure, but also improves fitness and lowers the risk of cardiovascular injury during exercise among hypertensive individuals.

Although endurance exercise training promotes beneficial effects on blood pressure regulation, approximately 50% of adults among those engaged in aerobic exercise program discontinued within 3 to 6 months (Owen et al., 2010). Dropout from this exercise program had been reported due to several factors, such as lack of time and medical condition (e.g. obesity or arthritis) (Owen et al., 2010).

2.5.2 Resistance Exercise Training and Blood Pressure

Resistance exercise training (strength training) refers to any type of training using resistance (e.g. dumbbells and elastic band) against the force of muscular contraction (Preedy, 2012). Resistance training, such as weightlifting, is generally designed to build muscle and increase strength (Kraemer et al., 2002). Since a study by MacDougall et al. (1985) demonstrated that systolic and diastolic blood pressure could reach values of 320 mmHg and 250 mmHg, respectively, during the double leg-press

in normotensive individuals, the resistance training has not previously been recommended for hypertensive patients.

Nevertheless, subsequent studies have demonstrated that resistance training can lower resting blood pressure (Wiley et al., 1992, Wood et al., 2001, Vincent et al., 2003). Furthermore, several meta-analysis studies have consistently reported that both resting systolic and diastolic blood pressure could be reduced by 3 to 4 mmHg following resistance exercise training (Kelley and Kelley, 2000, Cornelissen and Fagard, 2005a, Fagard, 2006). Interestingly, no incident of high blood pressure was observed after the resistance exercise training (Kelley and Kelley, 2000). Nonetheless, contrary to the findings obtained from endurance exercise training, the magnitude of blood pressure reduction following resistance exercise training had been similar in hypertensive and normotensive individuals (Cornelissen and Fagard, 2005a, Fagard, 2006). Taken together, these findings demonstrated that resistance exercise training could be incorporated into an exercise program for blood pressure management.

Based on the modes of resistance exercise training, the recent meta-analysis study reported that the reduction in resting systolic blood pressure was larger after isometric resistance training (-10.9 mmHg) compared to dynamic resistance training (-1.8 mmHg) (Cornelissen and Smart, 2013). Besides, a similar pattern of reduction was also observed for resting diastolic blood pressure (Cornelissen and Smart, 2013). Generally, dynamic resistance exercise involves movement at the joints, whereas isometric resistance exercise does not require any movement of the affected joint and the muscle length will remain unchanged (Hoffman, 2002). Thus, from the findings on resting blood pressure and the exercise characteristics, the isometric resistance

exercise training had been found to be superior to the dynamic resistance exercise training in controlling and preventing high blood pressure.

2.5.3 Isometric Resistance Exercise Training and Blood Pressure

Over several years, numerous studies have investigated the role of isometric resistance exercise training in reducing blood pressure (Wiley et al., 1992, Ray and Carrasco, 2000, Howden et al., 2002, Taylor et al., 2003, Peters et al., 2006, McGowan et al., 2007, Millar et al., 2008, Millar et al., 2013). In fact, a current meta-analysis study reported that the reduction in resting blood pressure had been largest after isometric resistance exercise training (systolic: −10.9 mmHg, diastolic: −6.2 mmHg) compared to endurance (systolic: −3.5 mmHg, diastolic: −3.7 mmHg) and dynamic resistance exercise training (systolic: −1.8 mmHg, diastolic: −2.5 mmHg) (Cornelissen and Smart, 2013).

Although the optimal protocol of the isometric resistance exercise training has not been established at present, the most common protocol of this exercise training is composed of four sets of 2 minutes' handgrip (McGowan et al., 2007, Millar et al., 2008) or leg contractions (Howden et al., 2002) at 30 to 50 % of maximal voluntary contraction (MVC) (Wiley et al., 1992, Ray and Carrasco, 2000) with 1 to 4 minutes of rest period between each contraction (Wiley et al., 1992, McGowan et al., 2007) that are conducted three to five times per week for 4 to 10 weeks (Devereux et al., 2010, Badrov et al., 2013). Relative to exercise trained-muscle, hand grip isometric exercise training (Wiley et al., 1992, Badrov et al., 2013) has been found to reduce resting blood pressure more than leg isometric exercise training (Baross et al., 2012).

In addition, with regard to exercise intensity, the isometric handgrip exercise training that was performed at 30% of MVC (Taylor et al., 2003) elicited greater reduction in resting blood pressure compared to a similar exercise at 50% of MVC (Peters et al., 2006). On the other hand, as for training frequency, Badrov et al. (2013) reported that the effect of the isometric exercise training in lowering blood pressure had been independent of the volume of training as both groups trained 3 and 5 times per week produced a similar magnitude of reduction in blood pressure. Interestingly, the effect of this exercise training on blood pressure could be seen after 4 to 5 weeks of training with long duration of training, such as 8 to 10 weeks, which had shown to elicit larger reductions in blood pressure (Millar et al., 2007).

It has also been demonstrated that the effect of the isometric exercise training is more pronounced in those with hypertension than individuals with normal blood pressur. In addition, several studies have shown that no harmful effect was detected when performing the isometric exercise as no overload was discovered in cardiovascular changes and the hemodynamic parameters were observed immediately after the end of the first session of this exercise training (Li et al., 2000, Auerbach et al., 2000, Araujo et al., 2011, Olher et al., 2013). When compared to aerobic exercise training, the isometric exercise training has the potential to ensure long-term adherence as it is easy to perform, does not require higher intensity training, and less exercise time (Millar et al., 2014). Therefore, based on all the findings obtained from previous research, the isometric exercise training could be a very effective training for hypertensive and normotensive individuals to control and prevent high blood pressure, respectively.

At present, the mechanism by which isometric exercise training elicits reduction in blood pressure has remained unclear (Millar et al., 2014). Wiles et al. (2010) suggested that the rise in blood pressure during isometric exercise will stimulate the baroreceptors, which are sensory afferent nerve endings located in the carotid sinus and the aortic arch. When the blood pressure is elevated, the baroreceptors are stretched and result in a reflex-mediated increase in parasympathetic nerve activity, as well as a decrease in sympathetic nerve activity (Wiles et al., 2010). Consequently, it causes a decline in the heart rate, while the diameter of blood vessels increases and further leads to a drop in the blood pressure (Wiles et al., 2010). Moreover, other studies have suggested that the reduction in blood pressure after isometric exercise training is related to the repeated power of hydrogen (pH) changes due to muscle fatigue and lactate production that act as a metaboreceptor stimulus (Devereux, 2010); augmentation in vasodilator substances, for instance, nitric oxide (NO) (Lopez et al., 2009); and reduction in peripheral vascular adaptations (Gregory, 2012).

On the other hand, data retrieved from *HE*alth, *RI*sk factors, exercise *T*raining *A*nd *Ge*netics (HERITAGE) Family Study suggest that reduction in blood pressure after exercise may be influenced by genetic factors (Wilmore et al., 2001). With that, several candidate genes for the reduction in blood pressure after exercise had been proposed and among these, the *ACE* gene was initially believed to influence blood pressure response to exercise due to its role in the RAS (Hagberg et al., 2000, Marteau et al., 2005, Dhanachandra Singh et al., 2014).

2.5.4 *ACE I/D* Gene Polymorphism, Isometric Exercise Training, and Blood Pressure

The variation that occurs in the *ACE* gene, which is known as *ACE I/D* gene polymorphism, had been proposed to influence blood pressure regulation (Rigat et al., 1990). Moreover, Rigat et al. (1990) reported that the ACE concentration in the RAS was lowest and highest among individuals with *I* and *D* alleles of the *ACE I/D* gene polymorphism, respectively. Therefore, individuals with two copies of *I* allele might have lower resting blood pressure compared to those with two copies of *D* allele as the lower level of ACE decreased the production of ANG II, a potent vasodepressor and aldosterone stimulating peptide, besides activating bradykinin, a potent vasodilator that leads to a drop in blood pressure (Coates, 2003). Hence, given that the *ACE I/D* gene polymorphism has an important role in blood pressure regulation, blood pressure response to exercise training may vary among individuals with different genotypes of *ACE I/D* gene polymorphism.

Besides, a few studies have attempted to investigate the influence of the *ACE I/D* gene polymorphism on blood pressure in response to endurance exercise training (Montgomery et al., 1997, Hagberg et al., 1999, Rankinen et al., 2000a, Zhang et al., 2002, Dengel et al., 2002, Kim, 2009). Furthermore, Hagberg et al. (1999) reported that after 9 months of endurance exercise training at 75 to 85 % of VO_2max, individuals with *I* allele had reduced systolic and diastolic blood pressure more than individuals with *D* allele (systolic: -10 vs. -5 mmHg; diastolic: -10 vs. -1 mmHg). Meanwhile, in a study that involved 64 Japanese with mild to moderate essential hypertension subjects, who were engaged in 10 weeks of exercise therapy on a bicycle

ergometer, the systolic blood pressure, the diastolic blood pressure, and the mean value for arterial pressure significantly decreased in individuals with *II* and *ID* genotypes, but not in those individuals with *DD* genotype (Zhang et al., 2002). In contrast, a study by Kim (2009) found that adult women with *DD* genotype had a greater reduction in DBP than those with *II* and *ID* genotypes following 12 weeks of combined aerobic and resistance exercise training. Despite the positive finding on the influence of the *ACE I/D* gene polymorphism in blood pressure, studies by Rankinen et al. (2000a), Montgomery et al. (1997), and Dengel et al. (2002) found that the *ACE I/D* gene polymorphism did not influence blood pressure in response to 20 weeks of endurance exercise training. Nonetheless, negative finding was also found for dynamic resistance exercise training, where the blood pressure response to a three-month program of dynamic resistance exercise training at 60%, 70%, and 80% of 1-RM, respectively, for each month did not differ for hypertensive women with different *ACE I/D* genotypes (Mota et al., 2013). The reasons for the discrepancy between these studies are unclear, but might be due to the differences in exercise protocol. For instance, a study by Kim (2009) involved mixed aerobic and resistance exercise training compared with only aerobic training (endurance training) in study by Hagberg et al. (1999).

Despite conflicting findings of the above-mentioned studies, the positive finding obtained for the effects of *ACE I/D* gene polymorphism on endurance training adaptation raises the possibility that the *ACE I/D* gene polymorphism might also influence blood pressure response to isometric exercise training. Besides, based on the latest article review on isometric exercise training-induced reductions in resting blood pressure, the role of genetic factors, specifically the *ACE I/D* gene polymorphism in eliciting hypotension effect following the isometric exercise training, has not been

reported yet (Lawrence et al., 2014). Therefore, future research is warranted to examine the effects of *ACE I/D* gene polymorphism on blood pressure response to isometric exercise training.

2.6 Research Gaps in the Field

The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and health remain inconclusive due to inconsistent findings in the current literature (Ma et al., 2013). These inconsistent findings were thought to be related to some potential limitations including insufficient sample size, ethnicity, and disparity data in the current literature (Zilberman-Schapira et al., 2012, Ma et al., 2013).

The number of participants has been reported as one of the potential limitation in this field (Zilberman-Schapira et al., 2012). In order to make a concrete conclusion, studies in this field are warranted to have a very large population samples (i.e. more than a few hundred) (Guilherme et al., 2014). This is difficult to overcome because each polymorphism exhibits different frequencies in different populations, indicate that the sample size necessary to detect the effects of particular gene polymorphism can vary according the tested population (Guilherme et al., 2014).

Ethnicity has also been suggested to contribute to inconsistent findings due to lack confirmation in different ethnic population (Zilberman-Schapira et al., 2012). For instance, with regard to *ACE I/D* gene polymorphism, while studies involving Caucasian population reported that possession of the *I* allele of *ACE I/D* gene polymorphism influences endurance performance (Hagberg et al., 1999, Kasikcioglu

et al., 2004, Cam et al., 2007), some studies with Asian samples demonstrated that possession of the *ACE I* allele did not appear to influence endurance performance (Zhaoa et al., 2003, Tobina et al., 2010). Based on these findings and previous reports that the distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms vary between different ethnic groups (Barley et al., 1994, Batzer et al., 1994, Batzer et al., 1996, Jayapalan et al., 2008), there is a possibility that the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on physical performance could also vary by ethnicity. Thus, to understand the potential effect of ethnicity factor on *ACE I/D* and *ACTN3 R/X* gene polymorphisms, population stratification according to the ethnic background is, therefore, an important issue to consider in this field.

In addition, to the best of author's knowledge, there are limited studies comparing the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on physical performance in Asian population, particularly Malaysian population, to Caucasian population for a comprehensive description concerning the ethnic variation on these polymorphisms. One of the first report on the association between *ACTN3 R/X* gene polymorphism and physical performance was based on a study by Yang et al. (2003) involving Australian Caucasian athletes. In that study, Yang and colleagues observed that elite athletes of endurance sports are predominantly with *X* allele while the *R* allele was found to be over-represented in strength/power athletes (Yang et al., 2003). Based on the study of Yang et al. (2003), it would also be interesting to compare the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on physical performance in Malaysian population to Australian population to determine if similar association exist among Malaysian population as what reported among Australian Caucasian population.

The disproportion in the current literature was also noted as most related articles had been based on Caucasian populations, while there are fewer reports for the Asian population (Ma et al., 2013). Therefore, more studies in other populations, particularly among the Asian population, are required to verify the findings observed in the Caucasian samples.

In addition, although isometric handgrip training has been reported to be more efficient in lowering resting blood pressure compared to other different modes of exercise training (Cornelissen and Smart, 2013), the mechanism by which isometric handgrip training induced reduction in resting blood pressure has remained unknown. Given that the *ACE I/D* gene polymorphism influenced blood pressure response to endurance training (Hagberg et al., 1999, Zhang et al., 2002, Kim, 2009), there is a possibility that the *ACE I/D* gene polymorphism might also influence blood pressure response to isometric exercise training. Therefore, future studies are warranted to investigate the effect of the *ACE I/D* gene polymorphism on blood pressure adaptation to isometric exercise training. Thus, in future it may be possible to predict who will likely benefit most from the isometric exercise training for blood pressure management.

CHAPTER 3

RESEARCH OVERVIEW

3.1 Introduction

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). Therefore, the main idea of this thesis had been to provide the evidence gathered from three studies in support of the roles of specific genetic factors represented by angiotensin I-converting enzyme (*ACE*) *I/D* and alpha-actinin-3 (*ACTN3*) *R/X* gene polymorphisms on human physical performance and health within the Malaysian population.

A population-based study was carried out in the first study to determine if the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms were differed by ethnicity as a standard reference before establishing the effects of these polymorphisms on physical performance within the Malaysian population. Therefore, 180 Asians (99 Malays (55%), 45 Chinese (24.7%), 23 Other Bumiputras (12.9%), and 13 Indians (7.4%)) 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, who were sedentary healthy individuals were selected as participants in the first study. A sample of DNA was retrieved via buccal cell from each participant and the *ACE I/D* and *ACTN3 R/X* genotypes were then identified through Polymerase Chain Reaction.

The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and their association with ethnicity within the Malaysian population were examined in the second study. One hundred eighty well-trained Malaysian athletes (148 males, 32 females) aged 20.5 ± 1.9 years, and 180 Malaysian sedentary controls from the first study, involved in the second study. A sample of DNA was retrieved via buccal cell from each participant and the *ACE I/D* and *ACTN3 R/X* genotypes were then identified through Polymerase Chain Reaction. The endurance performance and muscular strength of Malaysian athletes were evaluated with twenty meters Yo-Yo intermittent recovery level 2 and maximal voluntary contraction tests, respectively. Within the cohort of Malaysian athletes, 34 participants (25 males, 9 females) aged 19.8 ± 1.9 years were classified as endurance athletes, 41 participants (25 males, 16 females) aged 19.7 ± 1.8 years as strength/power athletes, and 105 participants (99 males, 6 females) aged 21.0 ± 1.8 years as intermittent athlete. In addition, given that a strong association between *ACTN3 R/X* gene polymorphism and physical performance was previously observed in Australian population (Yang et al., 2003), therefore, 33 intermittent athletes (all males) aged 20.7 ± 4.0 years, who were of Caucasian origin, had been recruited from the Australian population for well-matched comparison-group study to Malaysian population, represents by 33 Malay intermittent athletes (all males) aged 20.8 ± 1.7 years from the intermittent group of Malaysian athletes.

In the final study, an experimental study was conducted to determine the influence of *ACE I/D* gene polymorphism on training adaptation for health among normotensive men in Malaysia. Thirty normotensive, untrained males (*ACE* genotype: *II*=10, *ID*=10, and *DD*=10), undergone the most common protocol of isometric

handgrip (IHG) 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. Cardiovascular and muscular variables were measured on three consecutive days prior to commencing training and after 8 weeks of training, as well as after the initial training session. The criteria of the third study that only men were involved was based on the understanding that regulation of blood pressure in women is affected by the menstrual cycle. Thus, it would be difficult to isolate the influence of genetic makeup on physiological adaptation of isometric handgrip training in this study if women were involved without considering their individual menstrual cycle variation.

The flow chart of the overall research process is illustrated in Figure 3.1. The comprehensive methodologies used in each study are described in detail in the following chapters. The study protocols on Malaysian and Australian samples were approved by the Human Research Ethics Committee in Universiti Sains Malaysia (Appendix A) and the Human Research Ethics Committee in University of Sydney, respectively (Appendix B).

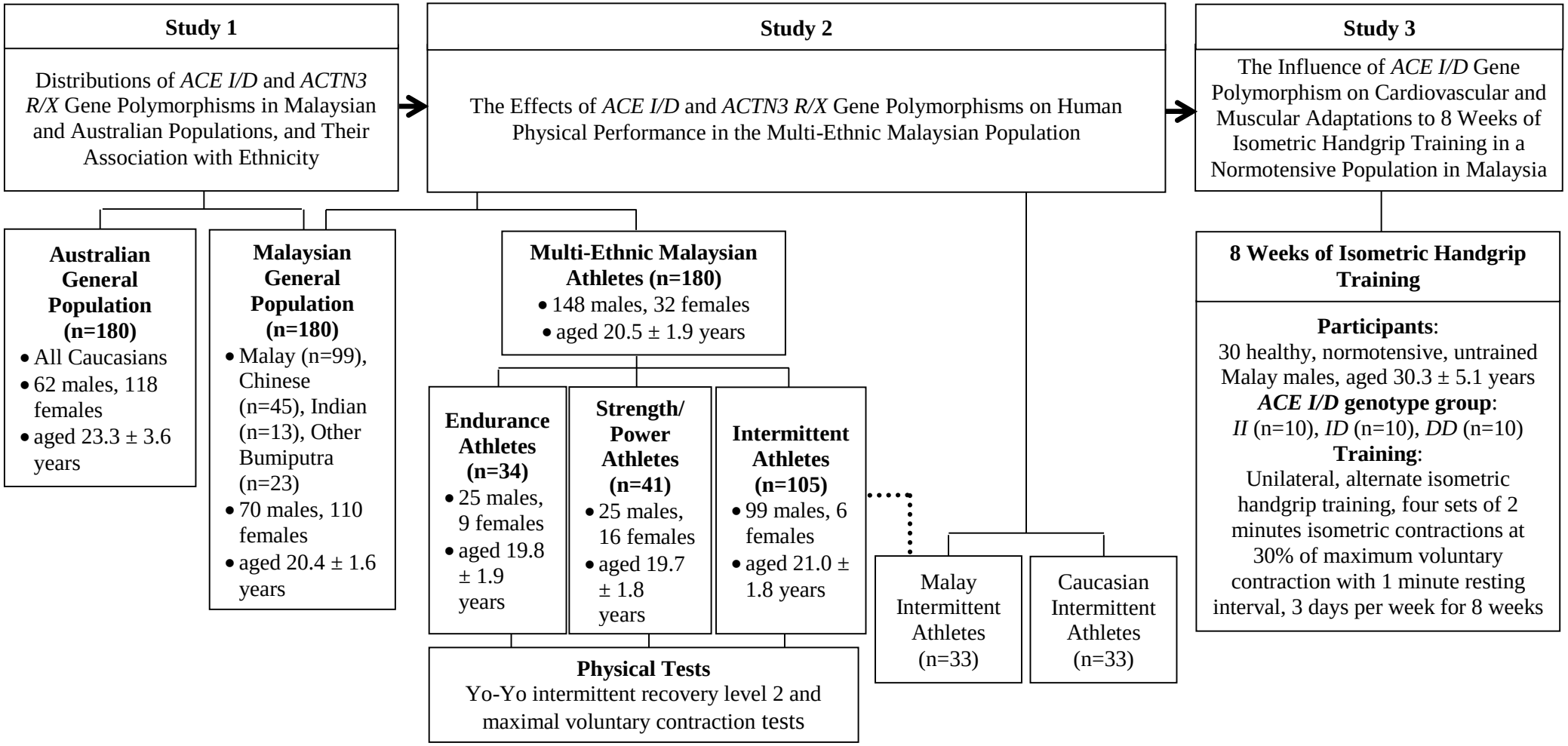


Figure 3.1 The general research process

3.2 Research Objectives and Hypotheses

3.2.1 Study 1: Distributions of *ACE I/D* and *ACTN3 R/X* Gene Polymorphisms in Malaysian and Australian Populations, and Their Association with Ethnicity

The objective of this study was to establish the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the Malaysian population. This study also determined whether the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms differ among ethnic groups.

3.2.2 Study 2: The Effects of *ACE I/D* and *ACTN3 R/X* Gene Polymorphisms on Human Physical Performance in the Multi-Ethnic Malaysian Population

The main focus of this study was 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. This study also determined if the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance differ by ethnicity. The ACE level was reported lower in individuals with two copies of *I* allele (Rigat et al., 1990) and led to a decrease in the conversion of ANG I to ANG II, which resulted in increased delivery of oxygenated blood to the working muscles (Sayed-Tabatabaei et al., 2006). Meanwhile, MacArthur et al. (2007) suggested that the loss of ACTN3 protein via the *X* allele leads to alterations in skeletal muscle metabolism towards more efficient aerobic metabolism. Therefore, possession of the *I* allele of *ACE I/D* and the *X* allele of *ACTN3 R/X* gene

polymorphisms were hypothesized to affect endurance performance. Meanwhile, based on the findings that the elevation level of the ACE activity in individuals with *D* allele of the *ACE I/D* gene polymorphism increased the production of ANG II (a potent growth factor in cardiac and vascular tissues) in the skeletal muscle renin-angiotensin system, which is the potential mechanism where muscle cell growth and hypertrophy may be activated (Geisterfer et al., 1998, Kai et al., 1998), and the *R* allele codes for functioning *ACTN3* gene that results in the production of ACTN3 protein (North et al., 1999), possession of the *D* allele of *ACE I/D* and the *R* allele of *ACTN3 R/X* gene polymorphisms were hypothesized to affect strength/power performance. The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance were hypothesized to vary between ethnic groups.

3.2.3 Study 3: The Influence of *ACE I/D* Gene Polymorphism on Cardiovascular and Muscular Adaptations to 8 Weeks of Isometric Handgrip Training in a Normotensive Population in Malaysia

This study was designed to examine the effect of *ACE I/D* gene polymorphism on cardiovascular and muscular adaptations to an 8-week isometric handgrip training among normotensive men with different *ACE I/D* genotypes. It was hypothesized that cardiovascular and muscular adaptations to an 8-week isometric handgrip training would vary between the *ACE I/D* genotype groups.

CHAPTER 4

DISTRIBUTIONS OF *ACE I/D* AND *ACTN3 R/X* GENE POLYMORPHISMS IN MALAYSIAN AND AUSTRALIAN POPULATIONS, AND THEIR ASSOCIATION WITH ETHNICITY

4.1 Introduction

Performance in sports has been suggested to be influenced by genetic factors (Guilherme et al., 2014). Among the candidate genes studied, the most studied genes related to sports performance have been the angiotensin I-converting enzyme (*ACE*) and alpha-actinin-3 (*ACTN3*) genes (Ma et al., 2013). The *ACE I/D* gene polymorphism in the *ACE* gene has been thought to confer a greater advantage in endurance activity due to its role in determining the level of ACE circulation in tissues (Rigat et al., 1990); a main component in the renin-angiotensin system (RAS) (Sayed-Tabatabaei et al., 2006). Meanwhile, the *ACTN3 R/X* gene polymorphism in the *ACTN3* gene has been thought to present an advantage to activities that require short bursts of intense strength and power because it codes for ACTN3 protein (North et al., 1999); a protein found only in the fast-twitch skeletal muscle fibre.

Moreover, research findings on the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance have remained inconsistent (Hagberg et al., 1999, Zhaoa et al., 2003, Kasikcioglu et al., 2004, Niemi and Majamaa, 2005, Cam et

al., 2007, Druzhevskaya et al., 2008, Eynon et al., 2009b, Tobina et al., 2010, Kothari et al., 2011, Gineviciene et al., 2011). Most studies involving the Caucasian population reported that possession of the *I* allele of *ACE I/D* gene polymorphism influences endurance performance (Hagberg et al., 1999, Kasikcioglu et al., 2004, Cam et al., 2007). Conversely, some studies in the Asian population revealed that possession of the *ACE I* allele did not appear to influence endurance performance, as the endurance performance was significantly higher in those with *ACE D* allele than those with *ACE I* allele (Zhaoa et al., 2003, Tobina et al., 2010). With regard to *ACTN3 R/X* gene polymorphism, studies involving Indian (Kothari et al., 2011), Finnish (Niemi and Majamaa, 2005), Israeli (Eynon et al., 2009b), and Russian (Druzhevskaya et al., 2008) athletes demonstrated that possession of the *ACTN3 R* allele may confer an advantage for strength/power performance. In contrast, Gineviciene et al. (2011) reported that possession of the *ACTN3 R* allele failed to confer any advantage to strength/power performance among Lithuanian athletes.

These contradictory results reported across different ethnicities were apparently consistent with the research findings on the effect of *ACE I/D* gene polymorphisms on 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). A previous meta-analysis study carried out by Kunz et al. (1998) demonstrated that *ACE I/D* gene polymorphism contributed to the susceptibility to diabetic nephropathy with a significant association among Asian patients, but not in Caucasian patients. In contrast, a meta-analysis study by Fujisawa et al. (1998) reported that the *ACE I/D* gene

polymorphism was associated with diabetic nephropathy in both Asian and Caucasian populations. Subsequently, in 2005, a study by Ng et al. (2005) in a large population-based sample clearly demonstrated that the association between *ACE I/D* gene polymorphism and diabetic nephropathy was more marked among type 2 diabetic patients in the Asian population than those from the Caucasian population. Ethnic difference in *ACE I/D* gene polymorphism was also observed in the susceptibility to hypertension. In a study that involved the Japanese population, *ACE I/D* gene polymorphism was not found to be associated with hypertension (Ishigami et al., 1995). In addition, studies concerning Caucasian samples also failed to identify any significant association between *ACE I/D* gene polymorphism and hypertension (Staessen et al., 1997, Sagnella et al., 1999). On the other hand, several studies reported that within the black population, there was a positive relationship between *ACE I/D* gene polymorphism and hypertension (Barley et al., 1996, Sagnella et al., 1999).

The reason for the existence of ethnic variation in the effects of these polymorphisms on physical performance and the susceptibility to certain diseases has remained unknown. The ethnic variation in the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms globally maybe partly responsible for these differences as well (Zilberman-Schapira et al., 2012). Moreover, studies showed that *I* allele frequency of the *ACE I/D* gene polymorphism was more frequent in the healthy Australian Aboriginal population (Lester et al., 1999) and least in European (Tiret et al., 1992). In healthy Caucasian populations, the *I* allele frequency ranged between 0.78 and 0.23, whereas the *D* allele frequency varied between 0.77 and 0.22. However, in healthy Asian populations,

the *I* and the *D* allele frequencies ranged from 0.76 to 0.42 and 0.58 to 0.24, respectively. On top of that, a study by Jayapalan et al. (2008) among different ethnic groups in Malaysia also found a significant difference in the distribution of *ACE I/D* gene polymorphism between ethnic groups in Malaysia, with the *I* allele tending to be more frequent in the Malay group, while a higher frequency of *D* allele was observed for the Indian group. As for the *ACTN3 R/X* gene polymorphism, the highest *R* allele frequency was found in Nigerian (Yang et al., 2007) and the lowest in Indian populations (Kothari et al., 2011). In a healthy Caucasian population, the frequencies of *R* and *X* alleles ranged from 0.61 to 0.50 and 0.50 to 0.39, respectively. Meanwhile, the *R* and *X* alleles frequencies in the healthy Asian population ranged from 0.53 to 0.39 and 0.61 to 0.47, respectively.

To date, the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the Malaysian population have not been well documented. The distribution of *ACE I/D* gene polymorphism in Malaysia was reported in 2008 (Jayapalan et al., 2008). However, the data retrieved from this previous study (Jayapalan et al., 2008) still need to be validated. In contrast, there has been no report thus far for *ACTN3 R/X* gene polymorphism distribution in the Malaysian population. Besides, it had been necessary to obtain information concerning distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the general population of Malaysia before establishing their effects on physical performance in this population. Since there was ethnic variation in the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms, it would also be interesting to compare the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the

Malaysian population to other population for a comprehensive description concerning the ethnic variation on these polymorphisms.

4.2 Aims

The aims of the present study were to (i) establish the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the multi-ethnic Malaysian population, and (ii) investigate ethnic variation on these 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. The distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms were hypothesized to vary between ethnic groups.

4.3 Methods and Participants

4.3.1 Study Design

This population-based study utilized two research designs, which were simple and comparative descriptive research designs. Simple descriptive research was employed to obtain information relating to the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the Malaysian population. Meanwhile, the comparative descriptive research was used to compare the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms among the populations.

4.3.2 Participants

The study group consisted of 180 Asians from Malaysia, and 180 Caucasians from Australia, who had provided informed consent to participate in this study. To limit variation due to genetic admixture, participants reported with mixed ancestry within three generations were excluded from this study. The number of participants for this study was based on the sample size calculation using the Power and Sample Size Calculation version 3.1.2 software (Dupont and Plummer, 1990) [Calculated sample size in each group = 164 participants; Research sample size = 164 participants + (164*10% (expected drop out)) = 180 participants]. The power of the study was set at 0.80 with the alpha level of 0.05 and the effect size of 0.25. Participants consent and information details forms were presented in Appendix C.

4.3.2.1 Malaysian population

One hundred and eighty participants from the Malaysian population were sedentary healthy individuals (70 males, 110 females), who reported having a sedentary lifestyle (two or fewer days a week of recreational exercise for less than 30 minutes a day for the preceding three months (Pate et al., 2008)), aged 20.4 ± 1.6 years. They were drawn from four ethnic groups in Malaysia. In order to obtain comprehensive results for the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the Malaysian population, the proportion of participants with different ethnic backgrounds had been set according to the current distribution ratio in Malaysia (Statistic, 2011). Based on the

respective ratio, 99 Malays (55%), 45 Chinese (24.7%), 23 Other Bumiputras (12.9%), and 13 Indians (7.4%) were selected as participants for this study. All study participants were students from several universities in Malaysia.

4.3.2.2 Australian population

In addition, in comparison to the Malaysian population, 180 sedentary healthy individuals of Caucasian origin (62 males, 118 females), who reported having a sedentary lifestyle (two or fewer days a week of recreational exercise for less than 30 minutes a day for the preceding three months (Pate et al., 2008), aged 23.3 ± 3.6 years had been recruited from the Australian population. All the participants were students at the University of Sydney, Sydney, Australia.

4.3.3 Deoxyribonucleic Acid (DNA) Sample Collection

After obtaining personal information from the participants, deoxyribonucleic acid (DNA) samples from each participant were obtained via buccal swab using a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy). The swabs were air dried and placed in sterile 1.5 ml microcentrifuge tubes and stored at -20°C until used for DNA isolation.

4.3.4 Genotype Determination

4.3.4.1 DNA Extraction

Genomic DNA was isolated from the swab samples using the GeneAll® Exgene™ Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co. Ltd, Seoul, South Korea). First, 400 µl of 1X phosphate buffered saline (PBS), 20 µl of Proteinase K (20 mg/ml), and 400 µl of blood lysis buffer were added into the tube with the swab. Immediately, the solutions were mixed with vigorous vortex. After that, the tube was incubated at 56˚C for 10 minutes and followed by a short spin to remove any drop from the inside of the lid. Thereafter, 400 µl of absolute ethanol was added to the lysates and was mixed well via vortex. The tube was subsequently centrifuged briefly and 700 µl of the mixture was carefully transferred to the SV column. The tube was then centrifuged for 1 minute at 6000 x g above (> 8000 revolutions per minute (rpm)). After discarding the supernatant, the SV column was reinserted into the new collection tube. Afterwards, 600 µl of column wash solution b buffer was added into the tube and was centrifuged for 1 min at 6,000 x g above (> 8000 rpm). After replacing the collection tube with a new one, 700 µl of column wash solution t buffer was added and the tube was centrifuged for 1 min at 6000 x g above (> 8000 rpm). Again, the pass-through was discarded and the SV column was reinserted into the collection tube. The tube was then centrifuged at full speed for 1 minute to remove the residual wash buffer, and the SV column was then placed in a fresh 1.5 ml Eppendorf tube. Lastly, 200 µl of elution buffer was added to the tube to increase the total DNA yield, and followed by an incubation process for 1 minute at room

temperature. The tube was centrifuged at full speed for 1 minute and was stored at -20^{0}C before applying it to molecular analysis.

4.3.4.2 Molecular Analysis for *ACE I/D* Gene Polymorphism

Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.8 µm of each primer (forward primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3': reverse primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3'), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 10.8 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the *ACE I/D* gene polymorphism was amplified under the following conditions; 7 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 62°C, and 1 minute at 72°C, with a final step of 7 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 490 base pair (bp) and 190 bp bands indicated the *ACE* insertion (*I*) and deletion (*D*) alleles, respectively. The PCR products for *ACE I/D* gene polymorphism were confirmed by sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia).

4.3.4.3 Molecular Analysis for *ACTN3 R/X* Gene Polymorphism

PCR was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.2 µm of each primer (forward primer: 5'-CTGTTGCCTGTGGTAAGTGGG-3': reverse primer: 5'-TGGTCACAGTATGCAGGAGGG-3'), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 12.3 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the *ACTN3 R/X* gene polymorphism was amplified under the following conditions; 2 minutes at 95°C, followed by 26 cycles of 30 seconds at 95°C, 30 seconds at 61.6°C, and 30 seconds at 72°C, with a final step of 5 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 291 bp band indicated the successful amplification of *ACTN3* gene. The PCR product was then confirmed by DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). In order to obtain the genotype of the *ACTN3 R/X* gene polymorphism, the amplified PCR product was digested with *Dde*I restriction enzyme (New England Biolabs, Beverly, MA, USA) in a final volume of 10 µl consisting of 1.0 µl of 10X NEBuffer3 (New England Biolabs, Beverly, MA, USA), 1 U of *Dde*I restriction enzyme (New England Biolabs, Beverly, MA, USA), and 8.5 µl of amplified PCR product. The reaction mix was incubated at 37°C for 45 minutes and the digestion product was electrophoresed on 2.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 205

bp and 86 bp bands indicated *R* allele, while the presence of 108 bp, 97 bp, and 86 bp bands indicated *X* allele.

4.4 Statistical Analysis

The descriptive data are presented as mean ± standard deviation (SD). Allele frequencies of *ACE I/D* and *ACTN3 R/X* gene polymorphisms were determined by direct counting. The Hardy-Weinberg equilibrium (HWE) test was used for genotyping quality control to describe that the genotype distribution of a population is large, self-contained, and randomly mating (Xu et al., 2002). Simple HWE calculator (http://www.koonec.com/wp-content/uploads/k-blog/HWE.xls.) was used to confirm that the observed *ACE I/D* and *ACTN3 R/X* genotype frequencies were in HWE for all groups ((i) Malaysian population (Malay, Chinese, Indian, and Other Bumiputra) and (ii) Australian population (Caucasian)). The chi-square (X^2) test was used to examine the difference in allele and genotype frequencies of the *ACE I/D* and *ACTN3 R/X* gene polymorphisms between the ethnic groups ((i) Malay vs. Chinese vs. Indian vs. Other Bumiputra, and (ii) Malaysian vs. Australian). All statistical evaluations were performed by using the IBM SPSS statistical version 20.0 (Armonk, New York, USA), with the level of significance set at $p < 0.050$.

4.5 Results

4.5.1 Hardy-Weinberg Equilibrium Test

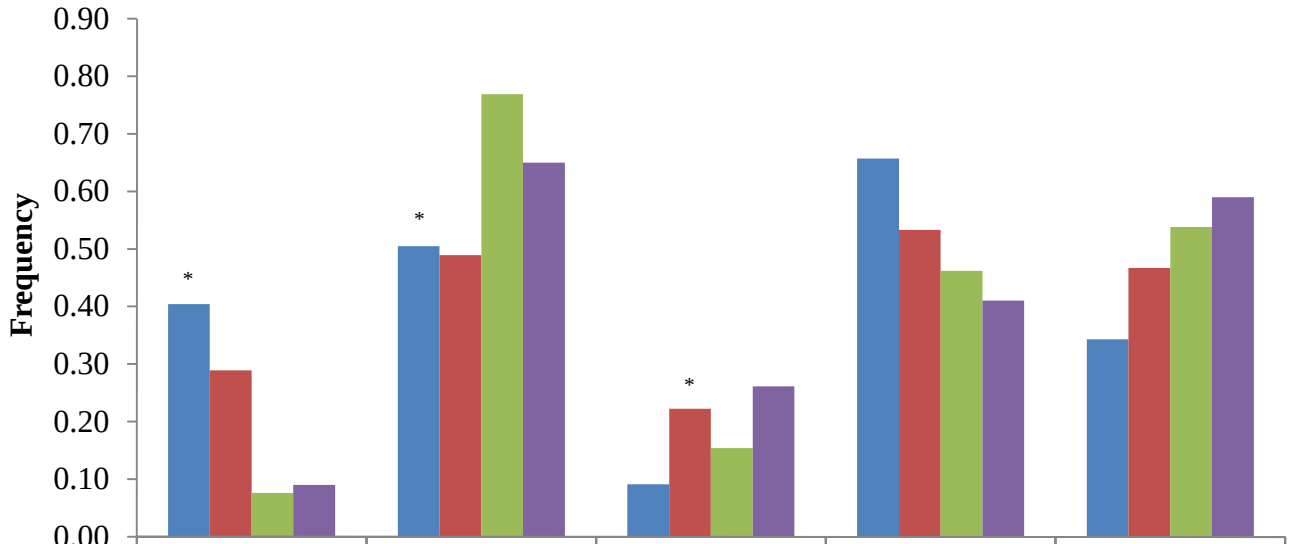
The distribution of *ACE I/D* and *ACTN3 R/X* genotypes for all groups had been in agreement with Hardy-Weinberg equilibrium ($p > 0.05$) (Appendix D).

4.5.2 The Distribution of *ACE I/D* Gene Polymorphism

4.5.2.1 Multi-Ethnic Groups in Malaysia

The distribution of *ACE I/D* genotype in the multi-ethnic population in Malaysia is presented in Figure 4.1. The Malay group had *ACE I/D* genotype frequencies of 0.40, 0.51, and 0.09, for *II*, *ID*, and *DD* genotypes respectively. Meanwhile, in the Chinese group, the frequencies of *II*, *ID*, and *DD* genotypes were 0.29, 0.49, and 0.22, respectively. The frequencies of *II*, *ID*, and *DD* genotypes for Indian and Other Bumiputra groups were 0.08, 0.77, and 0.15, as well as 0.09, 0.65, and 0.26, respectively. The statistical analysis showed that there was a significant difference in the distribution of *ACE I/D* genotype polymorphism between the ethnic groups ($X^2 = 16.828$, df = 6, $p = 0.010$). The Malay group differed significantly in *ACE I/D* genotype frequency when compared with the Other Bumiputra group ($X^2 = 10.594$, df = 2, $p = 0.005$), but not to those in the Chinese ($X^2 = 5.174$, df = 2, $p = 0.075$) and Indian ($X^2 = 5.319$, df = 2, $p = 0.070$) groups. The Chinese group did not differ significantly in *ACE I/D* genotype frequency when compared

with the Indian (X^2 = 3.542, df = 2, p = 0.170) and Other Bumiputra (X^2 = 3.656, df = 2, p = 0.161) groups. In addition, the Indian group did not differ significantly in *ACE I/D* genotype frequency when compared with the Other Bumiputra group (X^2 = 0.602, df = 2, p = 0.740). Among the four ethnic groups, the Malay and Other Bumiputra groups had the highest frequencies of *II* and *DD* genotypes, respectively. In contrast, the distribution of *ACE I/D* allele was not significantly different between Malay (*I* = 0.66; *D* = 0.34), Chinese (*I* = 0.53; *D* = 0.47), Indian (*I* = 0.46; *D* = 0.54), and Others Bumiputra (*I* = 0.41; *D* = 0.59) groups (X^2 = 6.882, df = 3, p = 0.076).



	II	*ID*	*DD*	*I*	*D*
	ACE I/D Genotype			*ACE I/D* Allele	
Malay (n=99)	0.40*	0.51*	0.09*	0.66	0.34
Chinese (n=45)	0.29	0.49	0.22	0.53	0.47
Indian (n=13)	0.08	0.77	0.15	0.46	0.54
Other Bumiputra (n=23)	0.09	0.65	0.26	0.41	0.59

Figure 4.1 *ACE I/D* allele and genotype frequencies in the multi-ethnic groups in Malaysia

Note.
*p = 0.005 for genotype frequency in Malay group vs. Other Bumiputra group

4.5.2.2 Malaysian and Australian Populations

The distributions of *ACE I/D* genotype in Malaysian and Australian populations are presented in Figure 4.2. In the Malaysian population, the frequencies of *II*, *ID*, and *DD* genotypes were 0.31, 0.54, and 0.15, respectively. Meanwhile, the frequencies of *II*, *ID*, and *DD* genotypes in the Australian population were 0.25, 0.47, and 0.28, respectively. Moreover, the statistical analysis showed that there was a significant difference in the distributions of *ACE I/D* genotype between Malaysian and Australian populations (X^2 = 9.516, df = 2, p = 0.009) with the frequencies of the *II* and *ID* genotypes higher among Malaysians compared to Australians. However, the distribution of *ACE I/D* allele were not significantly different between Malaysian (*I* = 0.58; *D* = 0.42) and Australian (*I* = 0.48; *D* = 0.52) populations (X^2 = 3.611, df = 1, p = 0.057).

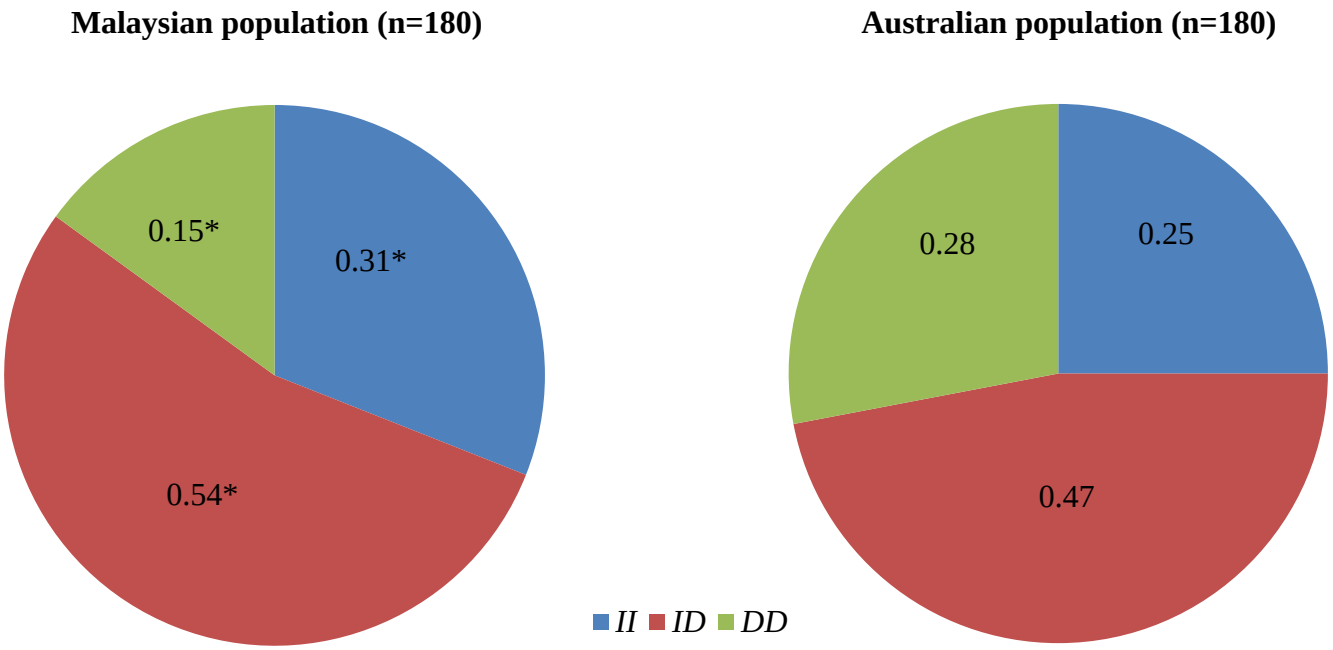


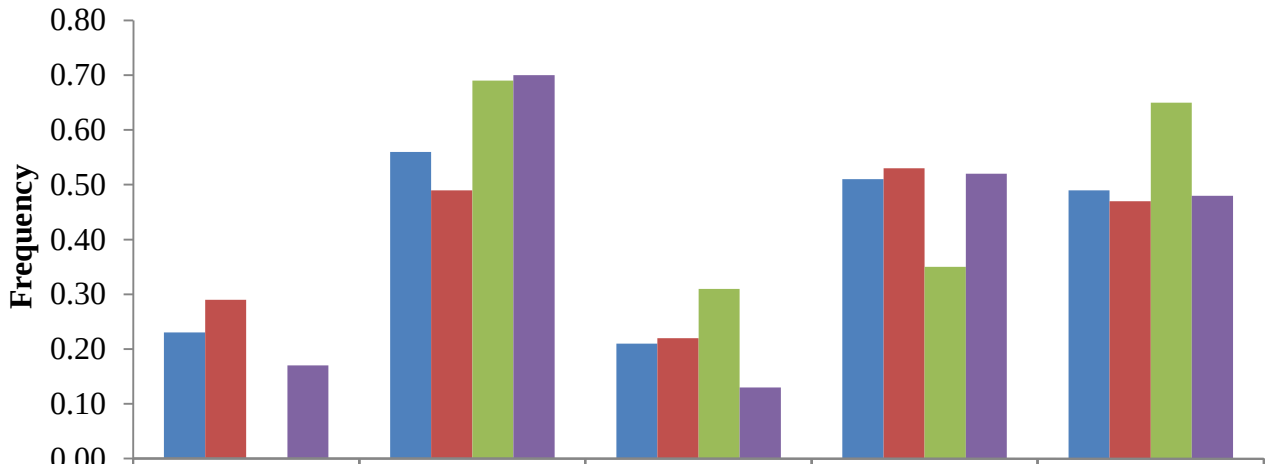
Figure 4.2 Frequencies of *ACE I/D* genotype in Malaysian and Australian populations

Note.
*p = 0.009 for genotype frequency in Malaysian population vs. Australian population

4.5.3 The Distribution of *ACTN R/X* Gene Polymorphism

4.5.3.1 Multi-Ethnic Groups in Malaysia

The *ACTN3 R/X* allele and genotype distributions of four ethnic groups in Malaysia are presented in Figure 4.3. The Malay group had *ACTN3 R/X* genotype frequencies of 0.23, 0.56, and 0.21, for *RR*, *RX*, and *XX* genotypes respectively. Meanwhile, in the Chinese group, the frequencies of *RR*, *RX*, and *XX* genotypes were 0.29, 0.49, and 0.22, respectively. The frequencies of *RR*, *RX*, and *XX* genotypes for Indian and Other Bumiputra groups were 0.00, 0.69, and 0.31, as well as 0.17, 0.70, and 0.13, respectively. The statistical analysis, however, showed insignificant difference between the ethnic groups with regard to *ACTN3 R/X* genotype frequency ($X^2 = 6.926$, df = 6, p = 0.328). The distribution of *ACTN3 R/X* allele was also not significantly different between Malay (*R* = 0.51; *X* = 0.49), Chinese (*R* = 0.53; *X* = 0.47), Indian (*R* = 0.35; *X* = 0.65), and Other Bumiputra (*R* = 0.52; *X* = 0.48) groups ($X^2 = 0.9383$, df = 3, p = 0.816).



	RR	*RX*	*XX*	*R*	*X*
	ACTN3 R/X Genotype			*ACTN3 R/X* Allele	
Malay (n=99)	0.23	0.56	0.21	0.51	0.49
Chinese (n=45)	0.29	0.49	0.22	0.53	0.47
Indian (n=13)	0.00	0.69	0.31	0.35	0.65
Other Bumiputra (n=23)	0.17	0.70	0.13	0.52	0.48

Figure 4.3 *ACTN3 R/X* allele and genotype frequencies in the multi-ethnic groups in Malaysia

4.5.3.2 Malaysian and Australian Populations

The distributions of *ACTN3 R/X* genotype in Malaysian and Australian populations are shown in Figure 4.4. In the Malaysian population, the frequencies of *RR*, *RX*, and *XX* genotypes were 0.22, 0.57, and 0.21, respectively. Meanwhile, the frequencies of *RR*, *RX*, and *XX* genotypes in the Australian population were 0.24, 0.57, and 0.19, respectively. Nevertheless, there was no significant difference in *ACTN3 R/X* genotype frequencies between Malaysian and Australian populations (X^2 = 0.413, df = 2, p = 0.814). In the Malaysian population, the frequencies of *R* and *X* alleles were 0.51 and 0.49, respectively. Meanwhile, the frequencies of *R* and *X* alleles in the Australian population were 0.53 and 0.47, respectively. The statistical analysis also showed that the difference in *ACTN3 R/X* allele frequency was insignificant between these two populations (X^2 = 0.1002, df = 1, p = 0.752).

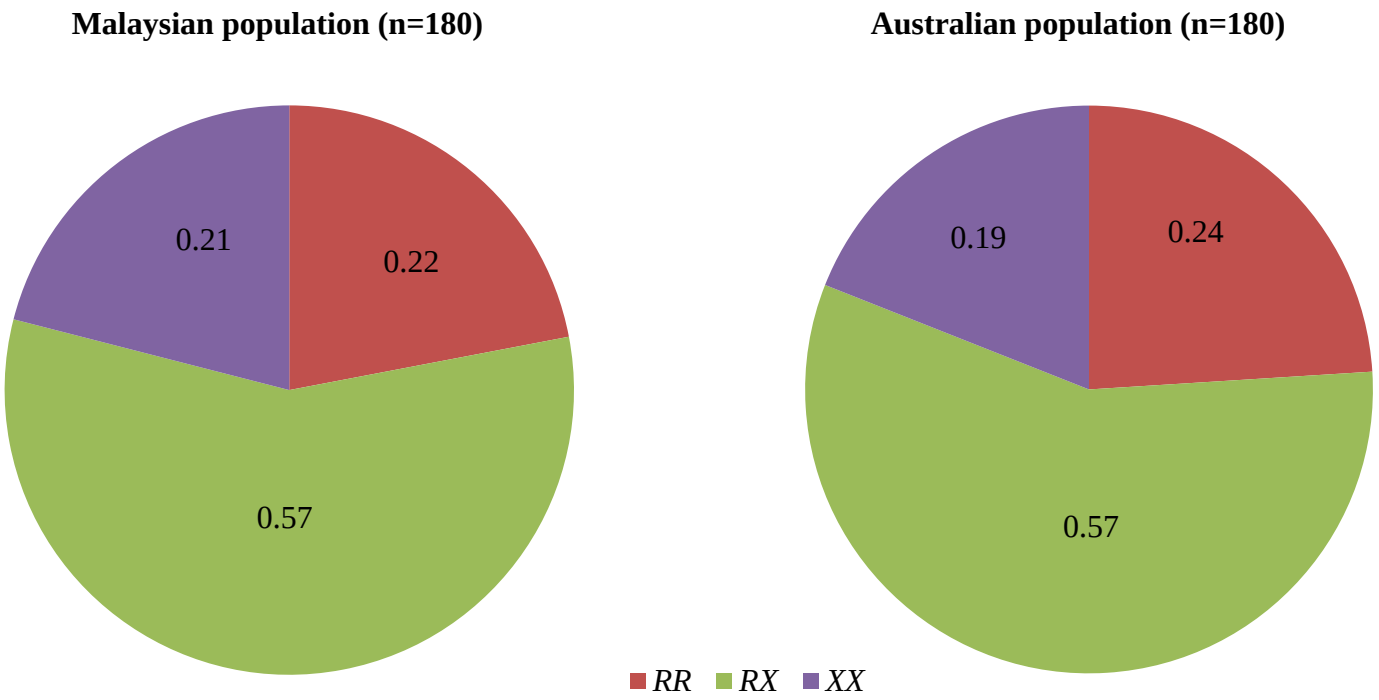


Figure 4.4 *ACTN3 R/X* genotype frequencies in Malaysian and Australian populations

4.6 Discussion

This population-based study established the distribution patterns of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the Malaysian population. In order to determine the exact distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms in the various populations in Malaysia, the number of participants with different ethnic groups was based on the current distribution ratio of ethnicity in Malaysia (Statistic, 2011), and included Other Bumiputra group, which had not been previously studied. This study was also designed to investigate ethnic variation in the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms by comparing the data from two different populations (Malaysians and Australians), and also between four ethnic groups in Malaysia.

The main finding of this study was that the distribution of *ACE I/D* gene polymorphism varied by ethnicity, as defined by a significant difference in the distribution of this polymorphism between Malaysian and Australian populations, as well as among the four ethnic groups in Malaysia. In contrast, the present study found that distribution of *ACTN3 R/X* gene polymorphism did not vary as much by ethnicity as no significant difference was observed in the distribution of this polymorphism among the ethnic groups.

As expected, a significant ethnic difference was observed in the distribution of the *ACE I/D* gene polymorphism between Malaysian and Australian populations. The results demonstrated that the *DD* genotype was significantly more frequent in the Australian population (0.28) than in the Malaysian population (0.15). The frequency of the *DD*

genotype in the Australian group was similar to a previous report for the Australian population (Lester et al., 1999, Lea et al., 2005). Meanwhile, the lower frequency of the *DD* genotype observed in the Malaysian population had been consistent with the findings obtained from previous studies conducted among other Asian populations (Saha et al., 1996, Huang et al., 2004, Movva et al., 2007).

The frequencies of *ACE II*, *ID*, and *DD* genotypes in Malaysian population were 0.31, 0.54 and 0.15, respectively. The results differed slightly from the previous report for the Malaysian population (Jayapalan et al., 2008). In the previous study, the *ACE I/D* genotype frequencies for the Malaysian population were 0.43, 0.43, and 0.14 for *II*, *ID*, and *DD* genotypes, respectively (Jayapalan et al., 2008). The reason for this inconsistent finding may be due to the different sample sizes involved in the study. The sample size for each ethnic group in the present study was based on the current distribution of these groups in Malaysia (Statistic, 2011), which had not been controlled for in the previous study (Jayapalan et al., 2008), thereby providing a more representative distribution of the *ACE I/D* gene polymorphism in the Malaysian population.

Within the Malaysian population, there was also a difference in the distribution of the *ACE I/D* gene polymorphism. Both Malay and Chinese groups showed to have higher frequency of *I* allele over the *D* allele, whereas the *D* allele appeared to be more prevalent than *I* allele in Indian and Other Bumiputra groups. The present result differed from the previous Malaysian study carried out by Jayapalan et al. (2008) in terms of allele and genotype frequencies though the similar trend of ethnic variation was observed. *I* allele

frequency was higher in the Malay group (0.66), followed by the Chinese (0.53), Indian (0.46), and the lowest in the Other Bumiputra group (0.41). The distribution pattern of *ACE I/D* gene polymorphism for the Malay group in the present study was remarkably similar with those in Japanese (Matsubara et al., 2002) and Taiwanese (Chuang et al., 1997) populations. The similar pattern between the Malay group and these populations matched with the Taiwan model, which hypothesised that the Malay group in the Malaysian population originated from the Austronesian group from Taiwan that have been thought to have migrated to the Malaysia Peninsular roughly 3,000 years ago (Comas et al., 1998). On the other hand, the distribution patterns of *ACE I/D* gene polymorphism for Chinese and Indian groups were slightly similar with the previous reports from populations in China (Saha et al., 1996) and India (Movva et al., 2007), respectively. This is of no surprise as Chinese in Malaysia mostly originated from Southern China while the Indians in Malaysia were mostly immigrants from Southern India (Gan et al., 2013). Nevertheless, there has been no any report on the frequency of *ACE I/D* gene polymorphism in the Other Bumiputra group, hence, this study had provided the first data set of this ethnic group. The frequency of the *I* allele in the Other Bumiputra group seemed to be among the lowest reported for Asian population, and was similar to that in Caucasian population (Ferrieres et al., 1999, Cam et al., 2005).

Apart from that, Batzer et al. (1994) suggested that the *ACE I/D* gene polymorphism is of African origin and the current ethnic variation on this polymorphism was due to the migration of modern humans out of Africa. During the migration of human, the frequencies of *ACE I/D* allele and genotype changed due to evolutionary factors, such

as natural selection and gene flow (Batzer et al., 1994). The *ACE I/D* gene polymorphism was used as markers in numerous population structure analyses as it has been considered to be a highly stable polymorphism, where there is no mechanism for deletion of this newly inserted element (Stoneking et al., 1997). The *D* allele is known as the ancestral form of this polymorphism, while the *I* allele is the most recent version of this polymorphism (Stoneking et al., 1997). The higher frequency of the *D* allele in a certain ethnic group is, therefore, indicative of the ancestral ethnic origin for this polymorphism (Stoneking et al., 1997). The frequencies of *D* allele observed in the Malaysian (0.42) and Australian (0.52) populations in the present study was relatively lower compared to previously reported of the black population (0.73) by Batzer et al. (1994), which appear to be consistent with this theory. Therefore, the finding of the present study supported the theory that the *ACE I/D* gene polymorphism is of African origin and the current ethnic variation on this polymorphism was due to the migration of modern humans out of Africa.

With regard to the present data obtained for *ACE I/D* gene polymorphism, ethnicity factor plays a significant role for the distribution of *ACE I/D* gene polymorphism, as previously suggested by Barley et al. (1994). These data indicate that the effect of *ACE I/D* gene polymorphism on human physical performance, as previously reported for the Caucasian population could be different in the Malaysian population.

In addition, with regard to *ACTN3 R/X* gene polymorphism, this is the first study reporting its distribution in the Malaysian population. The distributions of *ACTN3 RR*, *RX*, and *XX* genotypes were 0.22, 0.57, and 0.21, respectively. Meanwhile, the allele

distributions were 0.51 and 0.49 respectively, for *R* and *X* alleles. The allele frequency of *R* allele in the Malaysian population was closely similar to those reported for Indian population (Goel and Mittal, 2007). Moreover, demarcation data analysis of the Malaysian population based on ethnicity showed that the frequency of *R* allele in the Malay was 0.51, which was comparable with the findings obtained from Indian population (Goel and Mittal, 2007). The similar pattern between Malay group and Indian population had been concurrent with the finding retrieved from Comas et al. (1998), who reported that the Malay group in Malaysia are descendants of the Proto-Malays, who had admixed with Siamese, Javanese, Sumatran, Indian, Thai, Arab, and Chinese traders. On the other hand, the present results obtained for the Chinese and the Other Bumiputra groups were markedly similar to the previous report for the Asian population (Clarkson et al., 2005a). Meanwhile, the findings for the Indian group matched with the report by Kothari et al. (2011) for the Indian population.

When the data for the Malaysian population were compared to the Australian population, insignificant difference was observed for the genotype and the allele distributions of *ACTN3 R/X* gene polymorphism between these two populations. These findings are consistent with a previous study carried out by Goel and Mittal (2007), who demonstrated that the frequencies of both alleles and genotypes of *ACTN3 R/X* gene polymorphism in the Asian population had been similar to those of the Caucasian population. Within the Malaysian population, the statistical analysis also indicated insignificant difference in the distribution of this polymorphism between the four ethnic groups in Malaysia. A similar distribution between the Malaysian and the Australian

populations, as well as between the four ethnic groups in Malaysia, indicated that these studied populations may share similar positive selection of *ACTN3 R/X* gene polymorphism, as opposed in the previous study (MacArthur et al., 2007). In fact, it had been revealed that the effect of *ACTN3 R/X* gene polymorphism on human physical performance previously reported for the Caucasian population may also appear in the Malaysian population.

Despite the positive findings in the present study, the small sample size in certain ethnic groups is of particular relevance for genetic studies that often require sample size may have caused some of the analyses for *ACTN3 R/X* gene polymorphism to lose statistical power. Future studies with larger sample size may render more significant results.

4.7 Conclusion

In conclusion, this study demonstrated that the distribution of *ACE I/D* gene polymorphism varies by ethnicity, as defined by a significant difference in the distribution of this polymorphism between Malaysian and Australian populations, as well as between Malay and Other Bumiputra ethnic groups in Malaysia. Conversely, the distribution of *ACTN3 R/X* gene polymorphism did not vary by ethnicity. This study suggests that the effect of *ACE I/D* gene polymorphism on human physical performance may also differ by ethnicity, whilst the effects of *ACTN3 R/X* gene polymorphism on human physical performance may be similar across different human populations. The data from this study should, therefore, serve as a basis for the assessment of the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance in the Malaysian population. Therefore, further study is warranted for the assessment of the effects *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within the Malaysian population.

CHAPTER 5

THE EFFECTS OF *ACE I/D* AND *ACTN3 R/X* GENE POLYMORPHISMS ON HUMAN PHYSICAL PERFORMANCE IN THE MULTI-ETHNIC MALAYSIAN POPULATION

5.1 Introduction

Human physical performance is a complex human trait influenced by both environmental and genetic factors (Yu and Trent, 2010). Compelling evidence from twin studies reveals that genetic factors are more influential than environmental factors in determining human physical performance (Chatterjee and Das, 1995, Calvo et al., 2002, Maridaki, 2006, Alonso et al., 2014). Over the past several decades, several genetic variants have been identified to be related to human physical performance (Rankinen et al., 2001, Rankinen et al., 2002, Rankinen et al., 2004, Wolfarth et al., 2005, Rankinen et al., 2006, Bray et al., 2009, Loos et al., 2015) and among these, two genetic variants that have been most widely studied for human physical performance are angiotensin I-converting enzyme (*ACE*) *I/D* and alpha-actinin-3 (*ACTN3*) *R/X* gene polymorphisms (Ma et al., 2013).

ACE I/D gene polymorphism is responsible for the level of circulating and tissue ACE protein (Rigat et al., 1990), which is the main component of the renin-angiotensin system (RAS) (Sayed-Tabatabaei et al., 2006). ACE protein converts angiotensin I (ANG I) to angiotensin II (ANG II), which is a potent vasoconstrictor, and degrade bradykinin,

which is a potent vasodilator (Coates, 2003). *I* allele of the *ACE I/D* gene polymorphism produces less ACE protein compared to the *D* allele of this polymorphism (Rigat et al., 1990). The lower production of ACE protein decreases the conversion of ANG I to ANG II resulting in increased vasodilation and an increased delivery of oxygenated blood to the working muscles (Jones and Woods, 2003, Sayed-Tabatabaei et al., 2006). On the contrary, greater production of ACE protein increases the production of ANG II, which also acts as a muscle growth factor, resulting in increased muscle strength (Jones and Woods, 2003, Sayed-Tabatabaei et al., 2006). Given these different physiological characteristics, possession of the *ACE I* and *D* alleles may confer advantages for endurance and strength/power events, respectively.

ACTN3 R/X gene polymorphism codes for ACTN3 protein (North et al., 1999) which is one of the components of the Z disk of fast-twitch skeletal muscle fibre (Mills et al., 2001). While *R* allele of *ACTN3 R/X* gene polymorphism produces ACTN3 protein, *X* allele prevents the production of ACTN3 protein (North et al., 1999). The presence of ACTN3 protein gives static and stable function in maintaining the ordered myofibrillar array with greater coordination to aid generation of high muscle power and velocity during movements (Mills et al., 2001). Meanwhile, the lack of ACTN3 protein has resulted in alterations of skeletal muscle metabolism, explicit to aerobic metabolism (MacArthur et al., 2007). On the basis of the physiological function, possession of the *ACTN3 R* and *X* alleles may grant beneficial effects for strength/power and endurance activities, respectively.

The effects of possession of the *ACE I* and *ACTN3 X* alleles on endurance performance have been investigated in several case-control studies, with both alleles observed to be more prevalent among endurance-oriented athletes compared to other athletes or controls (Myerson et al., 1999, Nazarov et al., 2001, Tsianos et al., 2004b, Mayne, 2006, Ahmetov et al., 2008b, Min et al., 2009, Eynon et al., 2009b, Shang et al., 2010). In accordance with the findings from case-control studies, reports of several cross-sectional studies demonstrated that individuals with *ACE I* and *ACTN3 X* alleles exhibited higher endurance performance-related phenotype scores than those with *ACE D* and *ACTN3 R* alleles (Hagberg et al., 2002, Zhang et al., 2003, Heled, 2004, Lucia et al., 2007, Voroshin and Astratenkova, 2008, Goh et al., 2009, Ahmetov et al., 2011).

With regard to the effect of possession of the *ACE D* and *ACTN3 R* alleles on strength/power performance, several case-control studies reported that these two alleles are more frequently found in strength/power-oriented athletes compared to other athletes or controls (Nazarov et al., 2001, Yang et al., 2003, Niemi and Majamaa, 2005, Mayne, 2006, Costa et al., 2009b, Eynon et al., 2009b, Kothari et al., 2011, Kikuchi et al., 2012). Additionally, several cross-sectional studies showed that carriers of the *ACE D* and *ACTN3 R* alleles present greater levels of strength/power performance-related phenotype than *ACE I* and *ACTN3 X* alleles carriers (Clarkson et al., 2005a, Williams et al., 2005, Moran et al., 2006b, Vincent et al., 2007, Charbonneau, 2007, Giaccaglia et al., 2008, Ichinoseki-Sekine et al., 2010, Shang et al., 2012, Ahmetov et al., 2013, Erskine et al., 2014).

The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance, however, remain ambiguous as some studies failed to replicate the results of others (Hagberg et al., 1999, Zhaoa et al., 2003, Kasikcioglu et al., 2004, Clarkson et al., 2005a, Cam et al., 2007, Tobina et al., 2010, Ichinoseki-Sekine et al., 2010). For instance, studies involving Caucasian athletes revealed that individuals with *ACE II* genotype had higher maximal oxygen consumption (VO_2max) than other genotype carriers (Hagberg et al., 1999, Kasikcioglu et al., 2004). On the other hand, a study by Zhaoa et al. (2003) among an Asian population found that VO_2max value was higher in *ACE DD* genotype carriers compared to those with other *ACE I/D* genotypes. Furthermore, Cam et al. (2007) demonstrated that Caucasian athletes with *ACE II* genotype presented better endurance performance than other genotype carriers while in a study involving Japanese runners, the endurance performance was significantly higher in those with *ACE DD* genotype (Tobina et al., 2010). In a study among an Asian population, individuals with *ACTN3 R* allele exhibited a greater strength/power performance following resistance training than those with *ACTN3 X* allele (Ichinoseki-Sekine et al., 2010). Nevertheless, a study by Clarkson et al. (2005a) among Caucasian samples reported that individuals with *ACTN3 X* allele scored higher in strength/power performance following resistance training when compared to *ACTN3 R* allele carriers. These findings demonstrate that the influences of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance remain controversial and more importantly, suggest the potential ethnic variation in the effects of these polymorphisms on human physical performance.

The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance across different ethnic groups have yet to be largely examined. To date, the study by Ma et al. (2013) reported insufficient data among Asian populations. The findings from the first study of this research project showed that the distribution of *ACE I/D* gene polymorphism varied between the Malaysian and Australian populations. Therefore, there is a possibility that the effect of *ACE I/D* gene polymorphism on human physical performance in the Malaysian population would be different from Caucasian population. On the other hand, in the first study, the distribution of *ACTN3 R/X* gene polymorphism was similar between Malaysian and Australian populations, which indicate that the *ACTN3 R/X* gene polymorphism may confer similar effects on human physical performance between these two populations.

5.2 Aims

This study was designed 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. This study also determined if the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance differ by ethnicity. Possession of the *I* allele of *ACE I/D* and *X* allele of *ACTN3 R/X* gene polymorphisms were hypothesized to affect endurance performance. Meanwhile, possession of the *D* allele of *ACE I/D* and *R* allele of *ACTN3 R/X* gene polymorphisms were hypothesized to affect strength/power performance. It was also hypothesized that the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance will differ between ethnic groups.

5.3 Methods and Participants

5.3.1 Study Design

This study employed two different approaches to demonstrate the relevance of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance. One of the approaches was through case-control study in which the allele and genotype frequencies of *ACE I/D* and *ACTN3 R/X* gene polymorphisms were compared between: (i) the whole cohort of athletes and controls in the Malaysian population, (ii) multi-ethnic groups of athletes and controls in the Malaysian population, (iii) endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population, and (iv) Malay intermittent athletes and Australian intermittent athletes. The second approach was through cross-sectional study, in which endurance and strength/power performances were compared to different genotype groups of Malaysian athletes. The study protocols on Malaysian and Australian samples were approved by the Human Research Ethics Committee in Universiti Sains Malaysia (Appendix A) and the Human Research Ethics Committee in University of Sydney, respectively (Appendix B).

5.3.2 Participants

The participants were drawn from two different populations; a population of Asian (n=360) and Caucasian (n=33) living in Malaysia and Australia, respectively. Participants who reported having mixed ancestry within three generations were excluded from this

study. The number of participants for this study was based on the sample size calculation using Power and Sample Size Calculation version 3.1.2 software (Dupont and Plummer, 1990). The power of the study was set at 0.80 with 95% of confidence interval and the effect size of 0.25. Participants consent and information details forms were presented in Appendix C.

5.3.2.1 Malaysian Population

The study participants for Malaysian population comprised of 180 university athletes (148 male, 32 female) aged 20.5 ± 1.9 (mean ± SD) years and 180 sedentary healthy individuals (70 male, 110 female) aged 20.4 ± 1.6 years. All participants were students from several universities in Malaysia. The proportion of participants with different ethnic backgrounds had been set according to the current distribution ratio in Malaysia (Statistic, 2011). Based on the respective ratio and to ensure there was no ethnicity skew and to overcome any potential problems of population stratification, both athletes and control groups have same number of samples for each ethnic group with 99 Malays (55%), 45 Chinese (24.7%), 23 Other Bumiputras (12.9%), and 13 Indians (7.4%) in each group. All sedentary individuals reported of having sedentary lifestyle (two or fewer days a week of recreational exercise for less than 30 minutes a day for the preceding three months (Pate et al., 2008)). The athletes were trained for at least one year and represent the university in national sporting competition. Within the cohort of athletes, 34 participants (25 male, 9 female) aged 19.8 ± 1.9 years were classified as endurance athletes, 41 participants (25 male, 16 female) aged 19.7 ± 1.8 years as strength/power athletes, and 105 participants (99 male, 6 female)

aged 21.0 ± 1.8 years as intermittent athletes according to the type of exercise metabolism that predominates in the discipline they practice, time of competitive exercise performance and intensity of exertion in each sport using the classification described by Maciejewska-Karlowska et al. (2013) (Appendix E). Thirty three Malay intermittent athletes (all males) aged 20.8 ± 1.7 years in the intermittent group were randomly selected for well-matched comparison-group study to Australian population.

5.3.2.2 Australian Population

In addition, 33 intermittent athletes (all males) aged 20.7 ± 4.0 years, who were of Caucasian origin and trained for at least one year and represent the university in national sporting competition, had been recruited from the Australian population. All the participants in the Australian population were students at the University of Sydney, Sydney, Australia.

5.3.3 Anthropometric Measurements

Anthropometric measurements were collected from participants in the Malaysian population. Participant's body height was measured using a portable stadiometer (Seca 213, Seca Corporation, USA). Meanwhile, participant's body weight, body mass index, and body fat, were measured using an Omron KARADA Scan Body Composition & Scale (HBF-362, Omron Corporation, Japan).

5.3.4 Deoxyribonucleic Acid (DNA) Sample Collection

Deoxyribonucleic acid (DNA) sample from each participant was obtained via buccal swab using a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy). The swabs were air dried and placed in sterile 1.5 ml microcentrifuge tubes and stored at -20°C until used for DNA isolation.

5.3.5 Genotype Determination

5.3.5.1 DNA Extraction

Genomic DNA was isolated from the swab samples using the GeneAll® Exgene™ Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co. Ltd., Seoul, South Korea). First, 400 µl of 1X phosphate buffered saline (PBS), 20 µl of Proteinase K (20 mg/ml), and 400 µl of blood lysis buffer were added into the tube with the swab. Immediately, the solutions were mixed with vigorous vortex. After that, the tube was incubated at 56˚C for 10 minutes and followed by a short spin to remove any drop from the inside of the lid. Thereafter, 400 µl of absolute ethanol was added to the lysates and was mixed well via vortex. The tube was subsequently centrifuged briefly and 700 µl of the mixture was carefully transferred to the SV column. The tube was then centrifuged for 1 minute at 6000 x g above (> 8000 revolutions per minute (rpm)). After discarding the supernatant, the SV column was reinserted into the new collection tube. Afterwards, 600 µl of column wash solution b buffer was added into the tube and was centrifuged for 1

min at 6,000 x g above (> 8000 rpm). After replacing the collection tube with a new one, 700 µl of column wash solution t buffer was added and the tube was centrifuged for 1 min at 6000 x g above (> 8000 rpm). Again, the pass-through was discarded and the SV column was reinserted into the collection tube. The tube was then centrifuged at full speed for 1 minute to remove the residual wash buffer, and the SV column was then placed in a fresh 1.5 ml Eppendorf tube. Lastly, 200 µl of elution buffer was added to the tube to increase the total DNA yield, and followed by an incubation process for 1 minute at room temperature. The tube was centrifuged at full speed for 1 minute and was stored at -20^{0}C before applying it to molecular analysis.

5.3.5.2 Molecular Analysis for *ACE I/D* Gene Polymorphism

Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.8 µm of each primer (forward primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3': reverse primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3'), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 10.8 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the *ACE I/D* gene polymorphism was amplified under the following conditions; 7 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 62°C, and 1 minute at 72°C, with a final step of 7 minutes at 72°C. The

amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 490 base pair (bp) and 190 bp bands indicated the *ACE* insertion (*I*) and deletion (*D*) alleles, respectively. The PCR products for *ACE I/D* gene polymorphism were confirmed by sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia).

5.3.5.3 Molecular Analysis for *ACTN3 R/X* Gene Polymorphism

PCR was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.2 µm of each primer (forward primer: 5'-CTGTTGCCTGTGGTAAGTGGG-3': reverse primer: 5'-TGGTCACAGTATGCAGGAGGG-3'), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 12.3 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the *ACTN3 R/X* gene polymorphism was amplified under the following conditions; 2 minutes at 95°C, followed by 26 cycles of 30 seconds at 95°C, 30 seconds at 61.6°C, and 30 seconds at 72°C, with a final step of 5 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 291 bp band indicated the successful amplification of *ACTN3* gene. The PCR product was then confirmed by DNA sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia). In order to obtain the genotype of the *ACTN3 R/X* gene polymorphism, the amplified PCR

product was digested with *Dde*I restriction enzyme (New England Biolabs, Beverly, MA, USA) in a final volume of 10 µl consisting of 1.0 µl of 10X NEBuffer3 (New England Biolabs, Beverly, MA, USA), 1 U of of *Dde*I restriction enzyme (New England Biolabs, Beverly, MA, USA), and 8.5 µl of amplified PCR product. The reaction mix was incubated at 37°C for 45 minutes and the digestion product was electrophoresed on 2.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 205 bp and 86 bp bands is an indication for *R* allele while the presence of 108 bp, 97 bp, and 86 bp bands is an indication for *X* allele.

5.3.6 Physical Tests

Subsequent to DNA sampling, three physical tests (Yo-Yo intermittent recovery level 2, handgrip and leg strength tests) were administered to the Malaysian varsity athletes in order to determine their endurance and strength/power performances. The selection of the type of physical test used in this study was governed by the reason that the selected physical tests were valid and reliable for testing endurance and strength/power performances of the athletes. In addition, a study by Karakoc et al. (2012) found that Yo-Yo intermittent recovery level 2 is more suitable for testing endurance performances of the athletes over the other tests such as Yo-Yo endurance test (continuous) (YET). Meanwhile, the handgrip and leg strength tests are often used in physical fitness measures because of its ease of administration (Coldwells et al., 1994, Amaral et al., 2012). The validity and reliability of the each of the physical tests mentioned above have been

previously reviewed (handgrip strength (Anumula et al., 2014), leg strength (Coldwells et al., 1994), and Yo-Yo intermittent recovery level 2 (Krustrup et al., 2003) tests).

5.3.6.1 Yo-Yo Intermittent Recovery Level 2 Test

Before the test began, participants were fitted with a Polar transmitter belt (T61, Polar Electro Oy, Finland) onto their chest. In this test, the athletes started out shuttling from one end of the marked course to the other at a relatively slow pace and then quickly ramped up their speed according to the pace set by the recorded beeps. In each bout of intense running, they performed 10 seconds of active recovery and then returned to the start/finish line to await the cue for the next stage. Athletes were verbally encouraged during the test. A warning was given when they did not complete a successful out and back shuttle within the allocated time. Their maximum heart rate achieved immediately after running was recorded. The last speed level and number of shuttles reached before they received a second warning or voluntarily withdrew from the test was recorded as the score for the test. The endurance capacity of the athlete was computed by converting the score to the total distance covered using standard norm for Yo-Yo intermittent recovery level 2 test. In addition, to ensure that the athletes had given their maximum efforts during test, their heart rate achieved after running must be within 10 beats per minute (bpm) of their age-predicted maximum heart rate (Krustrup et al., 2003) (Athlete that did not achieve age-predicted maximum heart rate were excluded from the study). The procedure of the Yo-Yo intermittent recovery level 2 was consistent to the earlier method published by Bangsbo et al. (2008).

5.3.6.2 Handgrip Strength Test

The handgrip test was performed on a hand dynamometer (Takei A5401, Takei Scientific Instruments Co. Ltd., Japan) to determine an athlete's isometric handgrip strength. A standard procedure of measuring handgrip strength (kg) using a hand dynamometer followed the procedure described by Tomchuk (2011). The dynamometer was calibrated according to manufacturer's instruction, by hanging calibration weights across the handle of the dynamometer that fixed on a wooden support. The athletes held the dynamometer in the dominant hand (self-reported by the athletes), with the arm at right angles and the elbow by the side of the body. The handle of the dynamometer was adjusted if required. Prior to the start of the test, the dial on the dynamometer was reset to zero. When ready, the athletes squeezed the dynamometer with maximum isometric effort, which was maintained for about 5 seconds. Athletes were verbally encouraged during the test. The athlete performed the test three times with 10 to 20 seconds rest interval, and the average score was used for data analysis.

5.3.6.3 Leg Strength Test

Isometric leg strength (kg) was measured using a back and leg dynamometer (Takei A5402, Takei Scientific Instruments Co. Ltd., Japan). The procedure for this test followed the procedure described by Ashok (2008). The dynamometer was calibrated according to manufacturer's instruction by using calibration weights. Prior to the start of the test, the dial on the dynamometer was reset to zero. The athletes stood upright with

both feet on the base of the dynamometer. The chain length was adjusted until the athlete's knees were bent around 110 degrees. In this position, the athletes pulled the handle bar as hard as possible for 5 seconds, with the athletes were verbally encouraged during the test. The maximum reading indicated on the dynamometer was recorded. Each athlete performed the test three times with a pause of about 10 to 20 seconds between each trial and the average score was analysed for isometric leg strength.

5.4 Statistical Analysis

The descriptive data are presented as mean ± standard deviation (SD). The independent t-test was used to compare the means of two independent samples. Allele frequencies of *ACE I/D* and *ACTN3 R/X* gene polymorphisms were determined by direct counting. The Hardy-Weinberg equilibrium (HWE) test was used for genotyping quality control to describe that the genotype distribution of a population is large, self-contained, and randomly mating (Xu et al., 2002). As HWE may be violated in athletes without being caused by genotyping errors (Wittke-Thompson et al., 2005, Attia et al., 2009), the HWE test was tested for the control group in the Malaysian population. Simple HWE calculator (http://www.koonec.com/wp-content/uploads/k-blog/HWE.xls.) was used to confirm that the observed *ACE I/D* and *ACTN3 R/X* genotype frequencies were in HWE. The multivariate analysis was used to examine the association between ethnicity and studied polymorphisms factors on performance measured in this study (the Yo-Yo intermittent recovery level 2 performance, handgrip and leg strength). The chi-square (X^2) test was used to examine the difference in the *ACE I/D* and *ACTN3 R/X* allele and genotype

frequencies between the groups: (i) the whole cohort of athletes and controls in the Malaysian population, (ii) multi-ethnic groups of athletes and controls in the Malaysian population, (iii) endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population, and (iv) Malay intermittent athletes and Australian intermittent athletes. The mean of the anthropometric data among the athlete group and the mean of the Yo-Yo intermittent recovery level 2, handgrip strength and leg strength scores among the genotype groups were analysed using one-way analysis of variance (ANOVA) and followed by Bonferroni's post-hoc test when appropriate. All statistical evaluations were performed using the IBM SPSS statistical version 20.0, (Armonk, New York, USA), with the level of significance set at $p < 0.050$.

5.5 Results

5.5.1 Physical Characteristics of Participants

5.5.1.1 The Whole Cohort of Athletes and Controls in the Malaysian Population

Table 5.1 shows the descriptive statistics for the physical characteristics of the whole cohort of athletes and controls in the Malaysian population. There were significant differences in all variables between these two groups, with controls having lower mean values for height ($t(358) = 8.649$, $p < 0.001$), body weight ($t(358) = 7.882$, $p < 0.001$), and body mass index ($t(358) = 4.098$, $p < 0.001$) compared to athletes. Conversely, the mean value of body fat was higher in controls than in athletes ($t(358) = -4.244$, $p < 0.001$).

Table 5.1 Physical characteristics of athletes and controls in the Malaysian population

Variables	Athletes (n=180)	Controls (n=180)	p value
Height (cm)	168.8 ± 9.1*	160.7 ± 8.9	< 0.001
Body Weight (kg)	67.5 ± 13.6*	56.3 ± 12.4	< 0.001
Body Mass Index (kg/m^2)	23.6 ± 4.0*	21.8 ± 3.8	< 0.001
Body Fat (%)	18.6 ± 6.3*	21.5 ± 7.7	< 0.001

Note.
Data shown as mean ± SD
*Significantly different compared to controls ($p < 0.001$)

5.5.1.2 Endurance, Strength/Power, and Intermittent Athletes in the Malaysian Population

Physical characteristics of endurance, strength/power and intermittent athletes in the Malaysian population were presented in Table 5.2. All athletes were similar in body mass index ($F(2, 177) = 2.894$, $p = 0.058$) and body fat ($F(2, 177) = 0.635$, $p = 0.531$). There were significant differences in height ($F(2, 177) = 9{,}492$, $p < 0.001$) and body weight ($F(2, 177) = 6.027$, $p = 0.003$) between the athlete groups. Post hoc tests using the Bonferroni correction revealed that the mean values of height and body weight were significantly lower in the strength/power group when compared to the intermittent group (Height: $p < 0.001$, Body weight: $p = 0.001$), though it was not significantly different with the values among endurance group (Height: $p = 0.056$, Body weight: $p = 0.940$).

Table 5.2 Physical characteristics of endurance, strength/power and intermittent athletes in the Malaysian population

Variables	Endurance (n=34)	Strength/Power (n=41)	Intermittent (n=105)	p value
Height (cm)	168.5 ± 9.8	163.7 ± 10.4*	170.8 ± 7.9	< 0.001
Body Weight (kg)	63.6 ± 17.0	62.5 ± 9.2[#]	70.0 ± 13.4	0.003
Body Mass Index (kg/m^2)	22.1 ± 4.1	23.2 ± 3.0	24.0 ± 4.3	0.058
Body Fat (%)	18.6 ± 6.1	19.6 ± 7.3	18.7 ± 6.4	0.531

Note.
Data shown as mean ± SD
*Significantly different compared to intermittent athletes ($p < 0.001$)
[#] Significantly different compared to intermittent athletes ($p = 0.001$)

Yo-Yo intermittent recovery level 2 performance and leg strength value in athletes from different sporting disciplines were presented in Table 5.3. Yo-Yo intermittent recovery level 2 performance differed significantly among athletes from the three different sporting disciplines ($F(2, 177) = 4.340$, $p = 0.014$), with the endurance athletes had significantly higher performance scores than the strength/power ($p = 0.021$) and intermittent athletes ($p = 0.029$). Handgrip strength differed significantly among athletes from the three different sporting disciplines ($F(2, 177) = 3.994$, $p = 0.020$), with the intermittent athletes had significantly higher values of handgrip strength than the strength/power athletes ($p = 0.025$), but not significantly different compared to endurance athletes ($p = 0.336$). There was no significant difference was observed for the leg strength value among the sporting groups ($F(2, 177) = 0.538$, $p = 0.506$).

Table 5.3 Yo-Yo intermittent recovery level 2 performance and leg strength value in athletes from different sporting disciplines in the Malaysian population

Variables	**Endurance (n=34)**	**Strength/Power (n=41)**	**Intermittent (n=105)**	**p value**
Yo-Yo intermittent recovery level 2 performance (m)	429.4 ± 203.8	320.8 ± 197.5*	340.8 ± 148.7**	0.014
Handgrip Strength (kg)	37.7 ± 9.8	36.1 ± 10.2	40.8 ± 9.2[#]	0.020
Leg Strength (kg)	105.6 ± 28.2	108.9 ± 41.2	112.3 ± 33.5	0.506

Note.
Data shown as mean ± SD
*Significantly different compared to endurance athletes ($p = 0.021$)
**Significantly different compared to endurance athletes ($p = 0.029$)
[#]Significantly different compared to strength/power athletes ($p = 0.025$)

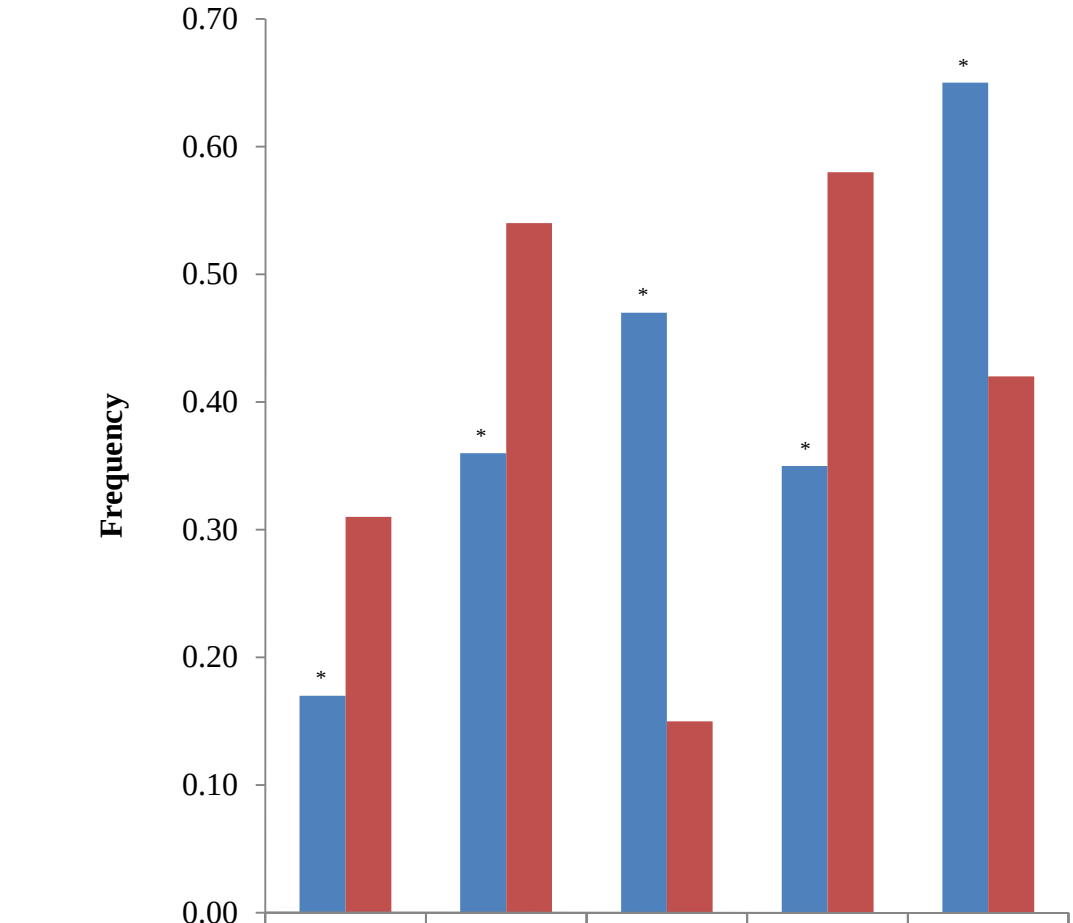
5.5.2 The Distribution of *ACE I/D* Gene Polymorphism

5.5.2.1 Hardy-Weinberg Equilibrium

ACE I/D genotype distribution for the control group in the Malaysian population was in agreement with the Hardy-Weinberg equilibrium ($p > 0.050$) (Appendix D).

5.5.2.2 The Whole Cohort of Athletes and Controls in the Malaysian Population

The allele and genotype frequencies of *ACE I/D* gene polymorphism in the whole cohort of athletes and controls in the Malaysian population are presented in Figure 5.1. The *ACE I/D* allele frequency in the whole cohort of athletes was significantly different from the whole cohort of controls ($X^2 = 18.776$, $df = 1$, $p < 0.001$) with the athletes had a lower frequency of *I* allele and higher frequency of *D* allele than controls. There was also a significant difference in *ACE I/D* genotype frequency between athletes and controls ($X^2 = 44.070$, $df = 2$, $p < 0.001$). The athletes had a lower frequency of *II* and *ID* genotypes than in controls. On the contrary, the frequency of *DD* genotype was significantly higher in athletes than controls.



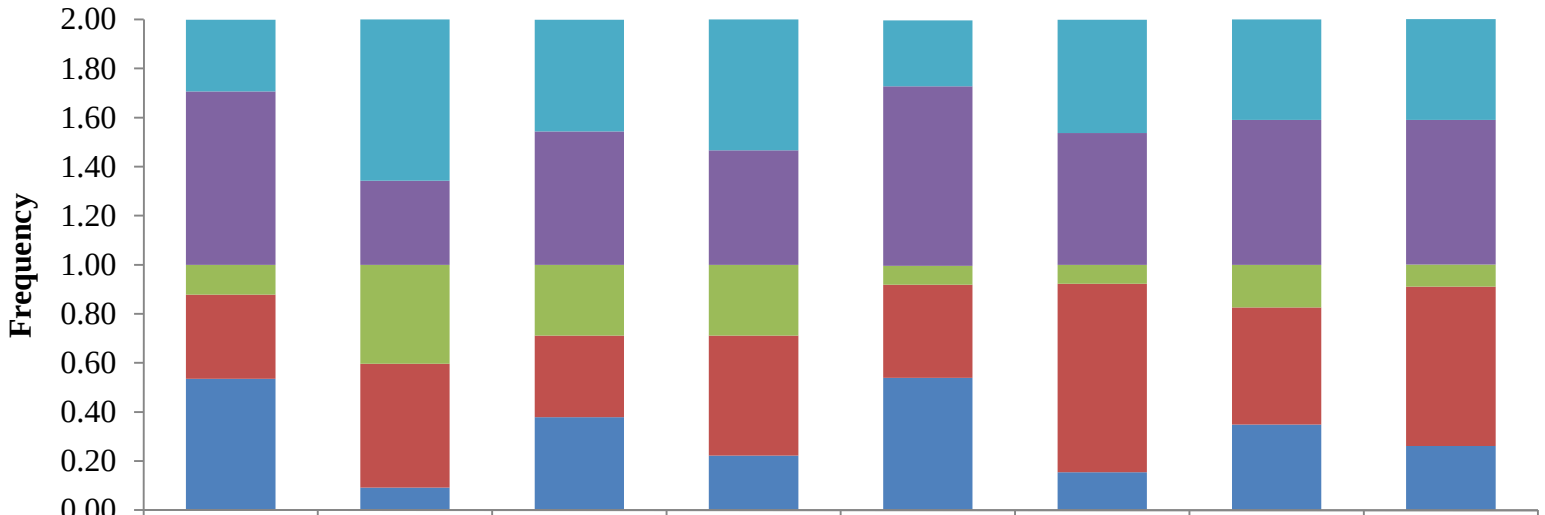
	II	*ID*	*DD*	*I*	*D*
	ACE I/D Genotype			*ACE I/D* Allele	
Athletes (n=180)	0.17*	0.36*	0.47*	0.35*	0.65*
Controls (n=180)	0.31	0.54	0.15	0.58	0.42

Figure 5.1 *ACE I/D* allele and genotype frequencies in the whole cohort of athletes and controls in the Malaysian population

Note.
*p < 0.001 for allele and genotype frequencies in athletes vs. controls

5.5.2.3 Multi-Ethnic Groups of Athletes and Controls in the Malaysian Population

The allele and genotype frequencies of *ACE I/D* gene polymorphism among multi-ethnic groups of athletes and controls in the Malaysian population are presented in Figure 5.2. Within athlete group, the distribution of *ACE I/D* genotype was not significantly different between the ethnic groups ($X^2 = 9.532$, df = 6, p = 0.146). The distribution of *ACE I/D* allele was also not significantly different between the ethnic groups ($X^2 = 5.1082$, df = 3, p = 0.164). Malay athletes differed from Malay controls in their allele ($X^2 = 26.248$, df = 1, $p < 0.001$) and genotype ($X^2 = 49.350$, df = 2, $p < 0.001$) frequencies, with Malay athletes had a higher frequency of *D* allele and *DD* genotype than in Malay controls. Chinese athletes did not differ from Chinese controls in their *ACE I/D* allele ($X^2 = 0.400$, df = 1, p = 0.527) and genotype ($X^2 = 3.139$, df = 2, p = 0.208) distributions. Similarly, Indian athletes did not differ from Indian controls in their *ACE I/D* allele ($X^2 = 0.65$, df = 1, p = 0.420) and genotype ($X^2 = 4.444$, df = 2, p = 0.108) frequencies. There were also insignificant differences in *ACE I/D* allele ($X^2 = 0$, df = 1, p = 1) and genotype ($X^2 = 0.012$, df = 2, p = 0.222) distributions between athletes and controls within Other Bumiputra group.



	Malay Athletes (n=99)	Malay Controls (n=99)	Chinese Athletes (n=45)	Chinese Controls (n=45)	Indian Athletes (n=13)	Indian Controls (n=13)	Other Bumiputra Athletes (n=23)	Other Bumiputra Controls (n=23)
ACE I/D Allele *I*	0.29*	0.66	0.46	0.53	0.27	0.46	0.41	0.41
ACE I/D Allele *D*	0.71*	0.34	0.54	0.47	0.73	0.54	0.59	0.59
ACE I/D Genotype *II*	0.12*	0.40	0.29	0.29	0.08	0.08	0.17	0.09
ACE I/D Genotype *ID*	0.34*	0.51	0.33	0.49	0.38	0.77	0.48	0.65
ACE I/D Genotype *DD*	0.54*	0.09	0.38	0.22	0.54	0.15	0.35	0.26

Figure 5.2 *ACE I/D* allele and genotype frequencies in multi-ethnic groups of athletes and controls in the Malaysian population

Note.
*$p < 0.001$ for allele and genotype frequencies in Malay athletes vs. Malay controls

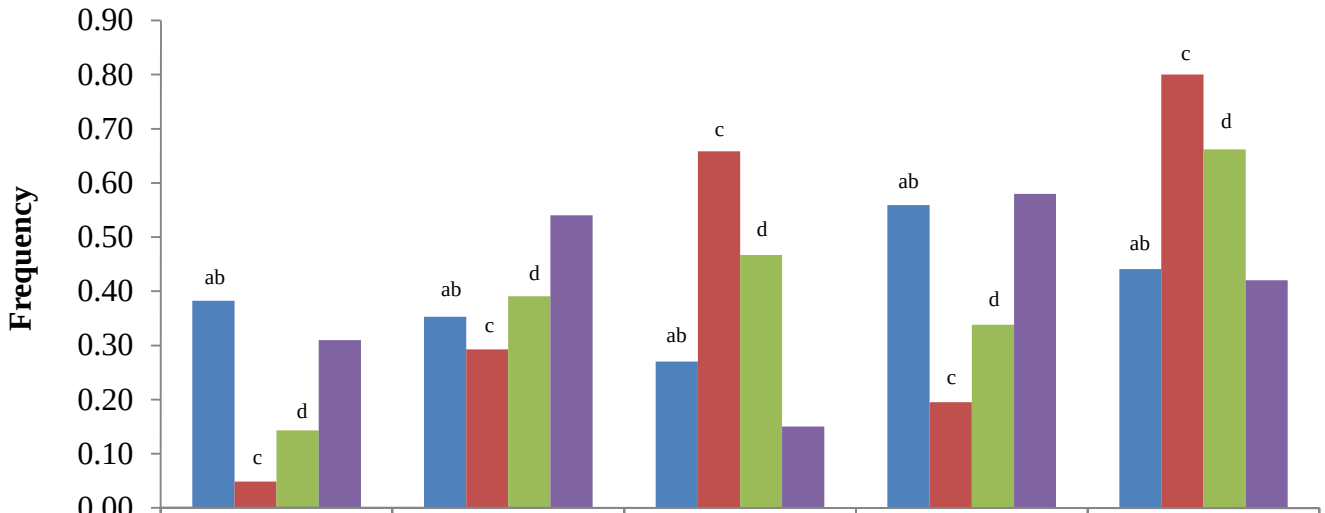
5.5.2.4 Endurance Athletes, Strength/Power Athletes, Intermittent Athletes, and Controls in the Malaysian Population

Considering the opposing effects of possession of the *I* and *D* alleles on particular sporting disciplines, the *ACE I/D* allele and genotype frequencies were compared between endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population as shown in Figure 5.3. The *ACE I/D* allele ($X^2 = 28.71$, df = 3, $p < 0.001$) and genotype ($X^2 = 63.354$, df = 6, $p < 0.001$) frequencies differed significantly across the four groups.

The endurance athletes differed significantly in *ACE I/D* allele and genotype frequencies when compared with the strength/power ($X^2 = 10.671$, df = 1, $p = 0.001$ for allele frequency, and $X^2 = 16.558$, df = 2, $p < 0.001$ for genotype frequency) and intermittent athletes ($X^2 = 5.0097$, df = 1, $p = 0.025$ for allele frequency, and $X^2 = 9.919$, df = 2, $p = 0.007$ for genotype frequency), but not to those in controls ($X^2 = 0.042$, df = 1, $p = 0.836$ for allele frequency, and $X^2 = 4.808$, df = 2, $p = 0.090$ for genotype frequency).

The strength/power athletes differed significantly in *ACE I/D* allele and genotype frequencies when compared with the controls ($X^2 = 19.5623$, df = 1, $p < 0.001$ for allele frequency, and $X^2 = 48.132$, df = 2, $p < 0.001$ for genotype frequency), but not to those in intermittent athletes ($X^2 = 3.0566$, df = 1, $p = 0.08$ for allele frequency, and $X^2 = 5.103$, df = 2, $p = 0.078$ for genotype frequency). Meanwhile, the intermittent athletes differed significantly in *ACE I/D* allele and genotype frequencies when compared with the controls

(X^2 = 14.6438, df = 1, $p < 0.001$ for allele frequency, and X^2 = 35.308, df = 2, $p < 0.001$ for genotype frequency). Among the four groups, the endurance athletes had the highest frequencies of *II* genotype, whilst, the strength/power athletes showed the highest frequencies of *DD* genotype.



Endurance athletes (n=34)	0.38[ab]	0.35[ab]	0.27[ab]	0.56[ab]	0.44[ab]
Strength/Power athletes (n=41)	0.05[c]	0.29[c]	0.66[c]	0.20[c]	0.80[c]
Intermittet athletes (n=105)	0.14[d]	0.39[d]	0.47[d]	0.34[d]	0.66[d]
Controls (n=180)	0.31	0.54	0.15	0.58	0.42

Figure 5.3 *ACE I/D* allele and genotype frequencies among endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population

Note.

[a]$p = 0.001$ for allele frequency, and $p < 0.001$ for genotype frequency in endurance athletes vs. strength/power athletes

[b]$p = 0.025$ for allele frequency, and $p = 0.007$ for genotype frequency in endurance athletes vs. intermittent athletes

[c]$p < 0.001$ for allele and genotype frequencies in strength/power athletes vs. controls

[d]$p < 0.001$ for allele and genotype frequencies in intermittent athletes vs. controls

5.5.2.5 Malay and Caucasian Intermittent Athletes

The genotype frequency of the *ACE I/D* gene polymorphism among Malay intermittent athletes and Caucasian intermittent athletes are shown in Figure 5.4. There was a significant difference in genotype frequency between Malay and Caucasian intermittent athletes ($X^2 = 8.471$, df = 2, p = 0.014) with the frequency of the *DD* genotype in Malay intermittent athletes significantly higher than in Caucasian intermittent athletes, respectively. However, allele frequency of *ACE I/D* gene polymorphism amongst the Malay intermittent athletes (*I* = 0.46; *D* = 0.54) was not significantly different from that amongst the Caucasian intermittent athletes (*I* = 0.56; *D* = 0.44) ($X^2 = 0.546$, df = 1, p = 0.460).

5.5.3 Interaction between Ethnicity and *ACE I/D* Gene Polymorphism on Endurance and Strength/Power Performances

There was no significant interaction between ethnicity and *ACE I/D* gene polymorphism on Yo-Yo intermittent recovery level 2 performance (F(2, 174) = 0.651, p = 0.523), handgrip strength (F(2, 174) = 0.716, p = 0.490) and leg strength (F(2, 174) = 0.414, p = 0.662).

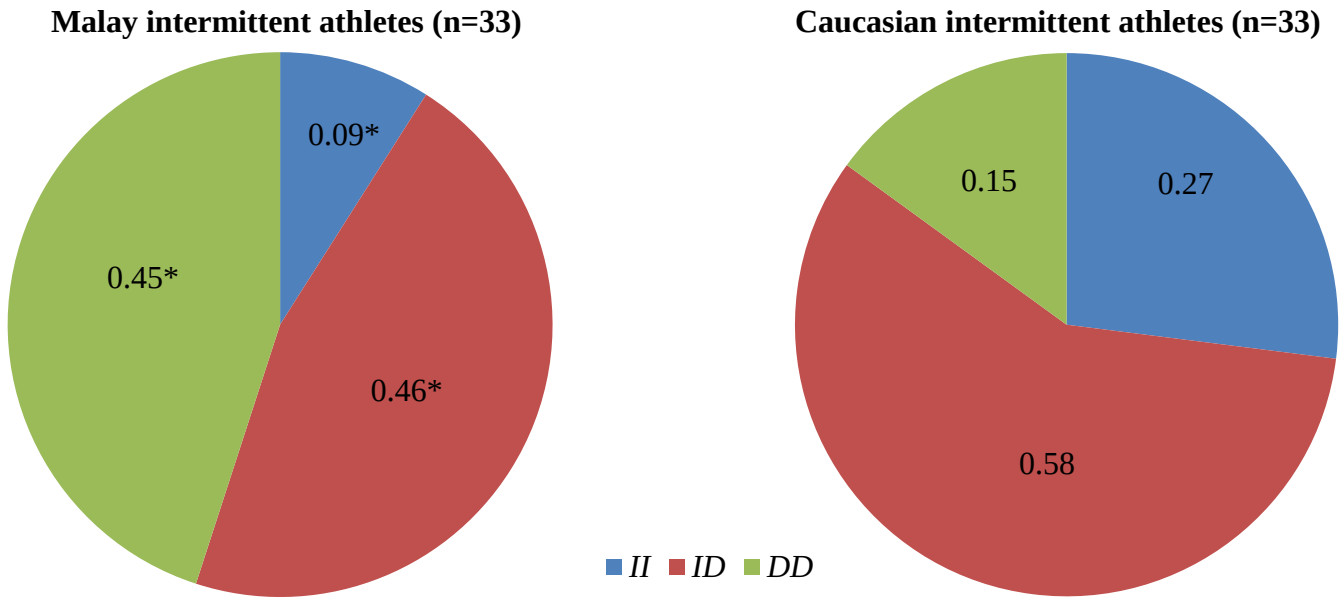


Figure 5.4 *ACE I/D* genotype frequency in Malay and Caucasian intermittent athletes

Note.
*p = 0.014 for genotype frequency in Malay intermittent athletes vs. Caucasian intermittent athletes

5.5.4 Effects of *ACE I/D* Gene Polymorphism on Endurance and Strength/Power Performances in the Malaysian Population

5.5.4.1 *ACE I/D* Gene Polymorphism and Endurance Performance in the Malaysian Population

The Yo-Yo intermittent recovery level 2 performances among athletes with different *ACE I/D* genotypes are presented in Table 5.4. The performance of Yo-Yo intermittent recovery level 2 was similar among athletes with different *ACE I/D* genotypes ($F(2, 177) = 0.006$, $p = 0.994$).

5.5.4.2 *ACE I/D* Gene Polymorphism and Strength/Power Performance (Handgrip Strength) in the Malaysian Population

The mean value of handgrip strength among athletes with different *ACE I/D* genotypes is presented in Table 5.4. There was no significant difference in handgrip strength across the genotype groups ($F(2, 177) = 1.818$, $p = 0.165$).

Table 5.4 Yo-Yo intermittent recovery level 2 performance and handgrip strength value in athletes with different *ACE I/D* genotypes

Variables	*II* genotype (n=30)	*ID* genotype (n=65)	*DD* genotype (n=85)	p value
Yo-Yo intermittent recovery level 2 performance (m)	350.7 ± 203.9	354.7 ± 170.2	352.5 ± 170.0	0.994
Handgrip strength (kg)	36.1 ± 8.5	39.5 ± 9.9	39.9 ± 9.9	0.165

Note.
Data shown as mean ± SD

5.5.4.3 *ACE I/D* Gene Polymorphism with Strength/Power Performance (Leg Strength) in the Malaysian Population

The mean values of leg strength among athletes with different *ACE I/D* genotypes are shown in Figure 5.5. There is a significant difference in leg strength across the genotype groups ($F(2, 177) = 3.122$, $p = 0.047$). Post hoc tests using the Bonferroni correction revealed that leg strength was significantly higher in the *DD* genotype group (113.8 ± 36.2) when compared to the *II* genotype group (96.2 ± 28.0) ($p = 0.048$), though it was not significantly different with the values among *ID* genotype group (112.2 ± 33.5) ($p = 0.104$).

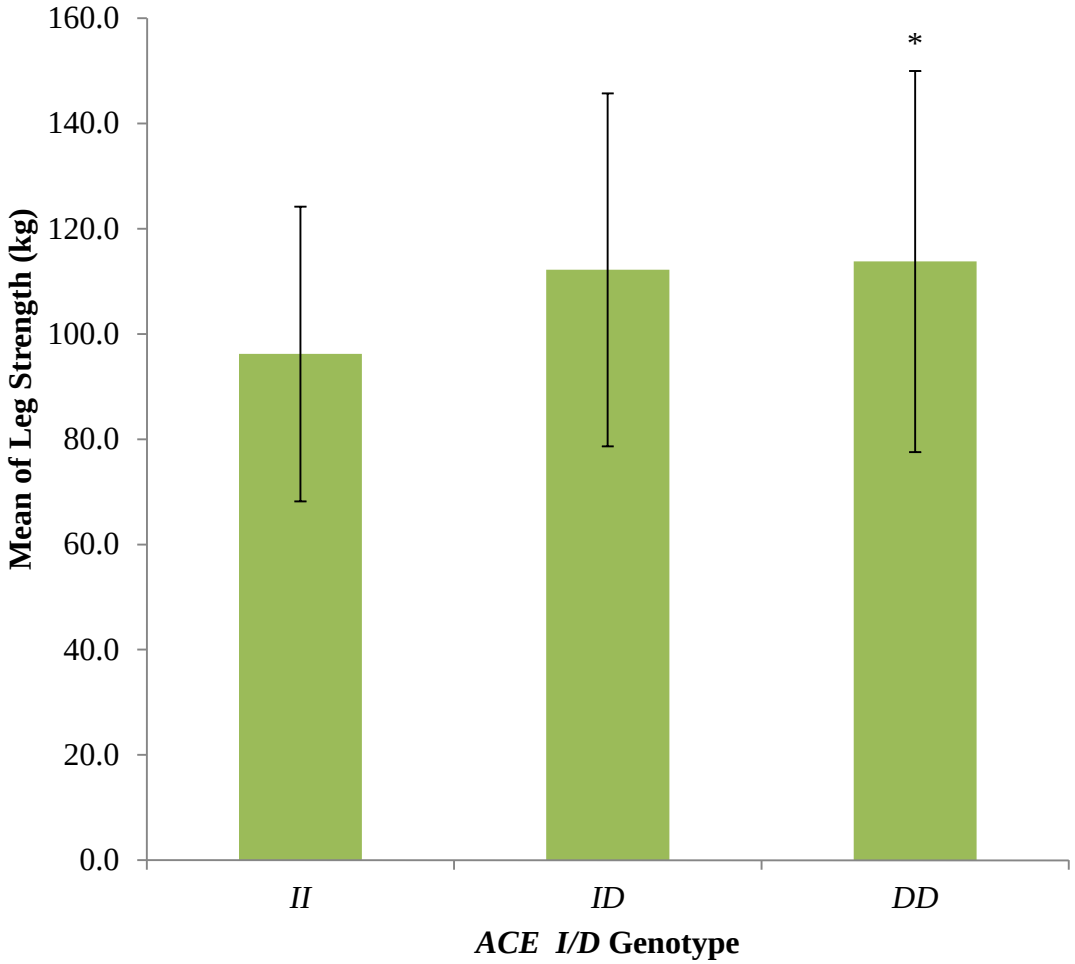


Figure 5.5 The mean value of leg strength in athletes with different *ACE I/D* genotypes in the Malaysian population

Note.
Data shown as mean ± SD
*Significantly different compared to *II* genotype (p = 0.048)

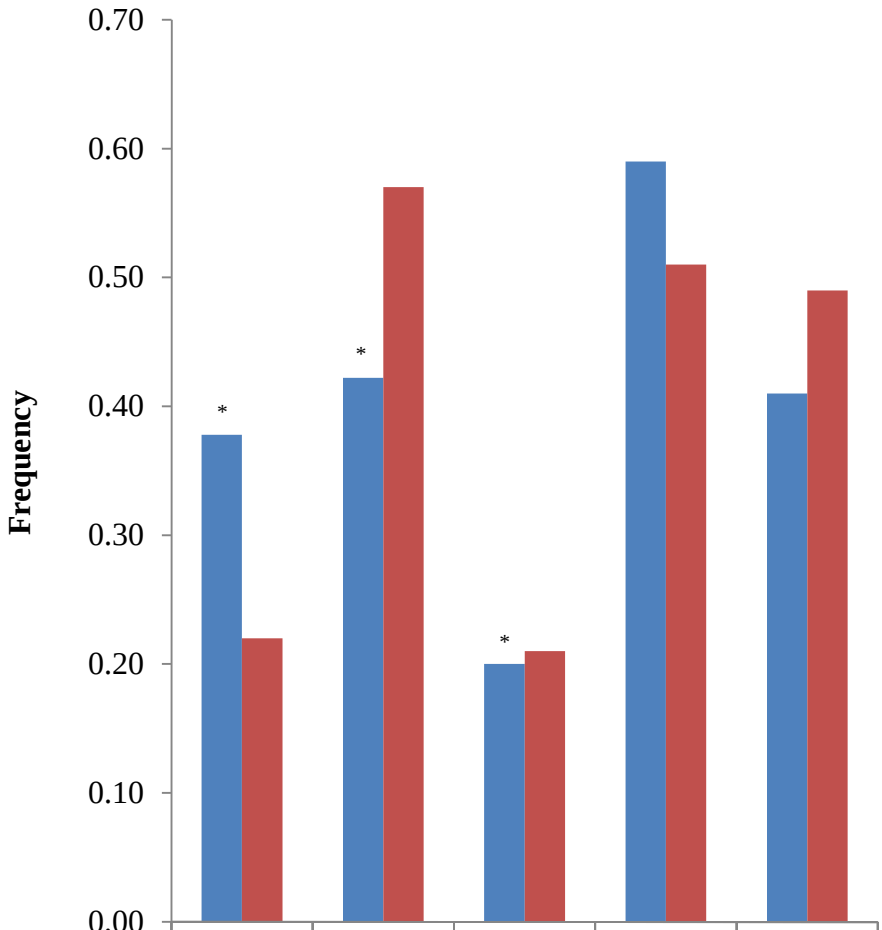
5.5.5 The Distribution of *ACTN R/X* Gene Polymorphism

5.5.5.1 Hardy-Weinberg Equilibrium

ACTN3 R/X genotype distribution for the control group in the Malaysian population was in agreement with the Hardy-Weinberg equilibrium ($p > 0.050$) (Appendix D).

5.5.5.2 The Whole Cohort of Athletes and Controls in the Malaysian Population

The allele and genotype frequencies of *ACTN3 R/X* gene polymorphism in the whole cohort of athletes and controls in the Malaysian population are presented in Figure 5.6. The frequency of *ACTN3 R/X* allele frequency in the whole cohort of athletes ($R = 0.59$; $X = 0.41$) was not significantly different from that the whole cohort of controls ($R = 0.51$; $X = 0.49$) ($X^2 = 2.1998$, df = 1, $p = 0.138$). However, there is a significant difference in *ACTN3 R/X* genotype frequency between athletes and controls ($X^2 = 11.111$, df = 2, $p = 0.004$) with the athletes had a higher frequency of *RR* genotype and lower frequencies of *RX* and XX genotypes compared to controls.



	RR	*RX*	*XX*	*R*	*X*
	ACTN3 R/X Genotype			*ACTN3 R/X* Allele	
Athletes (n=180)	0.38*	0.42*	0.20*	0.59	0.41
Controls (n=180)	0.22	0.57	0.21	0.51	0.49

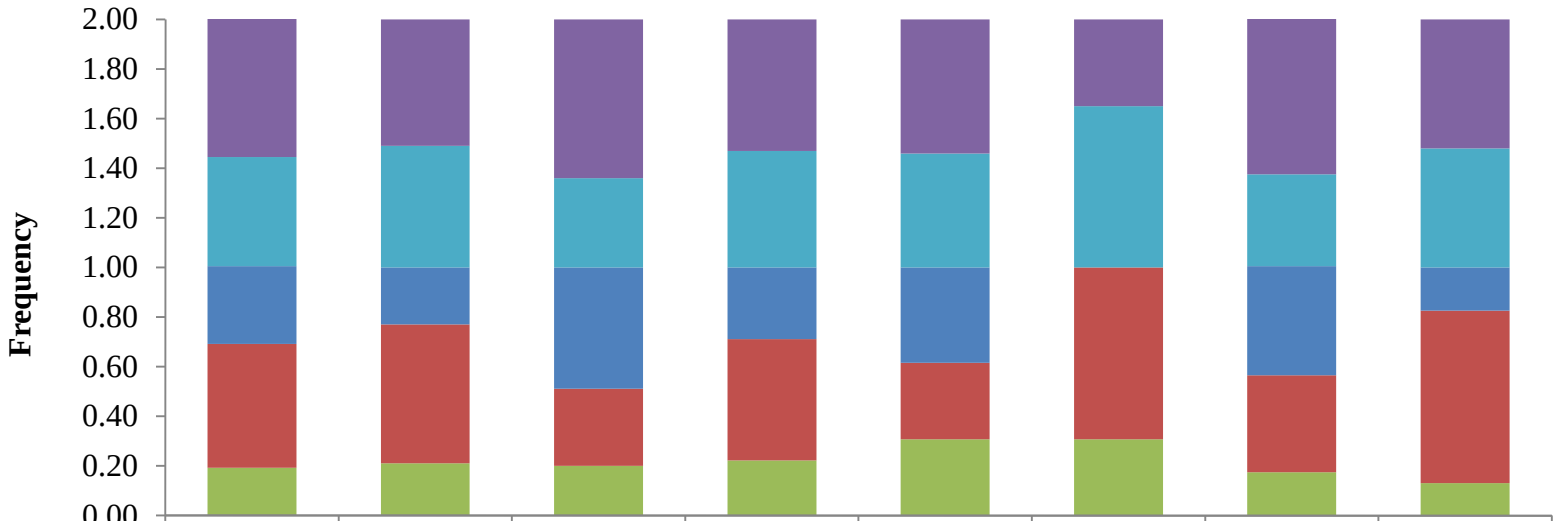
Figure 5.6 *ACTN3 R/X* allele and genotype frequencies in the whole cohort of athletes and controls in the Malaysian population

Note.
*p = 0.004 for genotype frequency in athletes vs. controls

5.5.5.3 Multi-Ethnic Groups of Athletes and Controls in the Malaysian Population

The allele and genotype frequencies of *ACTN3 R/X* gene polymorphism in the multi-ethnic groups of athletes and controls in the Malaysian population are presented in Figure 5.7. Within athlete group, the distribution of *ACTN3 R/X* genotype was not significantly different between the ethnic groups ($X^2 = 6.642$, df = 6, p = 0.355). The distribution of *ACTN3 R/X* allele was also not significantly different between the ethnic groups ($X^2 = 1.541$, df = 3, p = 0.672).

Malay athletes differed from Malay controls in their *ACTN3 R/X* genotype ($X^2 = 49.350$, df = 2, $p < 0.001$) frequency with Malay athletes had a higher frequency of *RR* genotype than in Malay controls. However, allele frequency was similar between the Malay athletes and their controls ($X^2 = 2.444$, df = 1 p = 0.117). Similarly, Indian athletes differed from Indian controls in their *ACTN3 R/X* genotype ($X^2 = 6.923$, df = 2, p = 0.031) frequency with Indian athletes had a higher frequency of *RR* genotype than in Indian controls. Conversely, allele frequency was similar between the Indian athletes and their controls ($X^2 = 0.619$, df = 1, p = 0.431). Chinese athletes did not differ from Chinese controls in their *ACTN3 R/X* allele ($X^2 = 1.147$, df = 1, p = 0.284) and genotype ($X^2 = 4.145$, df = 2, p = 0.126) distributions. There were also insignificant differences in *ACTN3 R/X* allele (($X^2 = 0.807$, df = 1, p = 0.369) and genotype ($X^2 = 4.674$, df = 2, p = 0.097) distributions between athletes and controls in Other Bumiputra group.



	Malay Athletes (n=99)	Malay Controls (n=99)	Chinese Athletes (n=45)	Chinese Controls (n=45)	Indian Athletes (n=13)	Indian Controls (n=13)	Other Bumiputra Athletes (n=23)	Other Bumiputra Controls (n=23)
ACTN3 R/X Allele *R*	0.56	0.51	0.64	0.53	0.54	0.35	0.63	0.52
ACTN3 R/X Allele *X*	0.44	0.49	0.36	0.47	0.46	0.65	0.37	0.48
ACTN3 R/X Genotype *RR*	0.31[a]	0.23	0.49	0.29	0.38[b]	0.00	0.44	0.17
ACTN3 R/X Genotype *RX*	0.50[a]	0.56	0.31	0.49	0.31[b]	0.69	0.39	0.70
ACTN3 R/X Genotype *XX*	0.19[a]	0.21	0.20	0.22	0.31[b]	0.31	0.17	0.13

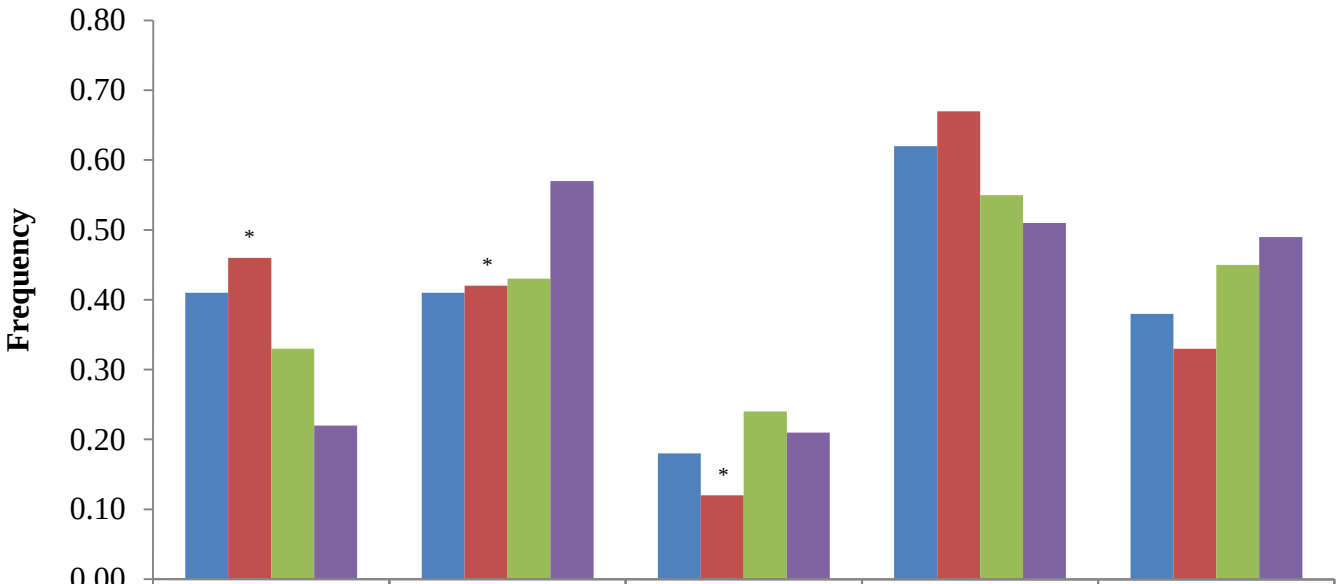
Figure 5.7 *ACTN3 R/X* allele and genotype frequencies in multi-ethnic groups of athletes and controls in the Malaysian population

Note.
[a]$p < 0.001$ for genotype frequency in Malay athletes vs. Malay controls
[b]$p = 0.031$ for genotype frequency in Indian athletes vs. Indian controls

5.5.5.4 Endurance Athletes, Strength/Power Athletes, Intermittent Athletes, and Controls in the Malaysian Population

Considering the opposing effects of possession of the *R* and *X* alleles on particular sporting disciplines, the *ACTN3 R/X* allele and genotype frequencies were compared between endurance athletes, strength/power athletes, intermittent athletes and controls in the Malaysian population as shown in Figure 5.8. The *ACTN3 R/X* allele frequency was similar between the four groups ($X^2 = 5.291$, df = 3, p = 0.152). However, the genotype frequencies differed significantly across the groups ($X^2 = 15.004$, df = 6, p = 0.020).

The endurance athletes did not differ significantly in *ACTN3 R/X* genotype frequency when compared with the controls ($X^2 = 5.504$, df = 2, p = 0.064), the strength/power ($X^2 = 0.490$, df = 2, p = 0.783) and intermittent ($X^2 = 0.903$, df = 2, p = 0.637) athletes. The strength/power athletes differed significantly in *ACTN3 R/X* genotype frequency when compared with the controls ($X^2 = 10.074$, df = 2, p = 0.006), but not to those in intermittent athletes ($X^2 = 3.298$, df = 2, p = 0.192). Meanwhile, the intermittent athletes did not differ significantly in *ACTN3 R/X* genotype frequency when compared with the controls ($X^2 = 5.781$, df = 2, p = 0.056). Among the four groups, the strength/power and intermittent athletes had the highest frequencies of *RR* and *XX* genotypes, respectively.



	RR	*RX*	*XX*	*R*	*X*
	ACTN3 R/X Genotype			*ACTN3 R/X* Allele	
Endurance athletes (n=34)	0.41	0.41	0.18	0.62	0.38
Strength/Power athletes (n=41)	0.46*	0.42*	0.12*	0.67	0.33
Intermittent athletes (n=105)	0.33	0.43	0.24	0.55	0.45
Controls (n=180)	0.22	0.57	0.21	0.51	0.49

Figure 5.8 *ACTN3 R/X* allele and genotype frequencies among endurance athletes, strength/power athletes, intermittent athletes, and controls in the Malaysian population

Note.

*p = 0.006 for genotype frequency in strength/power athletes vs. controls

5.5.5.5 Malay and Caucasian Intermittent Athletes

The genotype frequency of the *ACTN3 R/X* gene polymorphism among Malay intermittent athletes and Caucasian intermittent athletes are shown in Figure 5.9. There was a significant difference in genotype frequency between Malay and Caucasian intermittent athletes ($X^2 = 9.906$, df = 2, p = 0.007) with the frequency of *RR* and *XX* genotypes in Malay intermittent athletes higher than in Caucasian intermittent athletes. However, allele frequency of *ACTN3 R/X* gene polymorphism amongst the Malay intermittent athletes (*R* = 0.48; *X* = 0.52) was not significantly different from that amongst the Caucasian intermittent athletes (*R* = 0.56; *X* = 0.44) ($X^2 = 0.243$, df = 1, p = 0.622).

5.5.6 Interaction between Ethnicity and *ACTN3 R/X* Gene Polymorphism on Strength/Power and Endurance Performances

There was no significant interaction between ethnicity and *ACTN3 R/X* gene polymorphism on handgrip strength (F(2, 174) = 1.153, p = 0.334), leg strength (F(2, 174) = 1.308, p = 0.256), and Yo-Yo intermittent recovery level 2 performance (F(2, 174) = 1.003, p = 0.425).

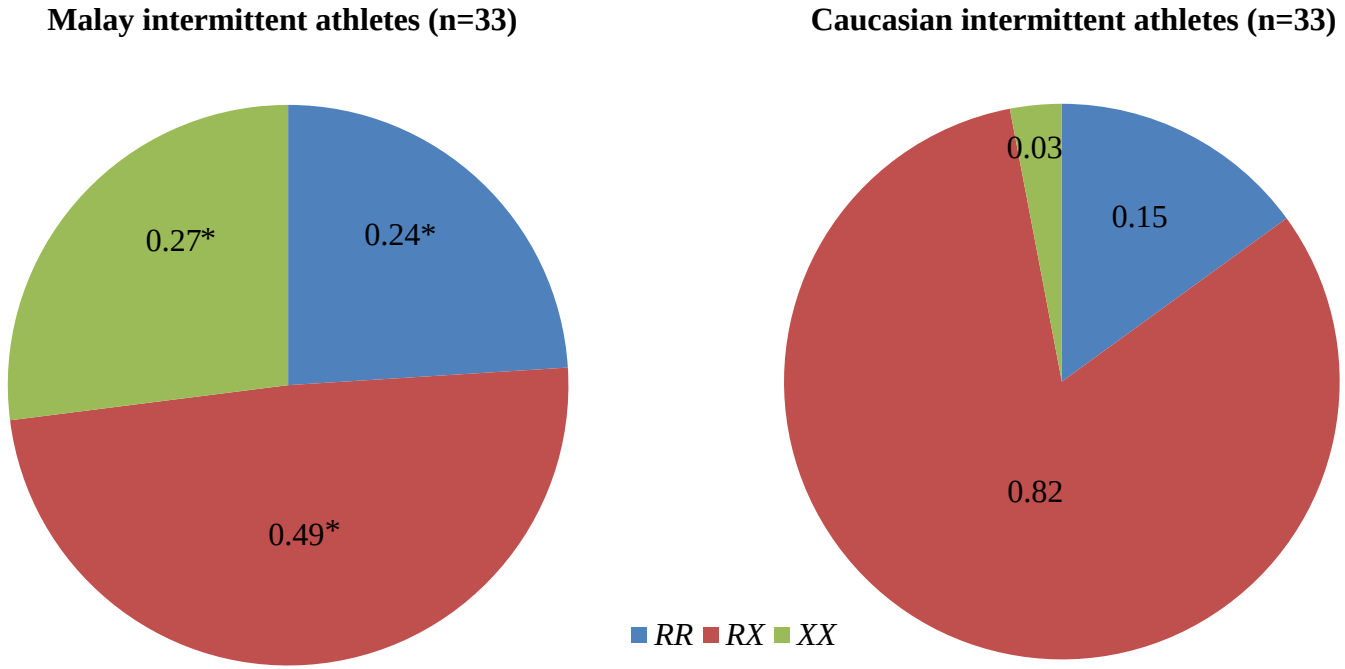


Figure 5.9 *ACTN3 R/X* genotype frequencies in Malay and Caucasian intermittent athletes

Note.
*p = 0.007 for genotype frequency in Malay intermittent athletes vs. Caucasian intermittent athletes

5.5.7 Effects of *ACTN3 R/X* Gene Polymorphism on Strength/Power and Endurance Performances in the Malaysian Population

5.5.7.1 *ACTN3 R/X* Gene Polymorphism and Strength/Power Performance (Handgrip Strength) in the Malaysian Population

The mean value of handgrip strength among athletes with different *ACTN3 R/X* genotypes is presented in Figure 5.10. There is a significant difference in handgrip strength across the genotype groups ($F(2, 177) = 3.647$, $p = 0.028$). Using a Bonferroni post hoc test, the handgrip strength recorded by the athletes with *RR* genotype (41.6 ± 8.4) is significantly higher than those with the *RX* genotype (37.4 ± 10.2) ($p = 0.031$), but not significantly different from athletes with the *XX* genotype (38.1 ± 10.2) ($p = 0.228$).

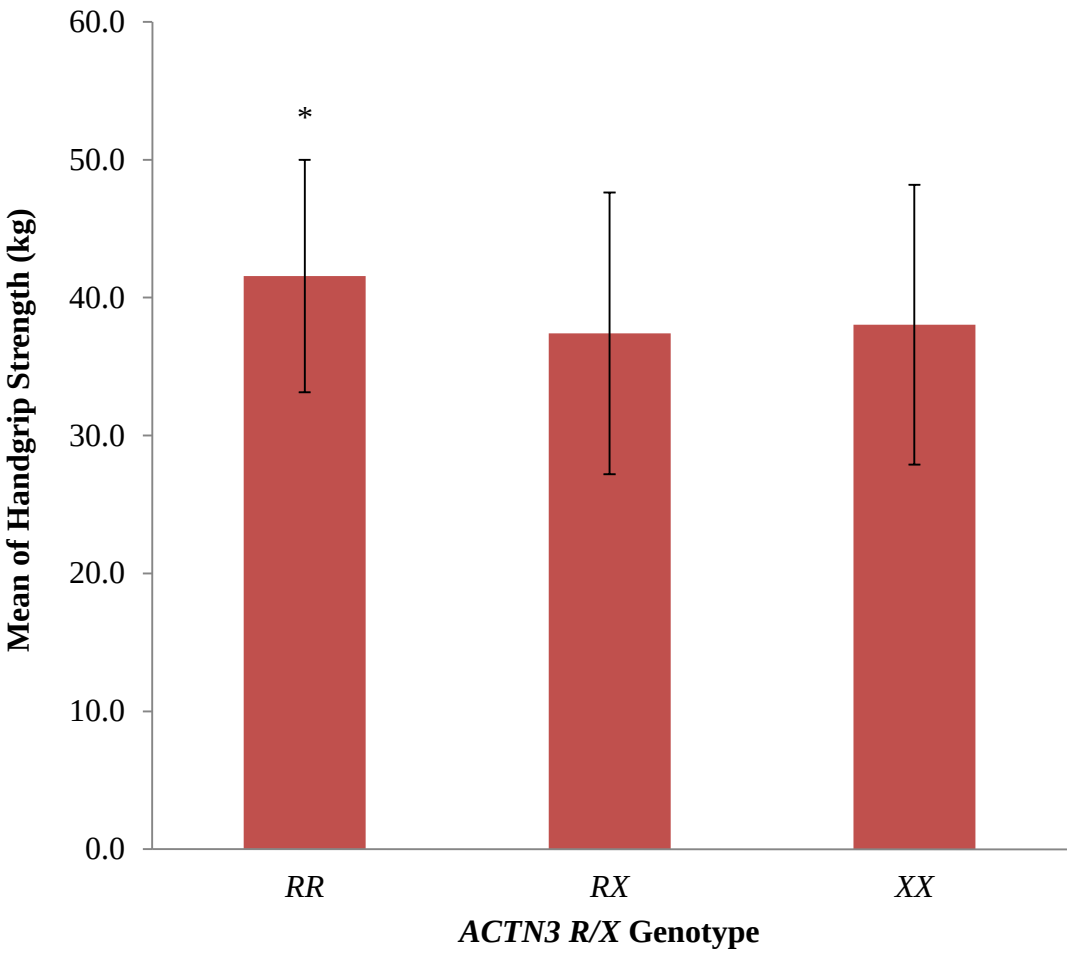


Figure 5.10 The mean value of handgrip strength in athletes with different *ACTN3 R/X* genotypes in the Malaysian population

Note.
Data shown as mean ± SD
*Significantly different compared to *RX* genotype (p = 0.031)

5.5.7.2 *ACTN3 R/X* Gene Polymorphism and Strength/Power Performance (Leg Strength) in the Malaysian Population

The mean value of leg strength among athletes with different *ACTN3 R/X* genotypes is presented in Figure 5.11. There is a significant difference in leg strength across the genotype groups ($F(2, 177) = 7.979$, $p < 0.001$). Athletes with *RR* genotype (123.0 ± 29.8) have a significantly higher leg strength than those with the *RX* genotype (102.6 ± 35.5) ($p = 0.001$) and *XX* genotype (102.6 ± 34.1) ($p = 0.010$).

5.5.7.3 *ACTN3 R/X* Gene Polymorphism and Endurance Performance in the Malaysian Population

The performance of Yo-Yo intermittent recovery level 2 was similar among athletes with *RR* genotype (377.7 ± 208.1), *RX* genotype (342.4 ± 138.9), and *XX* genotype (328.7 ± 174.8) ($F(2, 177) = 1.663$, $p = 0.385$).

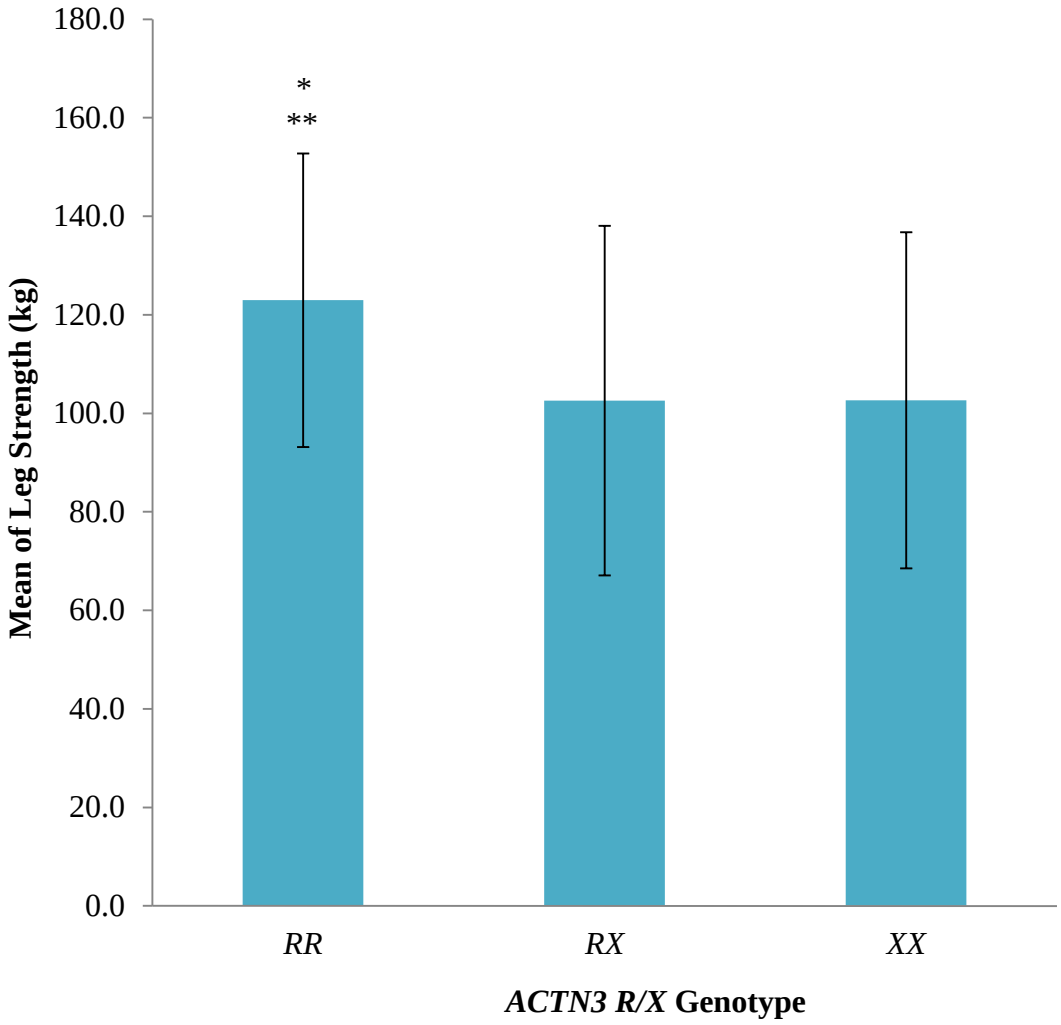


Figure 5.11 The mean value of leg strength in athletes with different *ACTN3 R/X* genotypes in the Malaysian population

Note.
Data shown as mean ± SD
*Significantly different compared to *RX* genotype (p = 0.001)
**Significantly different compared to *XX* genotype (p = 0.010)

5.6 Discussion

The effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance remain controversial with some studies showing positive effect (Ahmetov et al., 2008b, Cieszczyk et al., 2009, Pimenta et al., 2011, Cieszczyk et al., 2011, Eidera et al., 2013), with other studies showing negative effect (Ash et al., 2011, Holdys et al., 2011, Wang et al., 2013, Ahmetov et al., 2013). This inconclusive finding also appears when examined across the Caucasian (Hagberg et al., 1999, Kasikcioglu et al., 2004, Clarkson et al., 2005a, Cam et al., 2007), and Asian (Zhaoa et al., 2003, Tobina et al., 2010, Ichinoseki-Sekine et al., 2010) populations. These findings suggest that the *ACE I/D* and *ACTN3 R/X* gene polymorphisms may confer different effects in a specific population. However, the lack of reports in Asian population compared to the Caucasian population, as previously highlighted by Ma et al. (2013), warrants further investigation on the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance in Asian population.

This study was therefore designed to fill the gap in the literature and thus examine the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within Malaysian population and determine whether their effects differ by ethnicity. To the best of author's knowledge, there have been limited studies that have examined the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within multi-ethnic Malaysian population. The main finding of this study is that *ACE I/D* and *ACTN3 R/X* alleles and genotype frequencies did not vary much between the multi-ethnic groups of Malaysian athletes. These small variations did not have any influence on the human physical performance between

these ethnic groups. Also, the present study identified that possession of the *ACE D* allele and *ACTN3 R* allele were associated with increased strength/power performance within the Malaysian population. Meanwhile, possession of the *ACE I* allele and *ACTN3 X* allele did not appear to confer an advantage on endurance performance in the Malaysian population.

The athletes in the present study were drawn from different ethnic groups in Malaysia, thus it is probable that different composition of *ACE I/D* and *ACTN3 R/X* genes in different ethnic groups could be a factor that influence the performance of the athletes, which has been previously suggested by Zilberman-Schapira et al. (2012). Findings of the present study observed a similar distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms between multi-ethnic groups of Malaysian athletes. The present finding for *ACE I/D* gene polymorphisms had been contradicted with the findings obtained from the first study of this research project that the distribution of *ACE I/D* gene polymorphism varied between multi-ethnic groups in Malaysian. In addition, a multivariate analysis was performed on the present data to determine the interaction between the ethnic origin (Malay, Chinese, Indian and Other Bumiputra) and the *ACE I/D* and *ACTN3 R/X* gene polymorphisms on the endurance and strength/power performances. The analysed data showed that the *ACE I/D* and *ACTN3 R/X* gene polymorphisms did not interact with ethnicity to impact endurance and strength/power performances in the Malaysian population. Results of this study are consistent with previous research by Goh et al. (2009), who studied the impact of Asian ethnicity (Singaporean) on the effect of *ACE I/D* gene polymorphism on the aerobic capacity. Thus, the finding of the current study suggested that *ACE I/D* and *ACTN3 R/X* gene polymorphisms may have universal effect on human physical performance.

Previous research of the *ACE I/D* gene polymorphism in athletes demonstrated that the frequency of *ACE I* allele was significantly higher among endurance athletes than strength/power and intermittent athletes (Myerson et al., 1999, Nazarov et al., 2001, Tsianos et al., 2004b, Mayne, 2006, Min et al., 2009), suggesting that the presence of *ACE I* allele is required for endurance performance. In line with these studies, the present study observed the over-representation of the *ACE I* allele and *ACE II* genotype in the endurance group compared to the strength/power and intermittent groups, supporting the potential effect of possession of the *ACE I* allele on endurance performance.

Although the presence of *ACE I* allele was compatible with endurance athlete status in Malaysian population, the result of Yo-Yo intermittent recovery level 2 test demonstrated that the presence of *ACE I* allele is not the predictor of endurance performance in this population. The Yo-Yo intermittent recovery level 2 performance was found to be similar across the three *ACE I/D* genotype groups. The finding of this study contradicts that of earlier studies among Asian (Goh et al., 2009) and Caucasian (Cam et al., 2007, Voroshin and Astratenkova, 2008) samples, suggesting that individuals with *ACE II* genotype have better endurance performance compared to those with other *ACE I/D* genotypes.

The lack of an effect of possession of the *ACE I* allele on the Yo-Yo intermittent recovery level 2 performance observed in this study is consistent with previous results involving Caucasian samples (Rankinen et al., 2000c, Sonna et al., 2001). The reason for the lack of effect of possession of the *ACE I* allele observed in the present study remains unclear. Sonna and colleagues (2001) suggested that the

previous association reported for possession of the *ACE I* allele and endurance performance could be due to linkage with another gene in close proximity to the ACE locus, such as the human growth hormone gene. This is supported with evidence from previous studies on human growth hormone gene which showed that the increases in growth hormone production during exercise lead to increases in carbohydrate, fat, and protein metabolism (De Palo et al., 2001, Godfrey et al., 2003) and stimulate sweat secretion (Jorgensen et al., 2003), thereby influencing endurance performance. The finding of the present study suggests that possession of the *ACE I* allele may not the absolute criteria for success in endurance performance.

Furthermore, the present study identified an over-representation of the *ACE D* allele among strength/power athletes compared to the other athlete groups, which is consistent with the previous observations in other Asian (Kikuchi et al., 2012) and Caucasian (Nazarov et al., 2001, Mayne, 2006, Costa et al., 2009b) samples. In contrast to the finding observed for *ACE I* allele, the potentially favourable effect of possession of the *ACE D* allele on strength/power athletic status in Malaysian athletes is supported by the finding on leg strength. According to the present result, athletes with the *ACE DD* genotype exhibited greater leg strength than those with the *ACE II* and the *ACE ID* genotypes. This result is in line with previous studies that reported positive effects of possession of the *ACE D* allele on muscular strength parameters, such as isometric and isokinetic quadriceps muscle strength (Williams et al., 2005), and knee extensor strength (Giaccaglia et al., 2008). All of these positive findings indicate that possession of the *ACE D* allele might have an advantageous effect on short duration and high-intensity activities. A positive effect of possession of the *ACE D* allele on muscular strength observed in the present study supports the possible

mechanism for the effect of possession of the *ACE D* allele on muscular strength through the production of ANG II in the skeletal muscle (Charbonneau et al., 2008). A greater local ANG II production in the skeletal muscle has been reported to increase protein synthesis and cell hypertrophy (Jones and Woods, 2003), thereby inducing muscle contraction for maximal power (Rattigan et al., 1996).

The ACTN3 protein plays an important role in maintaining the ordered myofibrillar array for greater coordination to generate high muscle power and velocity during movements (Mills et al., 2001). Based on the physiological role of ACTN3 protein during muscle contraction, the present study hypothesised that athletes who have one or two copies of *ACTN3 R* allele (codes for ACTN3 protein) would predispose to strength/power sports and have greater muscular strength compared to athletes with *ACTN3 X* allele. As expected, the present study found a higher frequency *ACTN3 RR* genotype in the strength/power athletes compared to endurance and intermittent athletes. A higher frequency of *ACTN3 RR* genotype in strength/power athletes is in line with the previous finding among Asian (Kothari et al., 2011) and Caucasian (Yang et al., 2003, Eynon et al., 2009b) athletes.

The present study also found that athletes with *ACTN3 RR* genotype had greater hand and leg strength than athletes with other genotypes. This finding is consistent with the findings of earlier studies involving Caucasian (Clarkson et al., 2005a, Vincent et al., 2007, Erskine et al., 2014) and Asian populations (Shang et al., 2012), which demonstrated that the *ACTN3 RR* genotype carriers have greater strength/power capacity than other genotype carriers. These results confirm an advantageous effect of the presence of ACTN3 protein on muscle strength. This finding supports the previous

notion by Thomis et al. (1998) and Vincent et al. (2007), who reported that the likelihood of presenting the best performance in muscle strength depends on the genetic profile of the individual.

While the production of ACTN3 protein is coded by *ACTN3 R* allele, the incomplete sequence in the *ACTN3 X* allele prevents the production of this protein (North et al., 1999). The lack of ACTN3 protein has been reported to shift skeletal muscle metabolism to aerobic metabolism (MacArthur et al., 2007). Therefore, the present study hypothesized that the *ACTN3 X* allele is more frequent among athletes involved in endurance sports and athletes with *ACTN3 X* allele may have greater endurance capacity compared to those with *ACTN3 R* allele. The present data demonstrate a higher frequency of *ACTN3 XX* genotype among intermittent athletes than endurance and strength/power athletes. This result is not in agreement with the previous reports in the Caucasian population, which demonstrated a higher frequency of *ACTN3 XX* genotype in endurance athletes compared to strength/power and intermittent athletes (Eynon et al., 2009b, Ahmetov et al., 2011). This finding is contrary to those observed in the Chinese population (Shang et al., 2010). The reliability of a higher frequency of *ACTN3 X* allele in intermittent athletes could be explained by the fact that intermittent sport involves aerobic-anaerobic demand. Hence, an intermittent athlete requires both aerobic and anaerobic capacity. Therefore, the presence of *ACTN3 X* allele in intermittent athletes may confer a beneficial effect for aerobic activity.

In the present study, the endurance performance as measured by Yo-Yo intermittent recovery level 2 test was similar between the *ACTN3 R/X* genotype. This

finding is not equivalent to the previous study by Lucia et al. (2007). According to the study by Lucia et al. (2007), the carriers of the *ACTN3 XX* genotype had a higher endurance performance compared to carriers of *ACTN3 RR* and *ACTN3 RX* genotype. The contradictory finding between the present study and the study by Lucia et al. (2007) may be attributed to the difference in the study sample criteria. Lucia et al. (2007) had studied the effect of possession of the *ACTN3 XX* genotype on endurance performance among untrained people, whilst the present study focused on a group of athletes. Therefore, further studies involving both untrained and trained individuals may be needed to formulate a conclusive finding on the effect of possession of the *ACTN3 X* allele on endurance performance. Based on the present data, a deficiency of ACTN3 protein may not be critical to endurance performance in the Malaysian athletic population.

In light of the above-mentioned finding, the present study is the first to show a significant difference in the distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms between Malay intermittent athletes in the Malaysian population and Caucasian intermittent athletes in the Australian population. For well-matched comparison, the groups from these two populations contain adequate sample size of intermittent male athletes representing university level sporting competition. With respect to the *ACE I/D* gene polymorphism, there are significant variations in genotype frequencies in different ethnic groups with *ACE DD* genotype higher in those of Malay descent. Based on this data, the *ACE DD* genotype appeared to influence the performance of intermittent athletes in Malaysian population whilst this genotype is not extremely crucial for Australian athletes.

With respect to the *ACTN3 R/X* gene polymorphism, the genotype frequencies significantly differed between Malay and Caucasian intermittent athletes with *ACTN3 XX* genotype higher in those of Malay ethnicity compared to Caucasian athletes. In a previous study by Pimenta et al. (2011), athletes with *ACTN3 XX* genotype were reported to have higher concentrations of post-training cortisol when compared to *RR* and *RX* carriers. The high concentration of cortisol in athletes with *XX* genotype after training indicates to the increased level of muscle damage predicted from the increase of CK activity (Pimenta et al., 2011). Therefore, those athletes with *XX* genotype are speculated to have a high risk of muscle damage that may limit their potential to be excellent in competition (Pimenta et al., 2011). Based on this information, it is possible that the overrepresentation of athletes with *XX* genotype in Malay group may limits the potential to achieve successful results in intermittent competitions such as soccer and hockey when compared to Caucasian athletes. There is no clear explanation for the existence of the differences in the frequency of *ACE I/D* and *ACTN3 R/X* gene polymorphisms between Malay and Caucasian intermittent athlete populations, but it could not exclude that this difference could also be due to the different athlete development programs between these two cultures that may be influencing this shift.

The present study is presented with several limitations. The first limitation was related to the number of athletes used in this study as when the athletes were divided into three groups based on their sporting disciplines, the sample size decreased considerably. As in all such studies, extension to, and replication with a larger sample size is warranted. The studied groups in the Malaysian population were comprised of both females and males. Therefore, it remains unknown if the effects of the selected polymorphisms may differ by gender. As the aims of the present study was not related

to training effect, there is no data available on the training status of the athletes. Therefore, it is likely that performance measured in the present study due to the training effect which could mask gene-related effects on physical performance. The present study did not measure the physical performance of controls in the Malaysian population to reduce variability of the results. In addition, due to time constraints, there is no available data for the physical performance of Australian athletes. However, replicate study should be performed in future studies to validate the present findings.

Despite the above-mentioned limitation, confidence in the validity of the present findings is increased by the fact that each athlete group demonstrate different endurance and strength/power performances that are reliable with their physical characteristics. The population comparison between Malaysian athletes and Australian athletes were matched on sample size, gender, age, and sports discipline. Athlete and control groups in the Malaysian population were ethnically-matched to limit and control the effect of ethnicity. The genetic assessment was accurate and unbiased as the genotype distributions of the studied polymorphisms in the control group were in Hardy-Weinberg equilibrium.

5.7 Conclusion

In summary, this study demonstrates that the distributions of *ACE I/D* and *ACTN3 R/X* gene polymorphisms and their effects on the physical performance do not vary much between different ethnic groups of Malaysian athletes. This study confirmed the positive effects of possession of the *D* allele of *ACE I/D* and *R* allele of *ACTN3 R/X* gene polymorphisms on strength/power performance within Asian ethnicity as has been widely investigated among the Caucasian population. It is further concluded that possession of the *I* allele of *ACE I/D* and *X* allele of *ACTN3 R/X* gene polymorphisms are not the predictor for endurance performance within the Malaysian population. There is a different pattern in the distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms amongst Malays and Caucasian intermittent athletes that warrants further investigation on the athlete development programs between the two populations that may be influencing this variation. These preliminary data illustrate the importance of understanding the genetic makeup of Malaysian athletes and the effect of genetic factors on human physical performance.

CHAPTER 6

THE INFLUENCE OF *ACE I/D* GENE POLYMORPHISM ON CARDIOVASCULAR AND MUSCULAR ADAPTATIONS TO 8 WEEKS OF ISOMETRIC HANDGRIP TRAINING IN A NORMOTENSIVE POPULATION IN MALAYSIA

6.1 Introduction

Managing blood pressure is important in preventing hypertension, which is a major risk factor for heart attacks and strokes (Chobanian et al., 2003). It has been shown that lowering resting blood pressure can significantly reduce the risk of coronary disease by 21%, stroke by 37%, total cardiovascular mortality by 25%, and all-cause mortality rates by 13% (He and Whelton, 1999). With that, exercise has been suggested as the best and the most affordable way for managing blood pressure (Fagard, 2006).

Moderate-intensity aerobic exercise (about 40% to 50% of maximal oxygen consumption (VO_2max)) undertaken three to five times per week for 30 to 60 minutes per session is effective in reducing resting blood pressure in both normotensive and hypertensive individuals (Pescatello and Kulikowich, 2001, Cornelissen and Fagard, 2005a, Baynard et al., 2008, Goldberg et al., 2012). In a recent meta-analysis concerning exercise training for blood pressure, endurance exercise training was reported to reduce resting systolic and diastolic blood pressure by about 3.5 mmHg and 2.5 mmHg, respectively (Cornelissen and Smart, 2013).

Recently, in addition to aerobic exercise, resistance exercise training, which has not been previously recommended for blood pressure management in hypertensive patients (Palatini et al., 1989), has been shown to lower resting blood pressure in normotensive and hypertensive individuals (Wiley et al., 1992, Wood et al., 2001, Vincent et al., 2003, Collier et al., 2008). The reductions of 3 to 4 mmHg in resting systolic and diastolic blood pressure were observed following four weeks of resistance exercise training (Collier et al., 2008). Meanwhile, in another meta-analysis study conducted by Cornelissen and Smart (2013), the largest reductions in resting blood pressure had been reported following the isometric resistance exercise training (systolic: −10.9 mmHg, diastolic: −6.2 mmHg) compared to after endurance (systolic: −3.5 mmHg, diastolic: −3.7 mmHg) and dynamic resistance exercise training (systolic: −1.8 mmHg, diastolic: −2.5mmHg). Moreover, several earlier studies suggested that the isometric exercise training protocol consisting of four sets of 2-minute handgrip (McGowan et al., 2007, Millar et al., 2008) or leg contractions (Howden et al., 2002) at 30 % to 50 % of maximal voluntary contraction (MVC) (Wiley et al., 1992, Ray and Carrasco, 2000) with 1 to 4 minutes of rest period between each contraction (Wiley et al., 1992, McGowan et al., 2007) conducted three to five times per week for 4 to 10 weeks (Devereux et al., 2010, Badrov et al., 2013) to be more effective in lowering resting blood pressure than endurance and dynamic resistance exercise training. Relative to exercise trained-muscle, hand grip isometric exercise training (Wiley et al., 1992, Badrov et al., 2013) has been found to reduce resting blood pressure more than leg isometric exercise training (Baross et al., 2012). One possible explanation for this difference may have to do with greater increase in arterial pressure for hand grip than leg muscle contraction, that will stimulate the baroreceptors which in turn leads to

greater capacity for reduction in blood pressure following IHG training than leg isometric exercise training (Strange, 1999).

While the benefit of isometric exercise training for blood pressure management has been well documented, it has remained unclear on how factors that can influence blood pressure, such as gender and genetics, may influence efficiency of this isometric exercise program. Several twin studies previously reported that blood pressure is controlled by genetic factors (van den Bree et al., 1996, Hottenga et al., 2005). Given the fact that blood pressure has a genetic basis, research efforts have been directed towards identifying the candidate genes involved in blood pressure regulation (Sober et al., 2009, Arora and Newton-Cheh, 2010). Among the proposed candidate genes for blood pressure, the angiotensin I-converting enzyme (*ACE*) gene, has attracted much attention due to its role in the renin-angiotensin system (RAS); a physiological system that regulates blood pressure (Hagberg et al., 2000, Marteau et al., 2005, Dhanachandra Singh et al., 2014).

Within the *ACE* gene, the *ACE I/D* gene polymorphism showed a strong link with the level of ACE in the RAS (Tiret et al., 1992) and accounted for 47% of the total phenotypic variance of ACE (Rigat et al., 1990). On top of that, an initial report by Rigat et al. (1990) observed that ACE levels were progressively higher among individuals with *II*, *ID*, and *DD* genotypes of the *ACE I/D* gene polymorphism, respectively. Individuals with *II* genotype had been reported to have lower resting blood pressure than those with *ID* and *DD* genotypes (Wong et al., 2012). This is attributed to a lower ACE level in individuals with *II* genotype that decreases the formation of ANG II (a potent vasoconstrictor) and increases the production of

bradykinin (a potent vasodilator), leading to lower resting blood pressure (Coates, 2003).

The influences of the *ACE I/D* gene polymorphism on blood pressure in response to exercise training have been investigated in several studies, but the results have been inconsistent (Montgomery et al., 1997, Hagberg et al., 1999, Rankinen et al., 2000a, Zhang et al., 2002, Dengel et al., 2002, Kim, 2009, Mota et al., 2013). Some studies reported that the *ACE I/D* gene polymorphism did not influence blood pressure response to endurance (Montgomery et al., 1997, Rankinen et al., 2000a, Dengel et al., 2002) and dynamic resistance (Mota et al., 2013) exercise training. On the other hand, Hagberg et al. (1999) reported a greater drop in resting blood pressure among hypertensive men with *II* and *ID* genotypes compared to those with *DD* genotype after 9 months of endurance exercise training at 75 to 85 % of maximal oxygen consumption. Furthermore, a similar finding was also observed by Zhang et al. (2002), who investigated the impact of the *ACE I/D* gene polymorphism on blood pressure response to 10 weeks exercise therapy on a bicycle ergometer among 64 Japanese with mild to moderate essential hypertension. In contrast to the results obtained by Hagberg et al. (1999) and Zhang et al. (2002), Kim (2009) discovered that adult women with *DD* genotype had greater reduction in blood pressure (diastolic) than those with *II* and *ID* genotypes, following a 12-week combined aerobic and resistance exercise training. The reasons for these inconsistent results are unclear, but it may be due to the differences in sample sizes and insufficient exercise intensities for eliciting substantial changes in resting blood pressure. For instance, a study by Kim (2009) involved mixed aerobic and resistance exercise training compared with only aerobic training (endurance training) in study by Hagberg et al. (1999).

Despite the inconsistencies of some findings, to our knowledge, no study has investigated the effect of isometric exercise training on blood pressure response among individuals with different genotypes of the *ACE I/D* gene polymorphism. This investigation is important in determining if *ACE I/D* gene polymorphism has an influence on blood pressure in response to exercise training, which could help to identify individuals who would be more likely to benefit from this exercise program.

6.2 Aim

The aim of this study was 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. It was hypothesized that the *ACE I/D* gene polymorphism would influence the cardiovascular and muscular adaptations to an 8-week isometric handgrip training.

6.3 Methods and Participants

6.3.1 Study Design

This study employed a single-blind, repeated measures study design. Since the *ACE I/D* gene polymorphism was reported to be associated with an enhanced cardiovascular (Hagberg et al., 1999, Zhang et al., 2002, Kim, 2009) and muscular (Giaccaglia et al., 2008) responses to training, all participants underwent identical cardiovascular and muscular assessments before training (pre-training), after the initial training session (mid-training), and after 8 weeks of training (post-training). They performed isometric

handgrip (IHG) exercise three days per week for 8 weeks. All assessments and IHG training were conducted in a quiet temperature-controlled room (20 to 25ºC). A flowchart of the study design is presented in Figure 6.1.

6.3.2 Participants

At the initial phase of this study, a total of 50 healthy, normotensive, untrained males aged 30.3 ± 5.1 years old, who had been reported to having Malay ancestry within three generations and sedentary lifestyle (two or fewer days a week of recreational exercise for less than 30 minutes a day for the preceding three months (Pate et al., 2008)) were screened for *ACE I/D* gene polymorphism. Thirty of these initial participants, who comprised of 10 participants with each *II*, *ID*, and *DD* genotypes of *ACE I/D* gene polymorphism were then selected for isometric handgrip training. The number of participants for this study was based on the sample size calculation by using the Power and Sample Size Calculation version 3.1.2 software (Dupont and Plummer, 1990) [Calculated sample size = 27 participants; Research sample size = 27 participants + (27*10% (expected drop out)) = 30 participants]. The statistical power of the study was set at 0.80 with 95% of confidence interval and the effect size of 0.25.

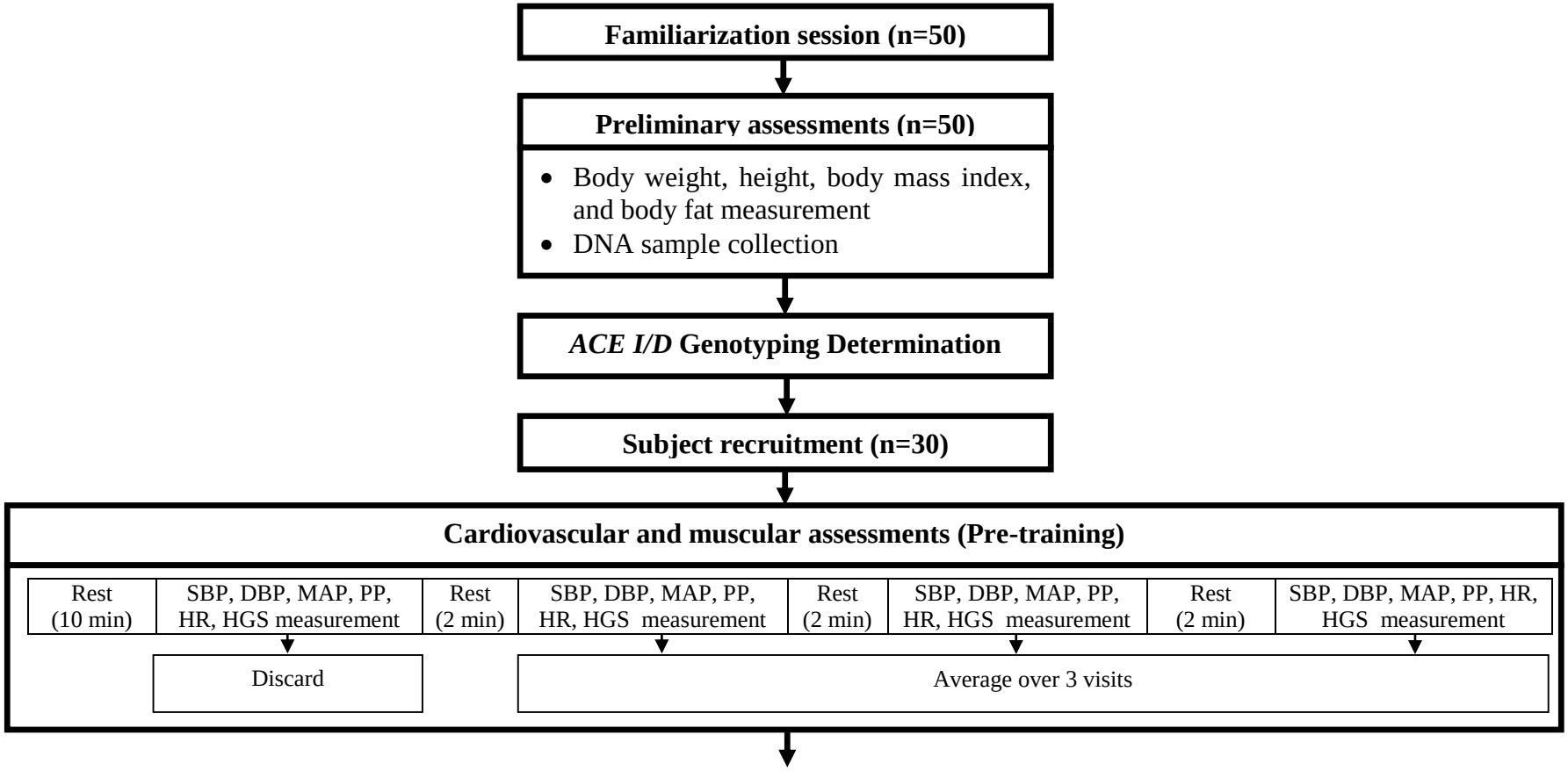


Figure 6.1 Flow chart of the study design

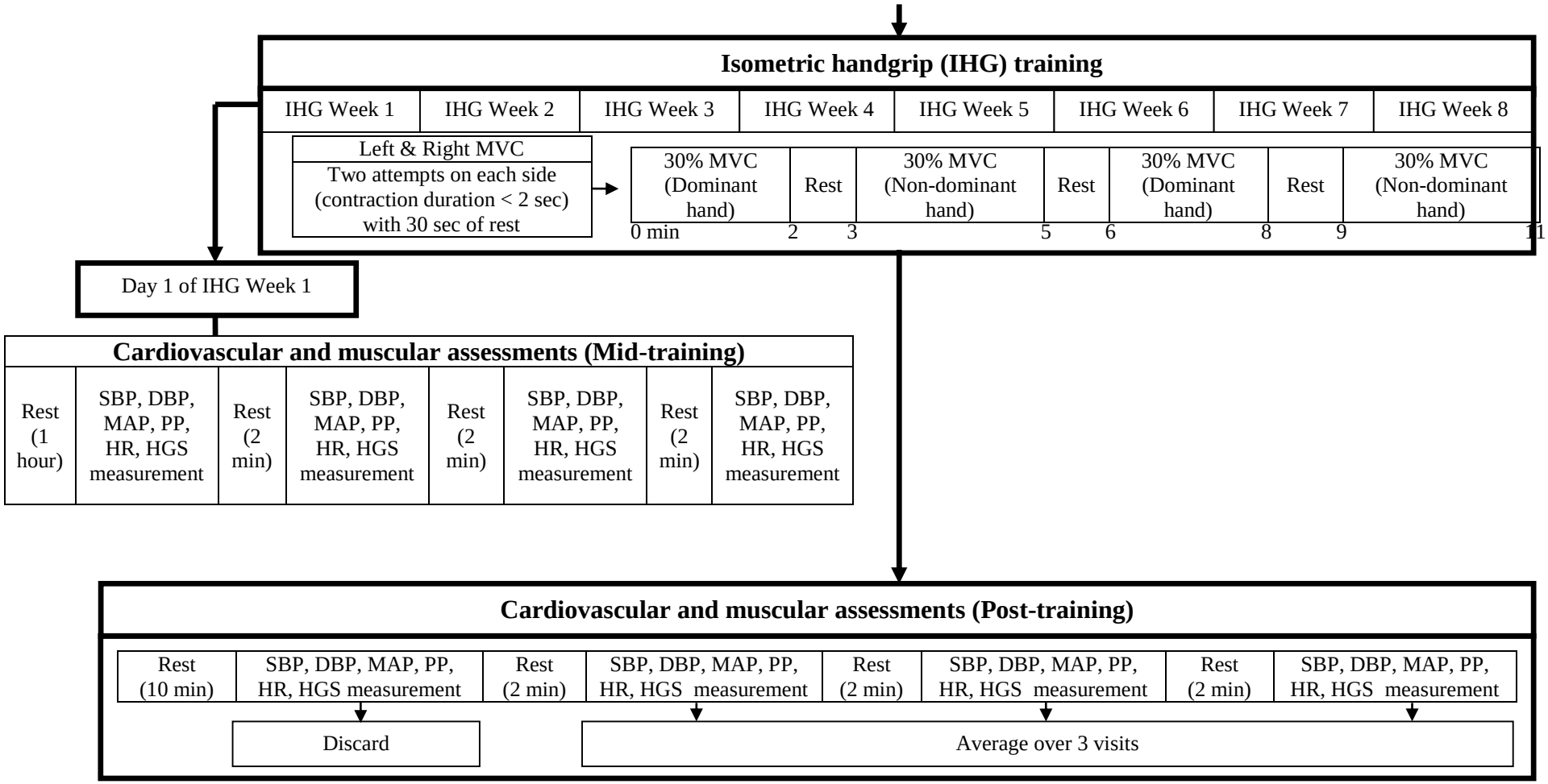


Figure 6.1 Continue

6.3.3 Familiarization Session

The participants were briefed of the testing protocols and familiarized with the instruments, as well as the procedures involved in the MVC test. After they had signed and completed the consent form, they were interviewed about their personal information, including gender, age, ethnicity, and health status. Participants consent and information details forms were presented in Appendix C.

6.3.4 Preliminary Assessments

During this session, anthropometric data of the participants, such as body weight, body height, body mass index, and body fat, were collected. Participant's body height was measured using a portable stadiometer (Seca 213, Seca Corporation, USA). Meanwhile, participant's body weight, body mass index, and body fat, were measured using an Omron KARADA Scan Body Composition & Scale (HBF-362, Omron Corporation, Japan). Immediately following this, deoxyribonucleic acid (DNA) sample from each participant was collected by using buccal swab with a sterile swab applicator (Classic Swabs by Copan Flock Technologies, Brescia, Italy). The swabs were air dried and placed in sterile 1.5 ml microcentrifuge tubes and stored at -20°C until used for DNA isolation.

6.3.5 *ACE I/D* Genotyping Determination

Genomic DNA was isolated from the swab samples by using the GeneAll® Exgene™ Cell SV kit following the manufacturer's protocol (GeneAll Biotechnology Co. Ltd., Seoul, South Korea). Polymerase chain reaction (PCR) was carried out in a final volume of 25 µl consisting of 2.5 µl of 10X standard reaction buffer (GeneAll Biotechnology Co. Ltd., Seoul, South Korea) (25 mm Mg^{2+}, 50 mm Tris-HCl, 50 mm KCl, 0.1 mm EDTA, 1 mm DTT, 0.5 mm PMSF, and 50% glycerol), 2.0 µl of dNTP mix (200 µm from each dNTP (dATP, dCTP, dGTP, and dTTP)), 0.8 µm of each primer (forward primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3': reverse primer: 5'-CTGGAGACCACTCCCATCCTTTCT-3'), 0.5 units of Taq DNA polymerase, 2.5 µl of dimethylsulfoxide, 10.8 µl of sterilize distilled water, and 5 µl of genomic DNA (2-8 ng/µl). The target fragment bearing the *ACE I/D* gene polymorphism was amplified under the following conditions; 7 minutes at 95°C, followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 62°C, and 1 minute at 72°C, with a final step of 7 minutes at 72°C. The amplified products were electrophoresed on 1.5% agarose gel that was pre-stained with ethidium bromide at 70 volts for 1 hour. The presence of 490 base pair (bp) and 190 bp bands indicated the *ACE* insertion (*I*) and deletion (*D*) alleles, respectively. The PCR products for *ACE I/D* gene polymorphism were confirmed by sequencing (First BASE Laboratories Sdn Bhd, Selangor, Malaysia).

6.3.6 Cardiovascular and Muscular Assessments

Prior to measurement of the study variables, the participants were asked to refrain from performing vigorous exercise and consuming caffeinated beverages within 24 hours before the assessments. A hand dynamometer (Takei A5401, Takei Scientific Instruments Co. Ltd., Japan) was used to measure the muscular variable, whilst the cardiovascular variables were assessed by using a non-invasive automated brachial oscillometry (Omron HEM907XL, Omron Healthcare, Inc., United States).

Cardiovascular (systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), and heart rate (HR)), and muscular (handgrip strength (HGS)) variables were measured on three consecutive days (at the same time (± 2 hours)) immediately prior to commencing training. During each visit, after 10 minutes of seated rest, all variables were measured on the dominant arm hand (self-reported by the participant) in the sitting position for four successive times with 2-minute rest intervals. The first of the four measurements of all variables in each visit was discarded (due to the white coat effect), while the remaining three measurements were averaged over the three visits to represent the pre-training value (Millar et al., 2008).

One hour after the initial training session (mid-training), the cardiovascular and the muscular variables were assessed based on the procedure described above to examine the acute effects of IHG exercise (Hecksteden et al., 2013). For this assessment, the first and the second measurements were discarded, while the last two measurements were

averaged to represent the mid-training value, as previously described in a study by Hecksteden et al. (2013).

In three consecutive days after 8 weeks of training (post-training), the cardiovascular and the muscular variables were again assessed based on the procedure described above. The measurements of cardiovascular and muscular variables were averaged in the same way as described for pre-training value to represent the post-training value (Millar et al., 2008).

6.3.7 Isometric Handgrip Training

The isometric handgrip (IHG) training protocol used in this study was based on the training protocol described by McGowan et al. (2007). Before every training session, the left and the right MVC values of the participants were assessed with two attempts on each side (contraction duration less than 2 seconds), separated by 30 seconds of rest. If the variance from the two recordings was less than 5%, the highest value was taken as the participant's MVC for that side. If the recordings differed by more than 5%, further attempts were made at 1-minute interval until a stable maximal value was obtained. All participants were trained using unilateral, alternate IHG exercise, three days per week for eight weeks (at the same time (± 2 hours)). In each session, the participants performed the IHG exercise while sitting with the working arm extended towards the front. The participants performed four trials of 2 minutes of IHG exercise at 30% of their MVC, with

a 1-minute rest period between each trial. The IHG exercise was performed using alternate hands starting with dominant hand.

6.4 Statistical Analysis

The descriptive data are presented as mean ± standard deviation (SD). Differences in pre- and post-training values were calculated as final (mid- or post-training) minus initial (pre-training) value. Positive and negative results indicated an increase and a decrease with IHG training, respectively. The mean of all variables at pre-, mid-, and post-training were compared with *II*, *ID*, and *DD* genotype groups via one-way analysis of variance (ANOVA), and was followed by Bonferroni's post-hoc test when appropriate. Paired sample t-test was used to compare mid- and post- training data with pre-training data of the entire cohort and each genotype group. All statistical evaluations were performed by using the IBM SPSS statistical version 20.0 (Armonk, New York, USA), with the level of significance set at $p < 0.050$.

6.5 Results

6.5.1 Characteristics of Participants

The physical characteristics of the participants according to *ACE I/D* genotype are shown in Table 6.1. All *ACE I/D* genotype groups were similar in age ($F(2, 27) = 2.834$, $p = 0.071$), height ($F(2, 27) = 0.133$, $p = 0.876$), body weight ($F(2, 27) = 0.066$, $p = 0.961$), body mass index ($F(2, 27) = 0.672$, $p = 0.519$), and body fat ($F(2, 27) = 0.937$, $p = 0.404$).

Table 6.1 Physical characteristics of participants according to *ACE I/D* genotype

Variables	***II*** **(n=10)**	***ID*** **(n=10)**	***DD*** **(n=10)**	**p value**
Age (years)	27.8 ± 6.2	32.9 ± 3.1	30.0 ± 4.5	0.071
Height (cm)	169.0 ± 6.2	170.4 ± 9.3	169.0 ± 5.4	0.876
Body Weight (kg)	72.6 ± 10.0	74.7 ± 24.0	72.4 ± 7.4	0.961
Body Mass Index (kg/m^2)	25.4 ± 3.1	27.1 ± 4.4	25.6 ± 3.2	0.519
Body Fat (%)	23.8 ± 3.5	26.0 ± 4.7	24.1 ± 3.7	0.404

Note.
Data shown as mean ± SD

6.5.2 Cardiovascular and Muscular Responses

Table 6.2 demonstrates the cardiovascular and muscular responses following 8 weeks of IHG training. SBP ($t(29) = 3.753$, $p = 0.001$), MAP ($t(29) = 3.008$, $p = 0.004$), PP ($t(29) = 2.401$, $p = 0.023$), and HR ($t(29) = 2.398$, $p = 0.023$), except DBP ($t(29) = 1.737$, $p = 0.093$), were significantly lower following IHG training than at pre-training. Meanwhile, HGS significantly increased after IHG training ($t(29) = -3.175$, $p = 0.004$).

Table 6.2 Cardiovascular and muscular responses to 8 weeks of IHG training

Variables	Pre-training	Post-training	Change (Δ) with training	p value
SBP (mmHg)	121.3 ± 9.1	117.1 ± 6.6*	-4.2 ± 6.1	0.001
DBP (mmHg)	76.2 ± 7.2	74.7 ± 7.9	-1.4 ± 4.5	0.093
MAP (mmHg)	91.2 ± 6.9	88.9 ± 6.8*	-2.4 ± 4.2	0.004
PP (mmHg)	45.1 ± 8.2	42.4 ± 6.8*	-2.8 ± 6.3	0.023
HR (bpm)	79.4 ± 7.7	76.8 ± 8.9*	-2.6 ± 5.9	0.023
HGS (kg)	43.5 ± 6.7	46.0 ± 7.5*	2.6 ± 4.4	0.004

Note.
Data shown as mean ± SD
*Significantly different compared to pre-training value ($p < 0.050$)

Table 6.3 demonstrates the cardiovascular and the muscular responses following a session of IHG exercise. SBP ($t(29) = 2.456$, $p = 0.020$) and MAP ($t(29) = 2.506$, $p = 0.018$), except DBP ($t(29) = 2.024$, $p = 0.052$), PP ($t(29) = 0.655$, $p = 0.518$), and HR ($t(29) = 0.608$, $p = 0.548$) had been significantly lower following a session of IHG exercise than at pre-training. Meanwhile, HGS ($t(29) = 0.8$, $p = 0.430$) did not increase significantly after IHG exercise.

Table 6.3 Cardiovascular and muscular responses to a session of IHG exercise

Variables	Pre-training	Mid-training	Change (Δ) with IHG exercise	p value
SBP (mmHg)	121.3 ± 9.1	118.1 ± 9.4*	-3.2 ± 7.2	0.020
DBP (mmHg)	76.2 ± 7.2	73.8 ± 8.5	-2.4 ± 6.5	0.052
MAP mmHg)	91.2 ± 6.9	88.5 ± 8.0*	-2.7 ± 5.8	0.018
PP (mmHg)	45.1 ± 8.2	44.3 ± 8.0	-0.9 ± 7.1	0.518
HR (bpm)	79.4 ± 7.7	78.5 ± 9.1	-0.9 ± 7.6	0.548
HGS (kg)	43.5 ± 6.7	43.2 ± 6.9	-0.3 ± 2.3	0.430

Note.
Data shown as mean ± SD
*Significantly different compared to pre-training value ($p < 0.050$)

6.5.3 *ACE I/D* Gene Polymorphism and Cardiovascular Response

6.5.3.1 Systolic Blood Pressure (SBP)

SBP among *ACE I/D* genotype groups at pre-, mid-, and post-training are shown in Table 6.4. SBP at the pre-training differed significantly between the *ACE I/D* genotype groups ($F(2, 27) = 4.005$, $p = 0.030$). SBP at pre-training in the *II* genotype group was significantly lower compared with the *ID* genotype group ($p = 0.043$). However, no significant difference in the SBP at pre-training between *II* and *DD* genotype groups ($p = 0.102$) was identified. There was also insignificant difference in SBP for mid-training ($F(2, 27) = 3.157$, $p = 0.059$) and post-training ($F(2, 27) = 2.018$, $p = 0.152$) between *ACE I/D* genotype groups.

Table 6.4 Systolic blood pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	115.3 ± 7.2*	125.0 ± 9.3	123.6 ± 8.2	0.030
Mid-training	113.2 ± 7.5	123.1 ± 8.9	117.9 ± 9.8	0.059
Post-training	113.8 ± 6.3	118.7 ± 4.9	118.8 ± 7.6	0.152

Note.
Data shown as mean ± SD
*Significantly different compared to *ID* genotype group ($p = 0.043$)

Figure 6.2 shows the changes in SBP following mid- and post-training in *ACE I/D* genotype groups. Following mid-training, SBP significantly decreased in the *DD* genotype group (Δ = -5.7 ± 6.6, t(9) = 2.734, p = 0.023), but not in the *ID* (Δ = -2.0 ± 8.4, t(9) = 0.738, p = 0.479) and *II* (Δ = -2.1 ± 6.8, t(9) = 0.986, p = 0.350) genotype groups. In post-training, SBP decreased significantly in the *ID* (Δ = -6.3 ± 7.3, t(9) = 2.750, p = 0.022) and *DD* (Δ = -4.7 ± 5.2, t(9) = 2.905, p = 0.017) genotype groups. However, SBP in the *II* genotype group was not significantly changed following post-training (Δ = -1.5 ± 5.3, t(9) = 0.909, p = 0.387).

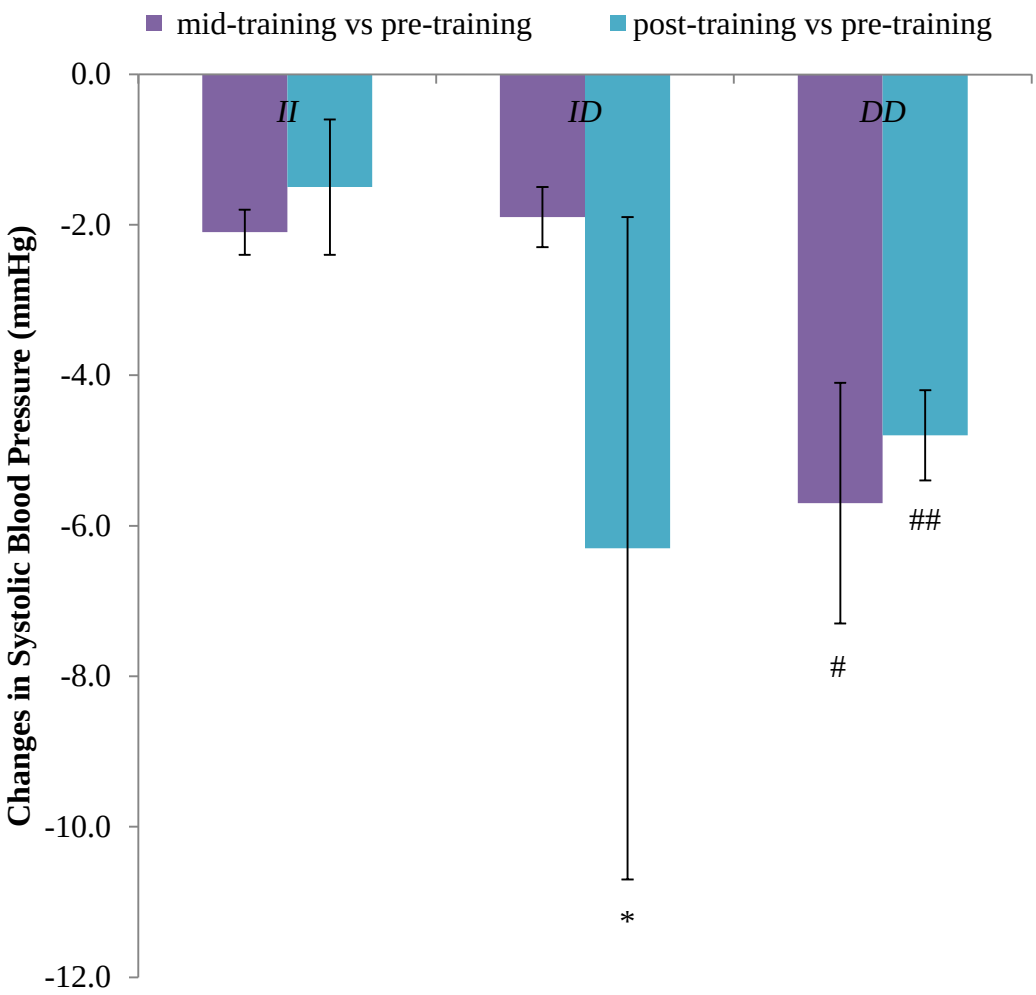


Figure 6.2 Changes in systolic blood pressure value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD
*Significant change from pre-training (p = 0.022)
#Significant change from pre-training (p = 0.023)
##Significant change from pre-training (p = 0.017)

6.5.3.2 Diastolic Blood Pressure (DBP)

Table 6.5 presents the DBP at pre-, mid-, and post-training among *ACE I/D* genotype groups. There were significant differences in DBP at pre-training ($F(2, 27) = 8.798$, $p = 0.001$), mid-training ($F(2, 27) = 6.292$, $p = 0.006$), and post-training ($F(2, 27) = 6.328$, $p = 0.006$) between the *ACE I/D* genotype groups. DBP at pre-training in the *II* genotype group was significantly lower compared with the *ID* ($p = 0.001$) and *DD* genotype groups ($p = 0.018$). At mid-training, the *II* genotype group had a significantly lower DBP than the *ID* genotype group ($p = 0.006$), but not for the *DD* genotype group ($p = 0.053$). Meanwhile, DBP at post-training in the *II* genotype group was significantly lower compared to *ID* ($p = 0.007$) and *DD* ($p = 0.038$) genotype groups.

Table 6.5 Diastolic blood pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	70.1 ± 4.6	0.6 ± 5.9*	77.8 ± 6.7**	0.001
Mid-training	67.3 ± 7.0$^{\neq}$	78.5 ± 5.2	75.6 ± 9.1	0.006
Post-training	68.6 ± 6.7	78.8 ± 6.7$^{\#}$	76.7 ± 6.9$^{\#\#}$	0.006

Note.
Data shown as mean ± SD
*Significantly different compared to *II* genotype group ($p = 0.001$)
**Significantly different compared to *II* genotype group ($p = 0.018$)
$^{\neq}$Significantly different compared to *ID* genotype group ($p = 0.006$)
$^{\#}$Significantly different compared to *II* genotype group ($p = 0.007$)
$^{\#\#}$Significantly different compared to *II* genotype group ($p = 0.038$)

Changes in DBP following mid- and post-training in *ACE I/D* genotype groups are portrayed in Figure 6.3. DBP in all genotype groups did not significantly change following mid-training (*II* = (Δ = -2.8 ± 5.6, t(9) = 1.576, p = 0.149), *ID* = (Δ = -2.1 ± 3.9, t(9) = 1.747, p = 0.115), and *DD* = (Δ = -2.3 ± 9.4, t(9) = 0.759, p = 0.467)). DBP in all genotype groups was also not significantly changed following post-training (*II* = (Δ = -1.4 ± 3.7, t(9) = 1.231, p = 0.250), *ID* = (Δ = -1.8 ± 5.7, t(9) = 0.971, p = 0.357), and *DD* = (Δ = -1.1 ± 4.4, t(9) = 0.801, p = 0.444)).

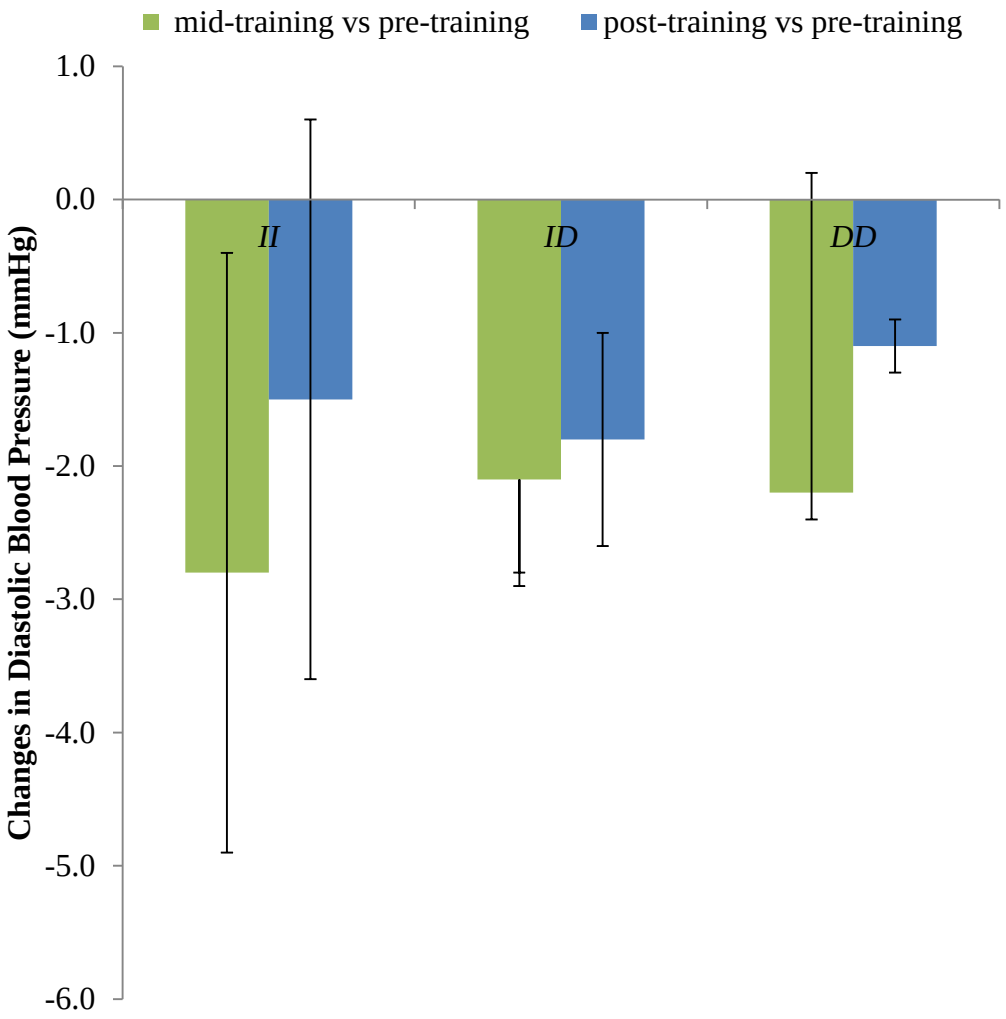


Figure 6.3 Changes in diastolic blood pressure value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD

6.5.3.3 Mean Arterial Pressure (MAP)

MAP at pre-, mid-, and post-training among *ACE I/D* genotype groups are shown in Table 6.6. MAP at pre-training ($F(2, 27) = 9.747$, $p = 0.001$), mid-training ($F(2, 27) = 6.395$, $p = 0.005$), and post-training ($F(2, 27) = 6.006$, $p = 0.007$) were significantly different between the *ACE I/D* genotype groups. At pre-training, MAP in the *II* genotype group was significantly lower than *ID* ($p = 0.001$) and *DD* ($p = 0.009$) genotype groups. At mid-training, *II* genotype group had a significantly lower MAP than the *ID* genotype group ($p = 0.005$), but not for the *DD* genotype group ($p = 0.085$). Meanwhile, MAP at post-training in the *II* genotype group was significantly lower compared to the *ID* ($p = 0.010$) and *DD* ($p = 0.035$) genotype groups.

Table 6.6 Mean arterial pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	85.2 ± 4.5	95.4 ± 5.8*	93.1 ± 6.0**	0.001
Mid-training	82.6 ± 6.4$^{\neq}$	93.3 ± 4.5	89.7 ± 8.8	0.005
Post-training	83.7 ± 5.3	92.1 ± 5.3$^{\#}$	90.7 ± 6.7$^{\#\#}$	0.007

Note.
Data shown as mean ± SD
*Significantly different compared to *II* genotype group ($p = 0.001$)
**Significantly different compared to *II* genotype group ($p = 0.009$)
$^{\neq}$Significantly different compared to *ID* genotype group ($p = 0.005$)
$^{\#}$Significantly different compared to *II* genotype group ($p = 0.010$)
$^{\#\#}$Significantly different compared to *II* genotype group ($p = 0.035$)

Changes in MAP following mid- and post-training in *ACE I/D* genotype groups are displayed in Figure 6.4. MAP in all genotype groups did not significantly change with mid-training (*II* = (Δ = -2.6 ± 5.0, t(9) = 1.599, p = 0.144), *ID* = (Δ = -2.1 ± 4.9, t(9) = 1.347, p = 0.211), and *DD* = (Δ = -3.4 ± 7.7, t(9) = 1.387, p = 0.199)). MAP in all genotype groups was also not significantly changed with post-training (*II* = (Δ = -1.5 ± 3.5, t(9) = 1.309, p = 0.223), *ID* = (Δ = -3.3 ± 5.3, t(9) = 1.953, p = 0.083), and *DD* = (Δ = -2.3 ± 3.7, t(9) = 1.987, p = 0.078)).

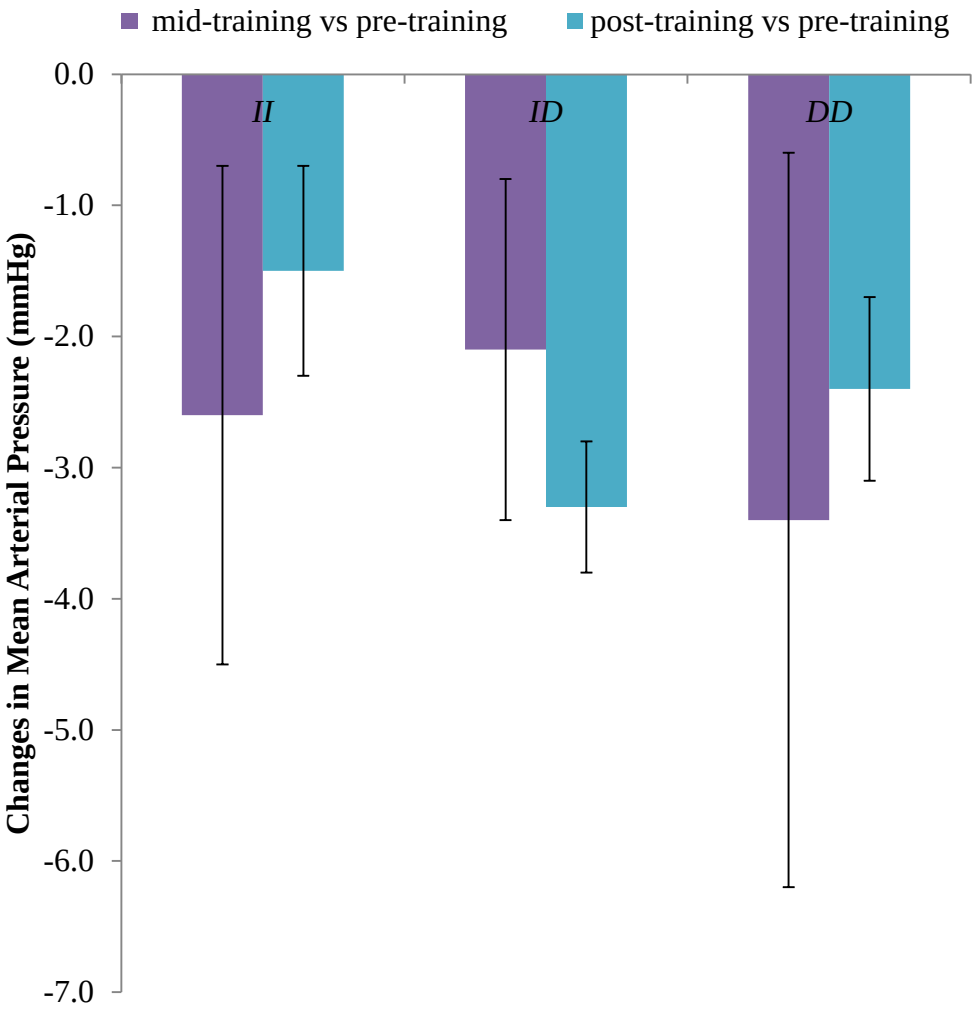


Figure 6.4 Changes in mean arterial pressure value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD

6.5.3.4 Pulse Pressure (PP)

Table 6.7 presents PP at pre-, mid-, and post-training among *ACE I/D* genotype groups. PP at pre-training ($F(2, 27) = 0.064$, $p = 0.938$), mid-training ($F(2, 27) = 0.488$, $p = 0.619$), and post-training ($F(2, 27) = 1.574$, $p = 0.226$) were not significantly different between the *ACE I/D* genotype groups.

Table 6.7 Pulse pressure at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	45.2 ± 7.3	44.4 ± 9.2	45.8 ± 8.7	0.938
Mid-training	45.9 ± 7.0	44.6 ± 10.3	42.4 ± 6.6	0.619
Post-training	45.2 ± 8.2	39.9 ± 6.4	42.1 ± 5.1	0.226

Note.
Data shown as mean ± SD

Changes in PP following mid- and post-training in *ACE I/D* genotype groups are displayed in Figure 6.5. PP in all genotype groups did not significantly change with mid-training (*II* = ($\Delta = 0.7 \pm 6.9$, $t(9) = -0.303$, $p = 0.769$), *ID* = ($\Delta = 0.2 \pm 6.5$, $t(9) = -0.092$, $p = 0.928$), and *DD* = ($\Delta = -3.4 \pm 7.9$, $t(9) = 1.364$, $p = 0.206$)). PP in all genotype groups was also not significantly changed with post-training (*II* = ($\Delta = -0.1 \pm 5.2$, $t(9) = 0.054$, $p = 0.958$), *ID* = ($\Delta = -4.6 \pm 7.2$, $t(9) = 2.009$, $p = 0.075$), and *DD* = ($\Delta = -3.6 \pm 6.0$, $t(9) = 1.899$, $p = 0.090$)).

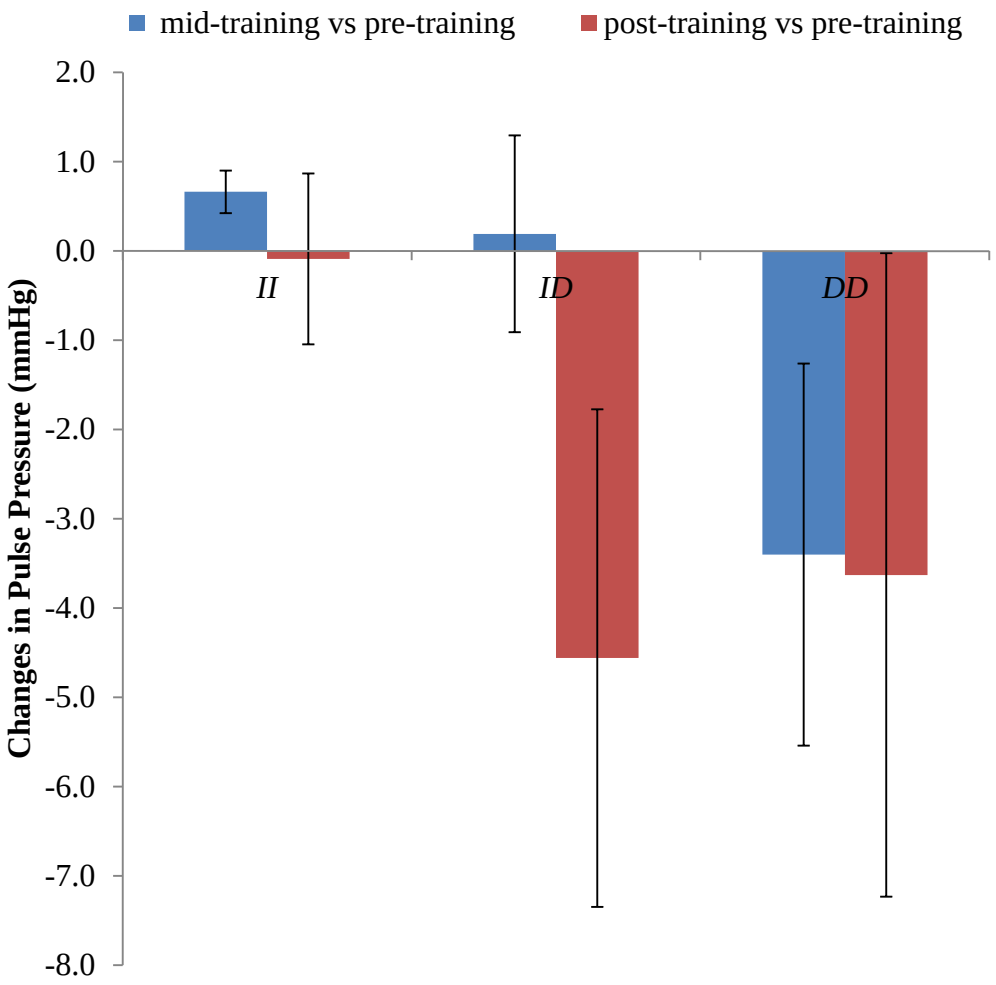


Figure 6.5 Changes in pulse pressure value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD

6.5.3.5 Heart Rate (HR)

Table 6.8 presents HR at pre-, mid-, and post-training among *ACE I/D* genotype groups. HR at pre-training ($F(2, 27) = 0.083$, $p = 0.920$), mid-training ($F(2, 27) = 0.891$, $p = 0.422$), and post-training ($F(2, 27) = 0.407$, $p = 0.669$) were not significantly different between the *ACE I/D* genotype groups.

Table 6.8 Heart rate at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	78.6 ± 6.5	79.8 ± 9.5	79.8 ± 7.7	0.920
Mid-training	77.0 ± 8.7	77.0 ± 10.2	81.7 ± 8.3	0.422
Post-training	78.9 ± 8.1	76.1 ± 9.3	75.5 ± 9.8	0.669

Note.
Data shown as mean ± SD

Changes in HR following mid- and post-training in *ACE I/D* genotype groups are presented in Figure 6.6. HR in all genotype groups did not significantly change with mid-training (*II* = ($\Delta = -1.6 \pm 6.8$, $t(9) = 0.726$, $p = 0.486$), *ID* = ($\Delta = -2.9 \pm 9.1$, $t(9) = 1.009$, $p = 0.339$), and *DD* = ($\Delta = 1.9 \pm 6.8$, $t(9) = -0.884$, $p = 0.400$)). HR decreased significantly following post-training in *ID* ($\Delta = -3.8 \pm 3.5$, $t(9) = 3.392$, $p = 0.008$) and *DD* ($\Delta = -4.3 \pm 4.2$, $t(9) = 3.246$, $p = 0.010$) genotype groups. However, HR in *II* genotype group did not significantly change following post-training ($\Delta = 0.4 \pm 8.1$, $t(9) = -0.144$, $p = 0.889$).

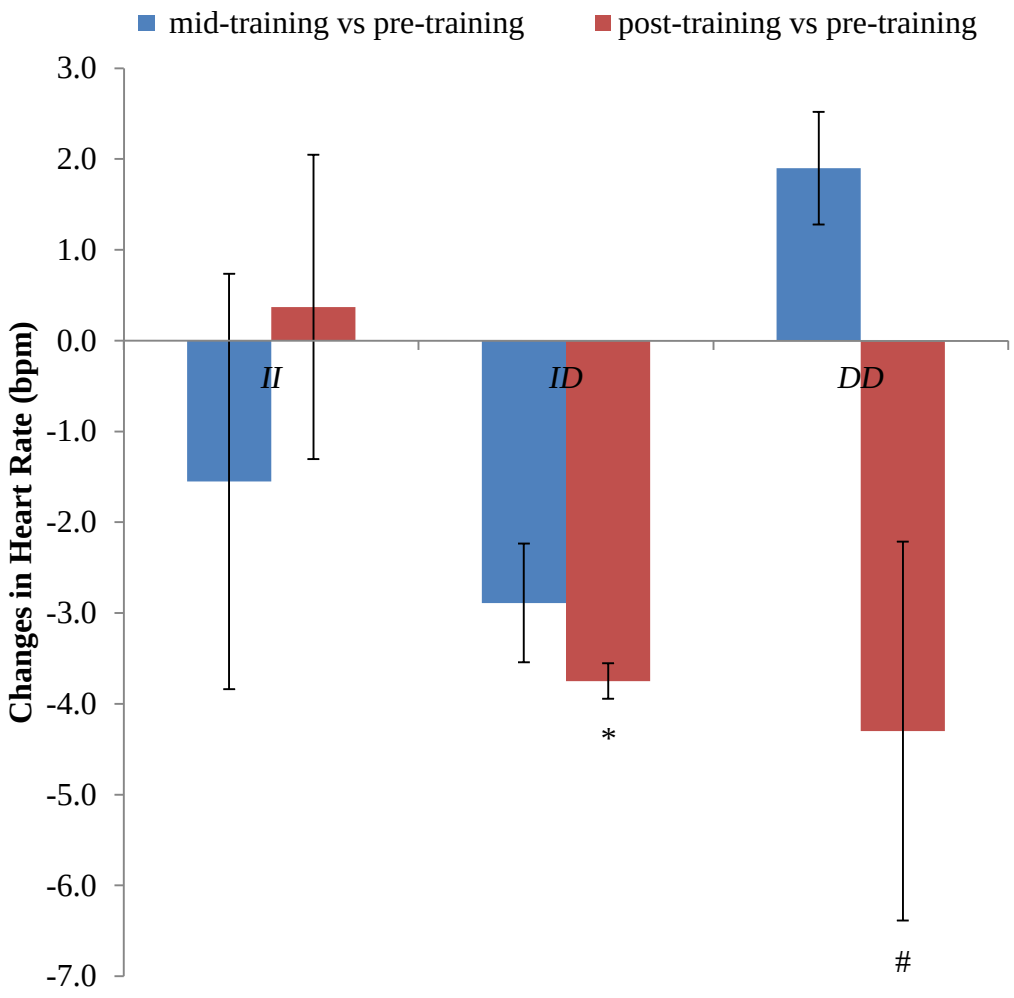


Figure 6.6 Changes in heart rate value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD
*Significant change from pre-training (p = 0.008)
Significant change from pre-training (p = 0.010)

6.5.4 *ACE I/D* Gene Polymorphism and Muscular Response

6.5.4.1 Handgrip Strength (HGS)

HGS at pre-, mid-, and post-training among *ACE I/D* genotype groups are shown in Table 6.9. HGS at pre-training ($F(2, 27) = 1.827$, $p = 0.180$), mid-training ($F(2, 27) = 2.306$, $p = 0.119$), and post-training ($F(2, 27) = 0.870$, $p = 0.431$) were not significantly different between the *ACE I/D* genotype groups.

Table 6.9 Handgrip strength at pre-, mid-, and post-training among *ACE I/D* genotype groups

Time course	*II* (n=10)	*ID* (n=10)	*DD* (n=10)	p value
Pre-training	43.4 ± 4.9	46.3 ± 8.0	40.8 ± 6.3	0.180
Mid-training	43.3 ± 5.7	46.3 ± 7.9	39.9 ± 6.0	0.119
Post-training	47.6 ± 4.6	47.1 ± 9.5	43.5 ± 7.8	0.431

Note.
Data shown as mean ± SD

Changes in HGS following mid- and post-training in *ACE I/D* genotype groups are presented in Figure 6.7. HGS in all genotype groups did not significantly change with mid-training (*II* = ($\Delta = -0.1 \pm 2.1$, $t(9) = 0.149$, $p = 0.885$), *ID* = ($\Delta = -0.1 \pm 1.4$, $t(9) = 0.136$, $p = 0.895$), and *DD* = ($\Delta = -0.8 \pm 3.1$, $t(9) = 0.844$, $p = 0.420$)). HGS increased significantly following post-training in the *II* genotype group ($\Delta = 4.2 \pm 4.1$, $t(9) = -3.212$, $p = 0.011$). Nevertheless, HGS in *ID* ($\Delta = 0.8 \pm 2.8$, $t(9) = -0.851$, $p = 0.417$) and *DD* ($\Delta = 2.7 \pm 5.6$, $t(9) = -1.542$, $p = 0.158$) genotype groups did not significantly change with IHG training.

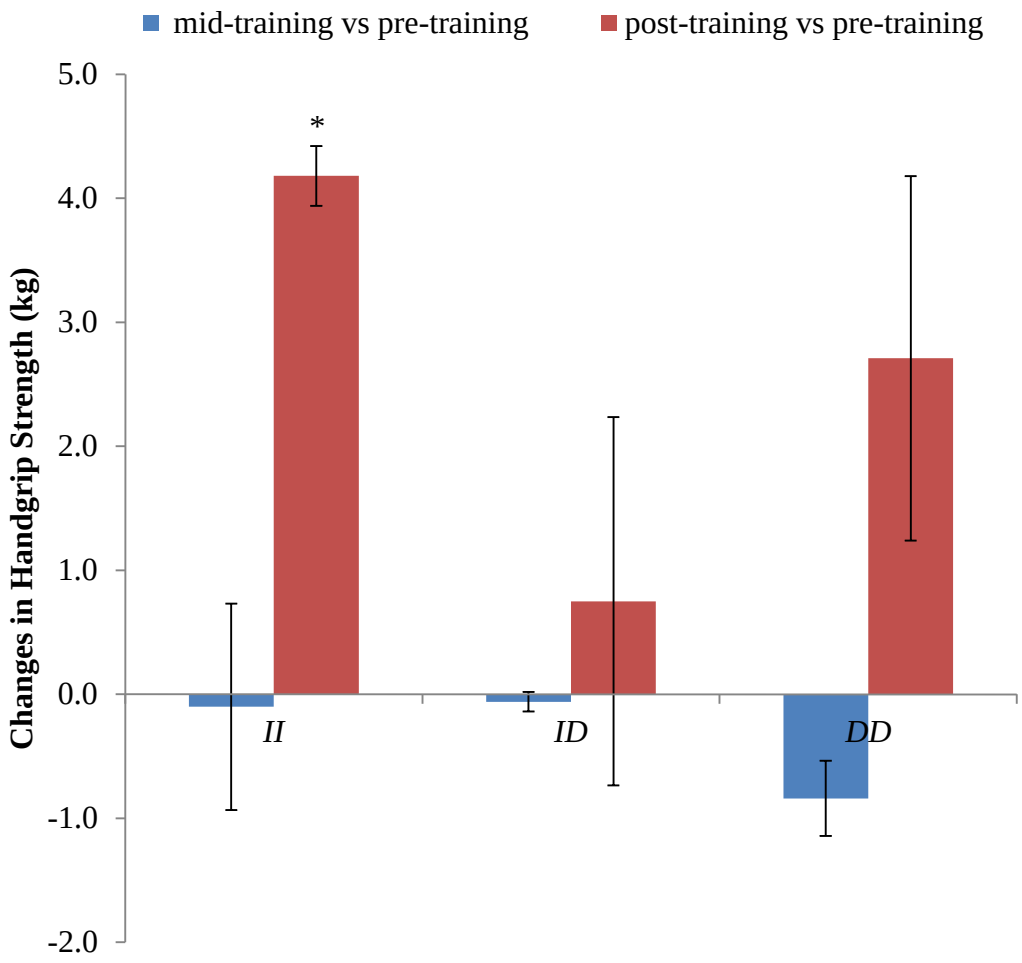


Figure 6.7 Changes in handgrip strength value to IHG training in *ACE I/D* genotype groups

Note.
Data shown as mean ± SD
*Significant change from pre-training (p = 0.011)

6.6 Discussion

This study examined if the *ACE I/D* gene polymorphism could influence cardiovascular and muscular responses to 8-weeks of IHG training among normotensive men in Malaysia. The main finding of this study was that the *ACE I/D* gene polymorphism influences cardiovascular and muscular adaptations to an 8-week IHG training in a normotensive population with the possession of *ACE D* and *ACE I* allele had an effect in reducing resting blood pressure and improved muscular strength, respectively, following the training.

Findings obtained from the current study showed that cardiovascular and muscular responses to IHG training varied between normotensive individuals with different *ACE I/D* genotypes. Consistent with the results retrieved from Kim (2009), this study revealed that normotensive men with *DD* and *ID* genotypes tended to have decreased resting SBP and HR with IHG training more than those with *II* genotype. Nevertheless, this finding was not comparable to the findings obtained from some previous studies (Hagberg et al., 1999, Zhang et al., 2002) that demonstrated individuals with *II* genotype had lower resting blood pressure than other genotypes after exercise. This inconsistency may be due to the differences in the mode of exercise performed. For example, a study by Kim (2009) involved mixed aerobic and resistance exercise training compared with only aerobic training (endurance training) in study by Hagberg et al. (1999). Several studies have demonstrated that the physiological adaptation for aerobic training was greater among

individuals with *II* genotype, and those with *DD* genotype responded better to resistance training (Cam et al., 2005, Defoor et al., 2006, Cam et al., 2007).

With regard to ACE activity, individuals with *DD* genotype had higher ACE activity compared to those with *II* genotype (Rigat et al., 1990). A higher level of ACE in circulating and skeletal muscle renin-angiotensin system (RAS) would increase the production of angiotensin II (ANG II) (Jones and Woods, 2003, Sayed-Tabatabaei et al., 2006). Nevertheless, ANG II has different effects on circulating and skeletal muscle RAS (Jones and Woods, 2003). In circulating RAS, ANG II binds to several receptors that construct the blood vessels to increase blood pressure (Sayed-Tabatabaei et al., 2006). However, ANG II in skeletal muscle RAS stimulates the production of angiotensin (ANG) (1-7) peptide; a potent vasodilator that causes a decrease in blood pressure (Jones and Woods, 2003). As this study employed an IHG training that particularly involved the contraction of skeletal muscle, the reduction in SBP and HR in individuals with *DD* genotype might be interpreted due to high production of ANG (1-7) during exercise. Nonetheless, the present study did not measure the components of skeletal muscle RAS, which warrants for future studies to confirm this possible mechanism.

The greater reduction in SBP and HR among *DD* genotype carrier could also be due to their higher baseline blood pressure values. This present result was consistent with the finding obtained by Millar et al. (2007), who demonstrated that normotensive individuals with higher baseline values of resting SBP and HR had a more pronounced reduction in these parameters after IHG training than those with lower baseline values.

This finding was also consistent with Wiley's et al. (1992), who observed a larger reduction in resting blood pressure in hypertensive patients following isometric exercise training compared with normotensive individuals. Collectively, these findings support the idea generated by Badrov et al. (2013), who had previously suggested that individuals with higher baseline blood pressure values might have greater capacity for reduction in blood pressure following IHG training compared to those with lower baseline blood pressure value. This speculation is supported by the fact that those with higher resting blood pressure, such as hypertensive patients, present greater sympathetic activity at rest (Schlaich et al., 2004) that could lead to greater hemodynamic responsiveness to sympathetic activation (Kaushik et al., 2004).

In the present study, the *ACE I/D* gene polymorphism was observed to influence muscular response to IHG training. The *II* genotype carriers exhibited a significantly greater increase in muscle strength after IHG training than *ID* and *DD* genotype carriers. This finding appears to contradict previous studies, which demonstrated that individuals with *DD* genotype obtained higher gains in muscle strength after exercise training than other genotype groups (Folland et al., 2000, Giaccaglia et al., 2008). The possible interpretation of the present data is that the lower ACE activity in participants with *ACE II* genotype may raise the production of bradykinin in skeletal muscle that increases local concentrations of nitric oxide, which in turn, raises mitochondrial efficiency, and thus, contractile function in skeletal muscle (Zhao et al., 1999). Moreover, previous study has shown that a high level of bradykinin in *II* genotype carriers resulted in improved tissue

oxygenation and a lesser rise in lactate that could lead to greater muscle efficiency (Kanazawa et al., 2002).

The findings obtained from the current study reaffirm previous reports of a reduction in resting BP after an 8-week IHG training among normotensive individuals (McGowan et al., 2007, Millar et al., 2008, Badrov et al., 2013). Similar to previous studies, this study found that an 8-week IHG training significantly decreased resting SBP, MAP, and PP by 4.2 mmHg, 2.4 mmHg, and 2.8 mmHg, respectively, (McGowan et al., 2007, Millar et al., 2008, Badrov et al., 2013). However, insignificant difference was observed in resting DBP after the IHG training program, which was similar to those reported previously among normotensive women (Badrov et al., 2013). However, the explanation for the lack of change in DBP following an 8-week IHG training has remained unclear. It may likely be due to the fact that DBP has a smaller range of values than SBP that limits the maximal change in DBP value (Pikilidou et al., 2013).

Concomitant with the reduction in resting BP, resting HR was also observed to be significantly lower after an 8-week IHG training. The decrease in resting HR in the present study was similar to the earlier finding by Singh et al. (2014). In fact, previous studies have suggested that the decrease in resting blood pressure and HR may be due to a reduction in sympathetic nerve activity during IHG exercise (Wiley et al., 1992, Mueller, 2007). When blood pressure is elevated during IHG exercise, the baroreceptors are stretched, resulting in a reflex-mediated increase in parasympathetic nerve activity, and a decrease in sympathetic nerve activity (Wiley et al., 1992). Consequently, it caused the

decline in the heart rate, while the diameter of blood vessels increased; leading to a drop in the blood pressure (Wiley et al., 1992).

Besides resting blood pressure and HR, the present study also showed that the 8-week IHG training improved muscle strength. The increase in muscle strength observed in this study had been consistent with those found in previous studies that demonstrated IHG exercise at 30% of MVC improved muscle strength (Howden et al., 2002, Mortimer and McKune, 2011). Considering all the changes in resting BP and HR, as well as muscle strength following IHG training, this training mode may be prescribed as part of lifestyle modification in maintaining a desirable blood pressure level, improving health quality of life, and reducing the risk of several diseases.

Comparable with the findings on cardiovascular response to IHG training, there was a significant reduction in cardiovascular variables immediately following a session of IHG exercise. Resting SBP and MAP were observed to significantly reduce by 3.2 mmHG and 2.7 mmHG, respectively, in response to IHG exercise. These findings indicated that IHG exercise may provide substantial benefits for hypertensive patients to lower their resting BP and HR for certain periods.

Although the present study has yielded some preliminary findings, there are some limitations that should be considered. First, the sample size in each genotype group may have been too small, and further larger studies are required to confirm these results. Second, the present study did not include a non-exercising control group to reduce

variability of the results as the present study was primarily designed to investigate the effects of *ACE I/D* gene polymorphism on IHG training adaptation where data were compared between genotype groups.

Despite these limitations, the current results remain valid and applicable as the participants in each were relatively homogeneous in terms gender, physical characteristics, and health status. The intervention was standardized participants with the same trained investigator conducted the training intervention. The participants underwent familiarization procedures to reduce the apprehension-induced variability of the baseline measurements. The present study was conducted as single-blind study where the genotype of the participants was withheld from the participants.

6.7 Conclusion

In conclusion, the present study demonstrated the influence of *ACE I/D* gene polymorphism on cardiovascular and muscular adaptations to an 8-week IHG training in a normotensive population. Hence, future studies on hypertensive patients are warranted to confirm the effects of *ACE I/D* gene polymorphism on cardiovascular and muscular responses to an 8-week IHG training.

CHAPTER 7

GENERAL CONCLUSION AND FUTURE DIRECTIONS

7.1 General Conclusion

The series of experiments presented in this thesis aimed to investigate the distribution of angiotensin I-converting enzyme (*ACE*) *I/D* and alpha-actinin-3 (*ACTN3*) *R/X* gene polymorphisms among an Asian population in Malaysia and its effect on human physical performance. The novelty of this entire research project was to demonstrate if *ACE I/D* and *ACTN3 R/X* gene polymorphisms influence physical performance and health in an Asian population in Malaysia which has received little previous attention compared to Caucasian populations. Specifically, this project addressed whether the distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms is different in the Asian population in Malaysia as compared to the Caucasian population. Further investigation was carried out to determine if human physical performance is affected by the *ACE I/D* and *ACTN3 R/X* gene polymorphisms within the Asian population in Malaysia as has been widely reported among the Caucasian population.

Based on the findings retrieved from the three presented studies, this thesis concludes that the *ACE I/D* and *ACTN3 R/X* gene polymorphisms play an important role in human physical performance and health within the Malaysian population. The specific

conclusions derived from the series of experiments presented in this thesis are depicted in the following:-

1. The distribution of *ACE I/D* gene polymorphism within the Malaysian population was different to the Australian population. The distribution of *ACE I/D* gene polymorphism also varied significantly between four ethnic groups in the Malaysian population. This finding highlighted that the current understanding on how *ACE I/D* gene polymorphism may influence human physical performance may be different in different ethnic population. Conversely, the distribution of *ACTN3 R/X* gene polymorphism did not differ between Malaysian and Australian populations as well as between four ethnic groups in the Malaysian population. This finding suggests that the *ACTN3 R/X* gene polymorphism may confer similar effects across different ethnic population.

2. Possession of the *D* allele of *ACE I/D* and *R* allele of *ACTN3 R/X* gene polymorphisms had a positive effect on strength/power performance in Malaysian population. This data confirmed the positive effects of possession of the *D* allele of *ACE I/D* and *R* allele of *ACTN3 R/X* gene polymorphisms on strength/power performance within the Asian ethnicity, as previously suggested for the Caucasian population (Clarkson et al., 2005b, Williams et al., 2005, Moran et al., 2006b, Vincent et al., 2007, Charbonneau, 2007, Giaccaglia et al., 2008, Ahmetov et al., 2013, Erskine et al., 2014). This finding highlights the importance of genetic

screening for athletes to ensure that they are suited to play a particular sport based on their genetic profile and for coaches to use when planning training programs.

On the other hand, possession of the *I* allele of *ACE I/D* and *X* allele of *ACTN3 R/X* gene polymorphisms did not influence endurance performance in the Malaysian population. This finding suggests that possession of the *I* allele of *ACE I/D* and *X* allele of *ACTN3 R/X* gene polymorphisms may not the absolute criteria for success as far as endurance performance is concerned.

The distribution of *ACE I/D* and *ACTN3 R/X* gene polymorphisms do not vary much between different ethnic groups of Malaysian athletes and did not have much influence on the physical performance between these ethnic groups. This finding indicated that *ACE I/D* and *ACTN3 R/X* gene polymorphisms may confer similar effects on human physical performance across different ethnic group in Malaysia.

3. The *ACE I/D* gene polymorphism influences cardiovascular and muscular adaptations to an 8-week isometric handgrip (IHG) training in a normotensive population. This study demonstrated that the normotensive men with *DD* and *ID* genotypes tended to have a decreased resting SBP and HR with IHG training more than those men with *II* genotype. On the contrary, the *II* genotype carriers exhibited a significantly greater increase in muscle strength after IHG training than those with *ID* and *DD* genotype carriers. This data may serve as a basis in

identifying individuals who would be more likely to benefit from the 8-week IHG training program.

7.2 Strengths and Limitations

Although this entire research project has reached its aims, there were some unavoidable limitations. Unforeseen difficulty in obtaining a large enough number of participants in each study may limit the generalizability of the current findings. As the second study are based on relatively mixed samples comprised of both genders, it is not clear whether the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance within the Malaysian population is related to specific gender.

Another shortcoming of the research is that the analyses of physical performance in the second study were restricted to the Malaysian athletes with no data available for the Malaysian controls in order to improve power by reducing variability. In addition, due to time constraint, there is no data available on the physical performance of Australian athletes. However, this is not considered to have any important impact on the main results of this study and further research could replicate the analyses of physical performance amongst the Australian athletes and Malaysian controls to further confirm the current finding. Another limitation of the research is the third study did not include a non-exercising control group to reduce variability of the results as the third study was primarily designed to investigate the effects of *ACE I/D* gene polymorphism on IHG training adaptation where data were compared between genotype groups. Nevertheless, future

studies involving non-exercising control group are warranted to confirm the present findings.

Despite the unavoidable limitations in the present research, a number of design features of this research lend support for the validity of the present findings. This research project followed recent recommendations on genomic research and exercise, that studies should use all the experimental approaches (case-control, cross-sectional and intervention studies) to demonstrate the relevance of *ACE I/D* and *ACTN3 R/X* gene polymorphisms to human physical performance and health within the Malaysian population (Guilherme et al., 2014). In the first and second study, the proportion of participants with different ethnic backgrounds in the Malaysian population had been set according to the current distribution ratio in Malaysia (Statistic, 2011), providing a more representative findings for the Malaysian population. In addition, participants within athlete and control groups in the Malaysian population were ethnically-matched to limit and control the potential effect of ethnicity. Malaysian and Australian populations were well-matched on age, gender, sample size and sporting discipline. Given that genotype distributions of studied polymorphisms were in Hardy-Weinberg equilibrium in the control group, the genetic assessment was accurate and unbiased. In the third study, the participants were relatively homogeneous in terms of age, sex, physical characteristic and health status with the intervention was standardized across the participants.

7.3 Future Directions

The current understanding on the influence of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and health remained controversial with inclusive findings especially when examined across different ethnicity. The series of experiments presented in this thesis has provided new insight regarding ethnicity and the *ACE I/D* and *ACTN3 R/X* gene polymorphisms effect on human physical performance and health. While several conclusions were drawn in this thesis, further studies are warranted in order to further confirm the effect of genetic across different ethnicities. Based on the current findings, further studies which address the following research question should be considered;

1. Are there any genetic variant interaction between the *ACE I/D* and *ACTN3 R/X* gene polymorphisms in influencing human physical performance?

2. Do the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance differ by gender?

3. What is the potential mechanism underlying the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance and health?

4. Do the effects of *ACE I/D* and *ACTN3 R/X* gene polymorphisms on human physical performance observed within the Malaysian population be of significance in other Asian populations?

5. Does the difference in the athlete development program between Malaysian and Australian population influence the distribution pattern of the *ACE I/D* and *ACTN3 R/X* gene polymorphisms in athletic population of these two populations?

6. Does the *ACE I/D* gene polymorphism drive similar cardiovascular and muscular changes in hypertensive patients following an 8-week IHG training program as those observed in normotensive participants in the current thesis?

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APPENDICES

APPENDIX A

RESEARCH APPROVAL FROM THE HUMAN RESEARCH ETHICS COMMITTEE (HREC) UNIVERSITI SAINS MALAYSIA

UNIVERSITI SAINS MALAYSIA

Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

Our. Ref. : USMKK/PPP/JEPeM [248.3.(5)]
Date : 26th April 2012

Universiti Sains Malaysia
Kampus Kesihatan,
16150 Kubang Kerian,
Kelantan. Malaysia.
T: 609 - 767 3000 *samb.* 2350 / 2352
F: 609 - 767 2351
E: jepem@kk.usm.my
www.crp.kk.usm.my

Dr. Ahmad Munir Che Muhamed
Advanced Medical and Dental Institute
No. 1-8, Persiaran Seksyen 4/1
Bandar Putra Bertam
13200 Kepala batas
Pulau Pinang.

The Human Research Ethics Committee, Universiti Sains Malaysia (FWA Reg. No: 00007718; IRB Reg. No: 00004494) has approved in principle the study mentioned below:

Title	Genetics Influence on Sport Performance: An Analysis on Malaysia Varsity Athletes.		
Protocol No	-	**Principle Investigator**	Dr. Ahmad Munir Che Muhamed
Date of approval Protocol received Reviewed by Committee Received Amended Protocol	26th April 2012 7th December 2011 28th March 2012 12th April 2012	**Co-Investigator(s)**	Assoc. Prof. Zafarina Zainuddin Prof. Rabindarjeet Singh Mrs. Hazwani Ahmad Yusof @ Hanafi
Research Center	Healthy Lifestyle Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia.	**Date of study start**	May 2012 – April 2014
Financial Support	Ministry of Higher Education Sport Grant.	**Number of Samples**	1, 444 subjects

The following item (✓) have been received and reviewed:-

(✓) **Ethical Approval Application Form**
(✓) **Study Protocol**
(✓) **Participant Information Sheet and Consent Form**

Investigator(s) are required to:
a) follow instructions, guidelines and requirements of the Human Research Ethics Committee, Universiti Sains Malaysia (JEPeM)
b) report any protocol deviations/violations to Human Research Ethics Committee (JEPeM)
c) comply with International Conference on Harmonization – Guidelines for Good Clinical Practice (ICH-GCP)
d) note that Human Research Ethics Committee (JEPeM) may audit the approved study.

PROFESSOR DR. MOHD SHUKRI OTHMAN
Chairman
Human Research Ethics Committee

UNIVERSITI SAINS MALAYSIA

Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

Our. Ref. : USM/JEPeM/2013(164)

Date : 30th October 2013

Universiti Sains Malaysia
Kampus Kesihatan,
16150 Kubang Kerian,
Kelantan. Malaysia.
T: 609 - 767 3000 *samb.* 2352 / 2362
F: 609 - 767 2351
E: jepem.usm@gmail.com
www.research.usm.my

Dr. Ahmad Munir Che Muhamed
Advanced Medical and Dental Institute (AMDI)
Universiti Sains Malaysia
Bertam
13200 Kepala Batas
PULAU PINANG.

Dear Dr,

APPLICATION FOR ETHICAL APPROVAL

Protocol Title: Genetics Influence on Sport Performance: An Analysis on Malaysia Varsity Athletes.

In Ref: USMKK/PPP/JEPeM (248.3[5])

I refer to your application received on 22nd October 2013.

I am pleased to inform you that the Human Research Ethics Committee, Universiti Sains Malaysia has reviewed your application and has approved in principle your application **to use the samples from Australian population (Caucasian)** for the above research title.

Thank you.

"ENSURING A SUSTAINABLE TOMORROW"

Yours sincerely,

(PROF. DR. HANS AMIN VAN ROSTENBERGHE)
Chairman
Human Research Ethics Committee

c.c Secretary of Human Research Ethics Committee, USM.

Universiti Sains Malaysia
Kampus Kesihatan,
16150 Kubang Kerian,
Kelantan. Malaysia.
T: 609 - 767 3000 *samb.* 2354/2362
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27th January 2015

Miss Hazwani Ahmad Yusof @ Hanafi
Ph D Student
Advanced Medical and Dental Institute
Universiti Sains Malaysia
Bertam
13200 Kepala Batas, Pulau Pinang.

JEPeM Code : USM/JEPeM/1406226
Protocol Title : The Influence of ACE I/D Gene Polymorphism on Blood Pressure Response Following Isometric Exercise Training.

Dear Miss.,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code **USM/JEPeM/1406226**, which should be used for all communication to the JEPeM-USM related to this study. This ethical clearance is valid from **January 2015** until **December 2015.**

The following documents have been approved for use in the study.

1. Research Proposal

In addition to the abovementioned documents, the following technical document was included in the review on which this approval was based:

1. Participant Information Sheet and Consent Form (English version)
2. Participant Information Sheet and Consent Form (Malay version)
3. Participant Information Sheet and Consent Form for Whole-Genome/Genetic Related Studies (English version)
4. Participant Information Sheet and Consent Form for Whole-Genome/Genetic Related Studies (Malay version)
5. Data Collection Form

Attached document is the list of members of JEPeM-USM present during the full board meeting reviewing your protocol.

While the study is in progress, we request you to submit to us the following documents:

1. Progress report using the **JEPeM-USM FORM 3(B) 2014: Continuing Review Application Form** every 1 year from date of approval (NOTE: In view of active ethical clearance, this report is mandatory even if the study has not started or is still awaiting release of funds.)
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using **JEPeM-USM FORM 3(A) 2014: Study Protocol Amendment Submission Form.**
3. Revisions in the informed consent form using the **JEPeM-USM FORM 3(A) 2014: Study Protocol Amendment Submission Form.**
4. Reports of adverse events (if any) including from other study sites (national, international) using the **JEPeM-USM FORM 3(G) 2014: Adverse Events Report.**

5. Notice of early termination of the study and reasons for such using **JEPeM-USM FORM 3(E) 2014.**
6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using **JEPeM-USM FORM 3(C) 2014: Final Report Form.**
9. Application for renewal of ethical clearance 90 days before the expiration date of this approval through submission of **JEPeM-USM FORM 3(B) 2014: Continuing Review Application Form.**

Please note that forms may be downloaded from the JEPeM-USM website: **www.jepem.kk.usm.my**

Jawatankuasa Etika Penyelidikan (Manusia), JEPeM-USM is in compliance with the Declaration of Helsinki, International Conference on Harmonization (ICH) Guidelines, Good Clinical Practice (GCP) Standards, Council for International Organizations of Medical Sciences (CIOMS) Guidelines, World Health Organization (WHO) Standards and Operational Guidance for Ethics Review of Health-Related Research and Surveying and Evaluating Ethical Review Practices, EC/IRB Standard Operating Procedures (SOPs), and Local Regulations and Standards in Ethical Review.

Thank you.

"ENSURING A SUSTAINABLE TOMORROW"

Very truly yours,

PROF. DR. HANS AMIN VAN ROSTENBERGHE
Chairperson
Jawatankuasa Etika Penyelidikan (Manusia) JEPeM
Universiti Sains Malaysia

Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM)
Human Research Ethics Committee USM (HREC)

Universiti Sains Malaysia
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Date of meeting: 19 October 2014
Venue : Meeting Room, Centre for Research Initiatives, Clinical and Health Sciences, USM Kampus Kesihatan.
Time : 9.00 a.m – 2.00 p.m
Meeting No : 293

Members of Committee of the Jawatankuasa Etika Penyelidikan (Manusia), JEPeM Universiti Sains Malaysia who reviewed the protocol/documents are as follows:

Member (Title and Name)		Occupation (Designation)	Male/ Female (M/F)	Tick (✓) if present when above items, were reviewed
Chairperson : Professor Dr. Hans Amin Van Rostenberghe		Chairperson of Jawatankuasa Etika Penyelidikan (Manusia), JEPeM USM	M	✓ (Chairperson)
Secretary: Mr. Mohd Bazlan Hafidz Mukrim		Research Officer	M	✓
Members :				
1.	Dato' Hj Ellias Zakaria	Lecturer, School of Humanity	M	✓
2.	Professor Wan Abdul Manan Wan Muda	Lecturer, School of Health Sciences	M	✓
3.	Professor Dr. Nik Hazlina Nik Hussain	Lecturer, School of Medical Sciences	F	✓
4.	Associate Professor Dr. Suzina Sheikh Abd Hamid	Lecturer, School of Medical Sciences	F	✓
5.	Dr. Soon Lean Keng	Lecturer, School of Health Sciences	F	✓
6.	Dr. Teguh Haryo Sasongko	Lecturer, Human Genome Centre, USM	M	✓
7.	Dr. Haslina Taib	Lecturer, School of Dental Sciences	F	✓
8.	Tn. Hj. Ismail Hassan	Community Representative	M	✓

The Jawatankuasa Etika Penyelidikan (Manusia), JEPeM of Universiti Sains Malaysia is in compliance with International Conference on Harmonization–Guidelines for Good Clinical Practice (ICH-GCP) guidelines and Declaration of Helsinki.

PROFESSOR DR. HANS AMIN VAN ROSTENBERGHE
Chairperson
Jawatankuasa Etika Penyelidikan (Manusia), JEPeM
Universiti Sains Malaysia

APPENDICES

APPENDIX B

RESEARCH APPROVAL FROM THE HUMAN RESEARCH ETHICS COMMITTEE (HREC) UNIVERSITY OF SYDNEY

Research Integrity
Human Research Ethics Committee

Friday, 15 November 2013

Dr Kieron Rooney
Exercise Health and Performance, Faculty of Health Sciences,
Email: kieron.rooney@sydney.edu.au

Dear Dr Kieron Rooney

I am pleased to inform you that the University of Sydney Human Research Ethics Committee (HREC) has approved your project entitled "**Establishing an estimation of frequencies of genotypes related to sport performance in a non-elite reference population"**.

Details of the approval are as follows:

Project No.: **2013/894**

Approval Date: **14 November 2013**

First Annual Report Due: **14 November 2014**

Authorised Personnel: **Rooney Kieron; Ahmad Yusof Hazwani; Gwinn Tom; Ruell Patricia; Che Muhammed Ahmad Munir; Zainuddin Zafarina;**

Documents Approved:

Date Uploaded	Type	Document Name
14/11/2013	Advertisements/Flyer	Amended advert
14/11/2013	Participant Info Statement	Amended PIS
06/09/2013	Participant Consent Form	Human ethics Consent form
06/09/2013	Other Type	Participant's information detail form

HREC approval is valid for four (4) years from the approval date stated in this letter and is granted pending the following conditions being met:

Condition/s of Approval

- Continuing compliance with the National Statement on Ethical Conduct in Research Involving Humans.
- Provision of an annual report on this research to the Human Research Ethics Committee from the approval date and at the completion of the study. Failure to submit reports will result in withdrawal of ethics approval for the project.
- All serious and unexpected adverse events should be reported to the HREC within 72 hours.
- All unforeseen events that might affect continued ethical acceptability of the project should be reported to the HREC as soon as possible.

- Any changes to the project including changes to research personnel must be approved by the HREC before the research project can proceed.

- Note that for student research projects, a copy of this letter must be included in the candidate's thesis.

Chief Investigator / Supervisor's responsibilities:

1. You must retain copies of all signed Consent Forms (if applicable) and provide these to the HREC on request.

2. It is your responsibility to provide a copy of this letter to any internal/external granting agencies if requested.

Please do not hesitate to contact Research Integrity (Human Ethics) should you require further information or clarification.

Yours sincerely

Professor Glen Davis
Chair
Human Research Ethics Committee

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007) and the CPMP/ICH Note for Guidance on Good Clinical Practice.

APPENDICES

APPENDIX C

PARTICIPANTS CONSENT AND INFORMATION DETAILS FORM

ATTACHMENT S

Subject Information and Consent Form
(Signature Page)

Research Title: Genetics Influence on Sport Performance: An analysis on Malaysian Varsity Athletes

Researcher's Name: Dr. Ahmad Munir Bin Che Muhamed

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Subject Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the researcher, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Subject Information and Consent Form to keep for myself.

_____ _____

Subject Name (Print or type) Subject Initials and Number

_____ _____

Subject I.C No. (New) Subject I.C No. (Old)

_____ _____

Signature of Subject or Legal Representative Date (dd/MM/yy)
(Add time if applicable)

_____

Name of Individual
Conducting Consent Discussion (Print or Type)

_____ _____

Signature of Individual Date (dd/MM/yy)
Conducting Consent Discussion

_____ _____

Name & Signature of Witness Date (dd/MM/yy)

Note: i) All subject who are involved in this study will not be covered by insurance.

ATTACHMENT G

Subject Information and Consent Form
(Signature Page)

Research Title: Genetics Influence on Sport Performance: An analysis on Malaysian Varsity Athletes

Researcher's Name: Dr. Ahmad Munir Bin Che Muhamed

To become a part this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Subject Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the researcher, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Subject Information and Consent Form to keep for myself.

_____ _____

Subject Name (Print or type) Subject Initials and Number

_____ _____

Subject I.C No. (New) Subject I.C No. (Old)

_____ _____

Signature of Subject or Legal Representative Date (dd/MM/yy)
(Add time if applicable)

_____

Name of Individual
conducting Consent Discussion (Print or Type)

_____ _____

Signature of Individual Date (dd/MM/yy)
Conducting Consent Discussion

_____ _____

Name & Signature of Witness Date (dd/MM/yy)

Note: i) All subject who are involved in this study will not be covered by insurance.
ii) Excess samples from this research will not be used for other reasons and will be destroyed with the consent from the Research Ethics Committee (Human), USM.

ATTACHMENT P

Subject's Material Publication Consent Form
Signature Page

Research Title: Genetics Influence on Sport Performance: An analysis on Malaysian Varsity Athletes

Researcher's Name: Dr. Ahmad Munir Bin Che Muhamed

To become a part this study, you or your legal representative must sign this page.
By signing this page, I am confirming the following:

- I understood that my name will not appear on the materials published and there have been efforts to make sure that the privacy of my name is kept confidential although the confidentiality is not completely guaranteed due to unexpected circumstances.
- I have read the materials or general description of what the material contains and reviewed all photographs and figures in which I am included that could be published.
- I have been offered the opportunity to read the manuscript and to see all materials in which I am included, but have waived my right to do so.
- All the published materials will be shared among the scientists and journalist worldwide.
- The materials will also be used in local publications, book publications and accessed by many local and international researchers worldwide.
- I hereby agree and allow the materials to be used in other publications required by other publishers with these conditions:
- The materials will not be used as advertisement purposes or as packaging materials.
- The materials will not be used out of contex – i.e.: Sample pictures will not be used in an article which is unrelated subject to the picture.

Subject Name (Print or type)	Subject Initials or Number
Subject I.C No. / Subject's Signature	Date (dd/MM/yy)
Name and Signature of Individual Conducting Consent Discussion	Date (dd/MM/yy)

Note: i) All subject who are involved in this study will not be covered by insurance.

THE UNIVERSITY OF SYDNEY

Discipline of Exercise and Sport Science
Faculty of Health Sciences

ABN 15 211 513 464

Dr. Kieron Rooney
Senior Lecturer

Room K120
C42 - K
The University of Sydney
NSW 2006 AUSTRALIA
Telephone: +61 2 9351 9135
Facsimile: +61 2 9351 9204
Email: kieron.rooney@sydney.edu.au
Web: http://www.sydney.edu.au/

PARTICIPANT CONSENT FORM

I, ...[PRINT NAME], give consent to my participation in the research project

TITLE: **Genetic Influence on Athletes Performance: An analysis on Malaysian and Australian Athletes**

In giving my consent I acknowledge that:

1. The procedures required for the project and the time involved have been explained to me, including any inconvenience, risk, discomfort or side effect and any questions I have about the project have been answered to my satisfaction.

2. I have read the Participant Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.

3. I understand that being in this study is completely voluntary – I am not under any obligation to consent.

4. I understand that my involvement is strictly confidential. I understand that any research data gathered from the results of the study may be published however no information about me will be used in any way that is identifiable.

5. I understand that I can withdraw from the study at any time, without affecting my relationship with the researcher(s) or the University of Sydney now or in the future.

6. I consent to:

- Cheek Swabs for DNA sampling YES ☐ NO ☐
- Personal Detail Questionnaire YES ☐ NO ☐
- My coach providing you a record of my playing history and performance YES ☐ NO ☐

.....
Signature

....
Please PRINT name

.....
Date

THE UNIVERSITY OF SYDNEY

ABN 15 211 513 464

Discipline of Exercise and Sport Science
Faculty of Health Sciences

Dr. Kieron Rooney
Senior Lecturer

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C42 - K
The University of Sydney
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Facsimile: +61 2 9351 9204
Email: kieron.rooney@sydney.edu.au
Web: http://www.sydney.edu.au/

PARTICIPANT CONSENT FORM

I, ...[PRINT NAME], give consent to my participation in the research project

TITLE: **Establishing an estimation of frequencies of genotypes related to sport performance in a non-elite reference population**
In giving my consent I acknowledge that:

1. The procedures required for the project and the time involved have been explained to me, including any inconvenience, risk, discomfort or side effect and any questions I have about the project have been answered to my satisfaction.
2. I have read the Participant Information Statement and have been given the opportunity to discuss the information and my involvement in the project with the researcher/s.
3. I understand that being in this study is completely voluntary – I am not under any obligation to consent.
4. I understand that my involvement is strictly confidential. I understand that any research data gathered from the results of the study may be published however no information about me will be used in any way that is identifiable.
5. I understand that I can withdraw from the study at any time, without affecting my relationship with the researcher(s) or the University of Sydney now or in the future.
6. I consent to:
   - Cheek Swabs for DNA sampling YES ☐ NO ☐
   - Personal Detail Questionnaire YES ☐ NO ☐

.....
Signature

....
Please PRINT name

.....
Date

ATTACHMENT E

Research Subject Information and Consent Form (Signature Page)

Research Title: The influence of ACE I/D gene polymorphism on blood pressure response following isometric exercise training

Researcher's Name: Hazwani Binti Ahmad Yusof @ Hanafi

I voluntarily consent to take part in this research study. I have fully discussed and understood the purpose, procedures and possible risks of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to my satisfaction.

Name of Subject (>12 years),	Signature	Date
_____	_____	_____

Name of Parent or Legally Accepted Representative	Signature	Date
_____	_____	_____

Translator Information
The study has been explained to the participant / legally acceptable representative in [specify the language used) language by [name of translator].

Witness Statement
I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him / her and clearly understands the nature, risks and benefits of his / her participation in the study.

Name of Witness	Signature	Date
_____	_____	

Investigator Statement
I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing this informed consent form clearly understands the nature, risks and benefits of her participation in the study.

Name of Investigator / Person administering consent	Signature	Date	Time Start	Time End
_____	_____	_____	_____	_____

ATTACHMENTF

Research Subject's Material Publication Consent Form
Signature Page

Research Title: The influence of ACE I/D gene polymorphism on blood pressure response following isometric exercise training

Researcher's Name: Hazwani Binti Ahmad Yusof @ Hanafi

To become a part this study, you or your legal representative must sign this page.

By signing this page, I am confirming the following:

- I understood that my name will not appear on the materials published and there have been efforts to make sure that the privacy of my name is kept confidential although the confidentiality is not completely guaranteed due to unexpected circumstances.
- I have read the materials or general description of what the material contains and reviewed all photographs and figures in which I am included that could be published.
- I have been offered the opportunity to read the manuscript and to see all materials in which I am included, but have waived my right to do so.
- All the published materials will be shared among the medical practitioners, scientists and journalist world wide.
- The materials will also be used in local publications, book publications and accessed by many local and international doctors world wide.
- I hereby agree and allow the materials to be used in other publications required by other publishers with these conditions:
- The materials will not be used as advertisement purposes nor as packaging materials.
- The materials will not be used out of contex – i.e.: Sample pictures will not be used in an article which is unrelated subject to the picture.

Research Subject Name (Print or type) Research Subject Initials or Number

Research Subject I.C No. Research Subject's Signature Date (dd/MM/yy)

Name and Signature of Individual Conducting Consent Discussion Date (dd/MM/yy)

PARTICIPANT INFORMATION DETAILS

BUTIRAN MAKLUMAT PESERTA

Life Style Science Cluster, Advanced Medical & Dental Institute, Universiti Sains Malaysia

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 THE MALAYSIAN POPULATION

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

A. Personal Details

Maklumat Peribadi

1. **Participant's I.D NO**:_____
   No. ID peserta

2. **Name of Participant**:_____
   Nama peserta

3. **I.D. NO**:_____
   No. kad pengenalan

4. **Gender**: _____ **5. Race**:_____
   Jantina Bangsa

6. **Current Address**:_____
   Alamat terkini
   _____

7. **Permanent Address**:_____
   Alamat tetap
   _____

8. **Email Address**:_____ **9. Phone No**:_____
   Alamat email No. Telefon

10. **Career**:_____ **11. Marital Status**:_____
    Kerjaya Status perkahwinan

12. **Education Level**:_____
    Tahap pengajian

B. Medical and Health Information

Maklumat Perubatan dan Kesihatan

1.	Have you ever had any of the following disease: Adakah anda pernah menghidapi mana-mana penyakit seperti berikut:		Yes Ya	No Tidak
	I.	**Heart Disease**/ Penyakit Jantung		
	II.	**Hypertension**/ Tekanan Darah Tinggi		
	III.	**Heart Attack**/ Serangan Jantung		
	IV.	**Stroke**/ Angin Ahmar		
	V.	**Asthma**/ Asma		
	VI.	**Diabetes**/ Kencing Manis		
	VII.	**Kidney Diseases**/ Penyakit Ginjal		
	VIII.	**Liver Diseases**/ Penyakit Hati		
	IX.	**Anemia**/ Anemia		
	X.	**Spinal injury**/ Kecederaan Tulang Belakang		
2.	**Do you engage in a diet program?** Adakah anda mengikuti program diet?			
3.	**Do you smoke?** Adakah anda merokok?			

***Thick (/) as appropriate**

Sila tanda (/) mana-mana yang berkenaan

4. **What types of exercise do you do (in the past 3 months)?**_____
   Apakah jenis senaman yang anda lakukan (dalam 3 bulan yang lalu)?

5. **How frequent do you exercise do (in the past 3 months)?**_____
   Berapa kerap kamu bersenam (dalam 3 bulan yang lalu)?

6. **Duration of exercise for each session (in minutes) do (in the past 3 months):_____**
   Tempoh masa senaman bagi setiap sesi (dalam minit) (dalam 3 bulan yang lalu)

7. **Are you an athlete? If yes, please answer the questions below:**_____
   Adakah anda seorang atlet?Jika Ya, sila jawab soalan di bawah:

   7.1 Sports involvement:_____
   Bidang sukan yang diceburi

   7.2 Current Level of competition:_____
   Tahap pertandingan sekarang

   7.3 Years playing at current level:_____
   Tahun bermain pada tahap sekarang

C. Analisis Filogeni dan Filogeografi di Semenanjung Malaysia.

A. Data Demografik

Nama: _____

No. KP: _____

Umur: _____ Agama:_____

Jantina: ☐ Lelaki ☐ Perempuan

No. Tel: _____
Alamat:
_____

_____

Etnik:

☐ Melayu ☐ Cina ☐ India

☐ Orang asli; Suku kaum.
Nyatakan:_____

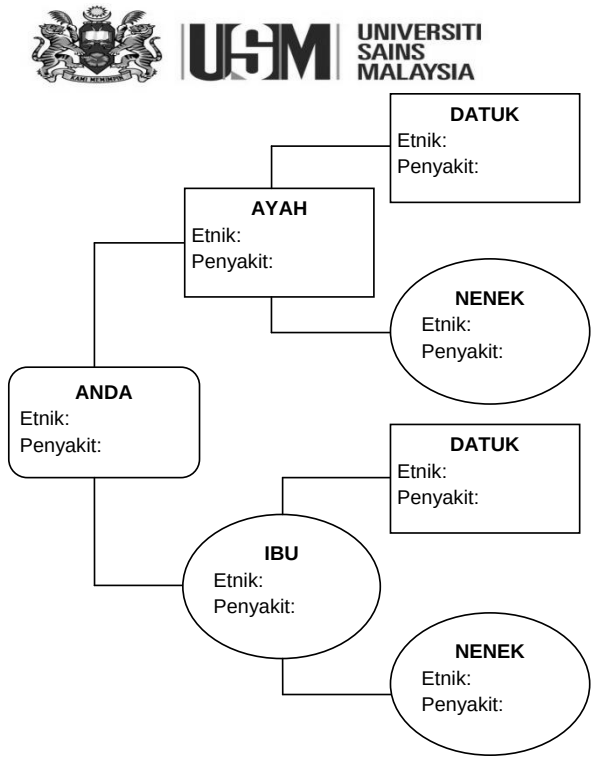
B. Kriteria Inklusi

	Ya	Tidak
1. Ketiga-tiga generasi mempunyai darah keturunan suku yang sama		
2. Tiada perkahwinan campuran dengan suku/bangsa lain untuk ketiga-tiga generasi		
3. Mengetahui sejarah keluarga dengan jelas untuk ketiga-tiga generasi		
4. Bertutur menggunakan dialek suku anda untuk urusan harian		

C. Sejarah penyakit jangkitan

	Ya	Tidak
1. Malaria		
2. TB		
3. Denggi		
4. Lain-lain. Nyatakan: ……		

Susur-galur keluarga:



PARTICIPANT INFORMATION DETAILS

Discipline of Exercise and Sport Science, Faculty of Health Sciences, University of Sydney, 75 East Street, Lidcombe, NSW 2141, Australia.

Establishing an estimation of frequencies of genotypes related to sport performance in a non-elite reference population

1.	**Participant I.D NO** (Filled in by researcher)	
2.	**Name**	
3.	**Gender**	
4.	**Date of Birth**	
5.	**Ethnicity (Country of Birth)**	
	Participant	
	Participant's Mother	
	Participant's Maternal grandmother	
	Participant's Maternal grandfather	
	Participant's Father	
	Participant's Paternal grandmother	
	Participant's Paternal grandfather	
6.	**Current Address**	
7.	**Email Address**	
8.	**Contact No**	

Have you ever had any kind of disease? e.g: Hypertension. If yes, please write down the name of the disease below.

_____
_____
_____
_____
_____
_____
_____

PARTICIPANT INFORMATION DETAILS

Discipline of Exercise and Sport Science, Faculty of Health Sciences, University of Sydney, 75 East Street, Lidcombe, NSW 2141, Australia.

Genetic Influence on Athletes Performance: An analysis on Malaysian and Australian Athletes

B. Personal Details

	Participant I.D NO (Filled in by researcher)	
1.	**Name**	
2.	**Gender**	
3.	**Date of Birth**	
4.	**Ethnicity (Country of Birth)**	
5.	Participant	
6.	Participant's Mother	
7.	Participant's Maternal grandmother	
8.	Participant's Maternal grandfather	
9.	Participant's Father	
10.	Participant's Paternal grandmother	
11.	Participant's Paternal grandfather	
12.	**Current Address**	
13.	**Email Address**	
14.	**Contact No**	
15.	**Sport**	
16.	**Current Level of competition**	
17.	**Years playing at current level**	

Comments (please list here information specific to your event example – if you are in a team sport list your most common positions played, if you are in an individual sport what is the distance you run / swim / cycle etc.):

_____
_____
_____
_____
_____
_____
_____

C. Injury History

1. Are you currently injured? YES ☐

NO ☐

If yes, Are you able to compete or are on training duties only?

_____

2. In the past 12 months have you been injured? YES ☐ NO ☐

If yes, what was the injury and were you able to compete or were limited to training duties only?

_____

3. Over the past 3 years how often have you been injured to the point of missing competition?

Not at all Sometimes Frequently

4. Over the past 3 years how often have you been injured but were still able to compete?

Not at all Sometimes Frequently

SUBJECT'S INFORMATION DETAILS

Lifestyle Science Cluster, Advanced Medical & Dental Institute, USM

The influence of ACE I/D gene polymorphism on blood pressure response following isometric exercise training

D. Personal Details

1.	**Subject's I.D NO** (Filled in by researcher)	
2.	**Gender**	
3.	**Age**	
4.	**Ethnicity**	
5.	Subject	
6.	Subject's Mother	
7.	Subject's Maternal grandmother	
8.	Subject's Maternal grandfather	
9.	Subject's Father	
10.	Subject's Paternal grandmother	
11.	Subject's Paternal grandfather	
12.	**Current Address**	
13.	**Email Address**	
14.	**Contact No**	
15.	**Career**	

B. Medical and Health Information

1.	**Have you ever had any of the following disease:**		**Yes**	**No**
	I.	**Heart Disease**		
	II.	**Hypertension**		
	III.	**Heart Attack**		
	IV.	**Stroke**		
	V.	**Asthma**		
	VI.	**Diabetes**		
	VII.	**Kidney Diseases**		
	VIII.	**Liver Diseases**		
	IX.	**Anemia**		
	X.	**Spinal injury**		
2.	**Do you smoke?**			

***Thick (/) as appropriate**

4. **What types of exercise do you do (in the past 3 months)?**_____
5. **How frequent do you exercise?**_____
6. **Duration of exercise for each session (in minutes):**_____

APPENDICES

APPENDIX D

HARDY-WEINBERG EQUILIBRIUM TEST

Malaysian general population (n=180)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	56	112	0	56	31.11	60.67	33.70	0.359	
ID	97	97	97	97	53.89	87.66	48.70	0.994	
DD	27	0	54	27	15.00	31.67	17.59	0.688	
Total	180	209	151	180	100.00	180.00	100.00	2.042	**0.150**
	360	**0.58**	**0.42**	360				(p-value) Chisq w 1 df	

Australian general population (n=180)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	45	90	0	45	25.00	42.05	23.36	0.207	
ID	84	84	84	84	46.67	89.90	49.94	0.387	
DD	51	0	102	51	28.33	48.05	26.69	0.181	
Total	180	174	186	180	100.00	180.00	100.00	0.775	**0.380**
	360	**0.48**	**0.52**	360				(p-value) Chisq w 1 df	

Malay general population (n=99)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	40	80	0	40	40.40	42.68	43.11	0.168	
ID	50	50	50	50	50.51	44.65	45.10	0.642	
DD	9	0	18	9	9.09	11.68	11.79	0.614	
Total	99	130	68	99	100.00	99.00	100.00	1.423	**0.230**
	198	**0.66**	**0.34**	198				(p-value) Chisq w 1 df	

Chinese general population (n=45)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	13	26	0	13	28.89	12.80	28.44	0.003	
ID	22	22	22	22	48.89	22.40	49.78	0.007	
DD	10	0	20	10	22.22	9.80	21.78	0.004	
Total	45	48	42	45	100.00	45.00	100.00	0.014	**0.900**
	90	**0.53**	**0.47**	90				(p-value) Chisq w 1 df	

Indian general population (n=13)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	1	2	0	1	7.69	2.77	21.30	1.130	
ID	10	10	10	10	76.92	6.46	49.70	1.938	
DD	2	0	4	2	15.38	3.77	28.99	0.830	
Total	13	12	14	13	100.00	13.00	100.00	3.899	**0.050**
	26	**0.46**	**0.54**	26				(p-value) Chisq w 1 df	

Other Bumiputra general population (n=23)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	2	4	0	2	8.70	3.92	17.06	0.943	
ID	15	15	15	15	65.22	11.15	48.49	1.328	
DD	6	0	12	6	26.09	7.92	34.45	0.467	
Total	23	19	27	23	100.00	23.00	100.00	2.738	**0.100**
	46	**0.41**	**0.59**	46				(p-value) Chisq w 1 df	

Malaysian general population (n=180)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	40	80	0	40	22.22	46.01	25.56	0.784	
RX	102	102	102	102	56.67	89.99	49.99	1.603	
XX	38	0	76	38	21.11	44.01	24.45	0.820	
Total	180	182	178	180	100.00	180.00	100.00	3.207	**0.070**
	360	**0.51**	**0.49**	360				(p-value) Chisq w 1 df	

Australian general population (n=180)

				Observed		HW - Expected			
ACTN3 R/X	n	allele R	allele X	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	44	88	0	44	24.44	50.14	27.85	0.752	
RX	102	102	102	102	56.67	89.72	49.85	1.680	
XX	34	0	68	34	18.89	40.14	22.30	0.939	
Total	180	190	170	180	100.00	180.00	100.00	3.371	**0.070**
	360	**0.53**	**0.47**	360				(p-value) Chisq w 1 df	

Malay general population (n=99)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	23	46	0	23	23.23	25.76	26.02	0.296	
RX	55	55	55	55	55.56	49.48	49.98	0.616	
XX	21	0	42	21	21.21	23.76	24.00	0.321	
Total	99	101	97	99	100.00	99.00	100.00	1.232	**0.270**
	198	**0.51**	**0.49**	198				(p-value) Chisq w 1 df	

Chinese general population (n=45)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	13	26	0	13	28.89	12.80	28.44	0.003	
RX	22	22	22	22	48.89	22.40	49.78	0.007	
XX	10	0	20	10	22.22	9.80	21.78	0.004	
Total	45	48	42	45	100.00	45.00	100.00	0.014	**0.900**
	90	**0.53**	**0.47**	90				(p-value) Chisq w 1 df	

Indian general population (n=13)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	0	0	0	0	0.00	1.56	11.98	1.558	
RX	9	9	9	9	69.23	5.88	45.27	1.649	
XX	4	0	8	4	30.77	5.56	42.75	0.437	
Total	13	9	17	13	100.00	13.00	100.00	3.644	**0.060**
	26	**0.35**	**0.65**	26				(p-value) Chisq w 1 df	

Other Bumiputra general population (n=23)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	4	8	0	4	17.39	6.26	27.22	0.816	
RX	16	16	16	16	69.57	11.48	49.91	1.781	
XX	3	0	6	3	13.04	5.26	22.87	0.972	
Total	23	24	22	23	100.00	23.00	100.00	3.569	**0.060**
	46	**0.52**	**0.48**	46				(p-value) Chisq w 1 df	

Control group in the Malaysian population (n=180)

				Observed		HW - Expected			
ACE I/D	n	allele *I*	allele *D*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
II	56	112	0	56	31.11	60.67	33.70	0.359	
ID	97	97	97	97	53.89	87.66	48.70	0.994	
DD	27	0	54	27	15.00	31.67	17.59	0.688	
Total	180	209	151	180	100.00	180.00	100.00	2.042	**0.150**
	360	**0.58**	**0.42**	360				(p-value) Chisq w 1 df	

Control group in the Malaysian population (n=180)

				Observed		HW - Expected			
ACTN3 R/X	n	allele *R*	allele *X*	Frequency	%	Frequency	%	cell Chi-sq.	p-value
RR	40	80	0	40	22.22	46.01	25.56	0.784	
RX	102	102	102	102	56.67	89.99	49.99	1.603	
XX	38	0	76	38	21.11	44.01	24.45	0.820	
Total	180	182	178	180	100.00	180.00	100.00	3.207	**0.070**
	360	**0.51**	**0.49**	360				(p-value) Chisq w 1 df	

APPENDICES

APPENDIX E

DETAIL INFORMATION OF ATHLETES

Endurance athletes (n=34)

No	Gender	Sports	Age	Height (cm)	Body Weight (kg)	Body Mass Index (kg/m2)	Body Fat (%)	Yo-Yo intermittent recovery level 2 performance (m)	Handgrip Strength (kg)	Leg Strength (kg)
1	Female	Long distance cycling	17	151.0	45.0	19.7	20.1	320.00	27.8	77.3
2	Female	Long distance cycling	18	154.6	45.8	19.2	16.1	480.00	28.6	77.5
3	Female	Long distance cycling	18	166.5	58.0	20.9	18.2	480.00	28.9	82.5
4	Female	Long distance cycling	18	154.0	47.7	20.1	15.7	560.00	26.4	98.5
5	Male	Long distance cycling	19	172.0	58.5	19.8	17.6	640.00	36.0	109.5
6	Male	Long distance cycling	17	172.0	59.1	20.0	14.5	480.00	34.7	114.3
7	Male	Long distance cycling	21	168.0	65.4	23.2	10.8	880.00	45.3	125.5
8	Male	Long distance cycling	18	171.5	65.7	22.3	16.6	400.00	44.6	137.0
9	Male	Long distance cycling	19	172.0	69.3	23.4	18.0	640.00	47.1	138.7
10	Male	Long distance cycling	20	161.0	60.4	23.3	8.0	880.00	45.8	158.0
11	Male	Long distance swimmer > 400 m	23	166.0	58.9	21.4	13.1	240.00	43.4	98.3
12	Male	Long distance swimmer > 400 m	21	168.6	74.7	26.3	22.6	240.00	36.8	116.0
13	Male	Long distance swimmer > 400 m	23	182.0	80.3	24.2	20.9	240.00	47.1	117.3
14	Male	Long distance swimmer > 400 m	23	168.7	55.8	19.6	11.8	400.00	34.2	122.7
15	Male	Long distance swimmer > 400 m	23	171.5	75.1	25.5	17.3	240.00	48.0	151.0
16	Female	Long distance track and field	20	151.5	44.4	19.3	21.8	200.00	22.1	41.2
17	Female	Long distance track and field	19	149.0	38.7	17.4	25.5	120.00	24.8	48.7
18	Female	Long distance track and field	20	154.0	49.2	20.7	24.0	200.00	21.9	51.3
19	Female	Long distance track and field	20	163.0	77.8	29.3	33.9	200.00	33.0	68.5
20	Male	Long distance track and field	19	165.0	62.7	23.0	20.5	440.00	40.8	87.8

21	Male	Long distance track and field	22	169.0	55.8	19.5	11.0	400.00	36.6	92.5
22	Male	Long distance track and field	23	182.1	72.5	21.9	16.5	400.00	46.5	93.5
23	Female	Long distance track and field	22	167.0	62.0	22.2	28.1	200.00	30.9	96.5
24	Male	Long distance track and field	20	168.0	58.3	20.7	24.0	440.00	40.2	98.6
25	Male	Long distance track and field	21	178.0	54.0	17.0	13.8	440.00	42.3	99.0
26	Male	Long distance track and field	20	173.0	61.1	20.4	24.3	440.00	37.7	100.8
27	Male	Long distance track and field	22	180.0	69.9	21.6	19.5	640.00	42.5	105.3
28	Male	Long distance track and field	24	176.5	70.8	22.7	16.4	440.00	48.1	111.8
29	Male	Long distance track and field	20	167.8	56.5	20.0	15.4	880.00	27.8	117.2
30	Male	Long distance track and field	17	173.7	61.1	20.3	12.8	480.00	37.8	122.8
31	Male	Long distance track and field	19	185.0	67.6	19.8	15.7	200.00	25.6	125.3
32	Male	Long distance track and field	19	176.5	96.9	31.1	27.4	480.00	40.2	129.5
33	Male	Long distance track and field	21	165.0	51.6	19.0	10.3	640.00	39.5	131.0
34	Male	Long distance track and field	18	186.7	133.0	38.1	30.9	240.00	70.2	143.3
		Mean ± SD	19.8 ± 1.9	168.5 ± 9.8	63.6 ± 17.0	22.1 ± 4.1	18.6 ± 6.1	429.4 ± 203.8	37.7 ± 9.8	105.6 ± 28.2

Strength/power athletes (n=41)

No	Gender	Sports	Age	Height (cm)	Body Weight (kg)	Body Mass Index (kg/m2)	Body Fat (%)	Yo-Yo intermittent recovery level 2 performance (m)	Handgrip Strength (kg)	Leg Strength (kg)
1	Male	Boxing	20	171.0	57.7	19.7	10.7	440.00	39.1	140.3
2	Male	Boxing	18	165.5	63.6	23.2	13.3	640.00	43.5	150.3
3	Male	Boxing	23	178.4	77.7	24.5	22.8	440.00	44.9	203.8
4	Female	Karate	19	166.0	56.5	20.5	21.9	160.00	30.1	109.8
5	Female	Taekwando	20	150.0	42.5	18.9	20.5	120.00	21.3	33.3
6	Female	Taekwando	20	160.0	57.6	22.5	27.1	160.00	29.1	44.5
7	Female	Taekwando	21	141.5	43.7	21.8	30.3	200.00	23.7	52.5
8	Female	Taekwando	21	165.5	51.2	18.7	24.3	120.00	20.0	54.7
9	Female	Taekwando	20	153.0	61.8	26.4	32.3	160.00	24.4	56.3
10	Female	Taekwando	21	156.5	56.8	23.2	28.0	160.00	25.4	61.7
11	Male	Taekwando	20	161.0	83.9	32.4	38.3	80.00	27.4	77.2
12	Female	Taekwando	19	136.0	68.9	28.3	33.5	200.00	25.5	79.3
13	Female	Taekwando	20	154.5	55.9	23.4	24.2	80.00	21.0	81.5
14	Female	Taekwando	23	156.0	53.1	21.8	22.7	320.00	22.7	83.3
15	Female	Taekwando	20	159.0	52.0	20.6	25.8	120.00	30.5	86.7
16	Male	Taekwando	21	165.5	64.7	23.6	17.8	120.00	31.8	87.3
17	Female	Taekwando	20	166.3	63.5	22.9	18.2	200.00	35.4	89.8
18	Male	Taekwando	20	171.6	58.1	19.8	11.1	320.00	38.6	91.5
19	Male	Taekwando	23	178.4	56.1	17.6	12.4	240.00	30.5	94.0
20	Male	Taekwando	23	169.5	71.5	24.9	18.7	120.00	40.5	96.8
21	Male	Taekwando	20	166.5	60.7	21.9	19.0	160.00	34.9	96.8
22	Male	Taekwando	20	173.5	73.3	24.3	15.9	280.00	47.2	104.2
23	Male	Taekwando	21	172.5	66.6	22.4	16.1	240.00	40.3	115.0
24	Male	Taekwando	20	170.0	62.1	21.5	12.1	360.00	36.6	124.0
25	Male	Taekwando	21	174.5	66.1	21.7	14.4	320.00	39.7	127.0
26	Male	Taekwando	18	168.5	75.2	26.5	19.4	280.00	48.0	131.7
27	Male	Taekwando	20	170.0	59.8	20.7	17.6	200.00	41.5	137.5
28	Male	Taekwando	20	168.5	64.4	22.7	15.8	320.00	35.4	143.5
29	Male	Taekwando	23	189.5	80.4	22.4	17.4	240.00	57.1	168.0
30	Female	Weight lifting	17	150.5	55.2	24.4	25.7	520.00	26.6	60.3

31	Female	Weight lifting	17	150.4	51.7	22.9	23.6	274.00	24.0	80.8
32	Female	Weight lifting	17	159.4	60.9	23.9	23.6	480.00	33.6	85.4
33	Female	Weight lifting	17	147.4	64.1	29.5	28.9	200.00	29.1	92.5
34	Male	Weight lifting	17	156.2	54.4	22.3	15.0	560.00	48.1	106.9
35	Male	Weight lifting	17	165.2	70.4	25.8	11.6	760.00	47.7	140.5
36	Male	Weight lifting	17	169.2	57.0	19.9	7.8	320.00	39.4	143.5
37	Male	Weight lifting	17	168.4	64.1	22.6	11.2	800.00	40.5	152.8
38	Male	Weight lifting	21	170.0	70.6	24.4	23.0	560.00	44.4	166.0
39	Male	Weight lifting	17	168.2	68.6	24.2	10.3	640.00	51.4	168.3
40	Male	Weight lifting	17	165.5	77.1	28.1	14.9	640.00	50.5	172.5
41	Male	Weight lifting	18	160.8	64.9	25.1	7.8	600.00	56.9	174.5
		Mean ± SD	19.7 ± 1.8	163.7 ± 10.4	62.5 ± 9.2	23.2 ± 3.0	19.6 ± 7.3	320.8 ± 197.5	36.1 ± 10.2	108.9 ± 41.2

Intermittent athletes (n=105)

No	Gender	Sports	Age	Height (cm)	Body Weight (kg)	Body Mass Index (kg/m2)	Body Fat (%)	Yo-Yo intermittent recovery level 2 performance (m)	Handgrip Strength (kg)	Leg Strength (kg)
1	Male	Badminton	24	167.5	55.8	19.9	23.8	120.00	32.1	77.7
2	Male	Badminton	23	166.0	61.3	22.2	18.2	280.00	48.6	82.5
3	Male	Badminton	24	157.2	55.6	22.5	11.0	200.00	41.3	83.0
4	Male	Badminton	23	176.0	67.8	21.9	17.6	280.00	35.5	97.0
5	Male	Badminton	20	182.5	72.3	21.7	13.6	280.00	50.5	118.3
6	Male	Badminton	20	167.5	65.5	23.3	14.3	280.00	48.3	136.7
7	Male	Basketball	22	169.5	63.9	22.2	14.1	480.00	39.4	63.2
8	Male	Basketball	22	169.0	74.4	26.0	17.1	320.00	23.9	65.2
9	Male	Basketball	22	165.2	67.5	24.8	19.9	320.00	32.4	80.3
10	Male	Basketball	23	176.6	63.5	20.4	18.4	160.00	47.2	123.5
11	Male	Basketball	23	179.1	69.6	21.7	22.5	120.00	33.7	129.2
12	Male	Cricket	21	170.5	82.1	28.2	23.7	160.00	47.9	107.0
13	Male	Cricket	21	162.6	56.5	21.4	16.8	440.00	44.7	116.7
14	Male	Cricket	20	152.2	59.9	25.9	19.7	640.00	41.3	122.7
15	Male	Cricket	19	178.1	72.0	22.7	16.3	440.00	47.5	130.5
16	Male	Cricket	21	167.5	80.3	28.6	23.3	240.00	52.2	135.0
17	Male	Cricket	22	177.6	71.4	22.7	18.0	160.00	46.4	138.8
18	Male	Cricket	20	165.5	66.5	24.3	14.1	440.00	55.7	151.0
19	Male	Cricket	19	172.1	71.9	24.3	17.0	440.00	53.4	154.5
20	Male	Football	19	166.0	68.3	24.8	13.6	482.00	23.6	43.5
21	Male	Football	20	149.0	44.3	20.0	11.8	480.00	26.1	68.5

22	Male	Football	21	165.0	55.4	20.3	15.8	600.00	32.6	80.7
23	Male	Football	25	169.3	56.3	19.7	16.7	240.00	31.2	84.0
24	Male	Football	19	167.0	59.4	21.3	8.9	640.00	36.5	85.4
25	Male	Football	19	168.0	64.8	23.0	13.1	400.00	39.8	86.2
26	Male	Football	20	180.0	69.8	21.5	17.9	440.00	37.0	88.3
27	Male	Football	19	171.5	64.5	21.9	13.7	560.00	31.9	91.8
28	Male	Football	21	171.5	69.8	23.7	19.6	440.00	39.2	92.3
29	Male	Football	20	183.5	70.9	21.1	9.8	480.00	46.6	94.0
30	Male	Football	22	172.5	98.1	33.0	30.4	240.00	40.8	96.0
31	Male	Football	21	179.0	70.4	22.0	15.7	240.00	30.3	104.7
32	Male	Football	20	167.5	70.7	25.2	19.7	280.00	39.2	111.8
33	Male	Football	24	170.0	57.3	19.8	13.1	440.00	37.0	115.3
34	Male	Football	24	177.5	70.3	22.3	14.0	640.00	36.3	115.7
35	Male	Football	21	172.0	70.6	23.9	16.4	240.00	41.3	117.0
36	Male	Football	20	172.5	60.3	20.3	9.4	200.00	41.7	120.3
37	Male	Football	21	169.5	64.4	22.4	14.8	280.00	38.7	121.8
38	Male	Football	25	164.0	66.5	24.7	17.7	440.00	43.8	122.5
39	Male	Football	21	170.5	71.2	24.5	18.9	440.00	47.1	124.5
40	Male	Football	21	182.0	71.4	21.6	14.4	320.00	38.5	125.3
41	Male	Football	19	168.0	72.2	25.6	18.6	440.00	46.7	127.8
42	Male	Football	22	172.5	71.4	24.0	18.9	640.00	36.7	130.2
43	Male	Football	20	172.5	63.7	21.4	16.7	440.00	46.0	130.8
44	Male	Football	22	170.0	67.9	23.5	21.7	280.00	36.1	135.7
45	Male	Football	24	161.0	56.0	22.7	15.9	640.00	47.7	145.7
46	Male	Football	19	168.5	65.4	23.0	13.5	640.00	46.5	153.0

47	Male	Football	19	174.0	80.1	26.5	22.2	640.00	50.1	160.0
48	Male	Football	21	172.5	72.9	24.5	19.4	400.00	41.8	163.7
49	Male	Futsal	22	177.0	66.5	21.2	13.2	400.00	30.1	58.0
50	Male	Futsal	23	159.5	85.7	33.7	27.9	160.00	22.9	67.5
51	Male	Futsal	20	164.5	51.4	19.0	5.8	400.00	26.1	78.5
52	Male	Futsal	20	177.5	70.6	22.4	16.2	301.00	31.2	84.8
53	Male	Futsal	21	160.5	76.7	29.8	26.8	280.00	22.4	89.0
54	Male	Futsal	23	174.0	62.4	20.6	16.7	240.00	34.1	90.8
55	Male	Futsal	20	172.5	68.1	22.9	14.6	360.00	36.4	99.7
56	Male	Futsal	21	172.5	61.1	20.5	11.9	400.00	26.0	100.7
57	Male	Futsal	24	179.5	64.3	20.0	17.0	560.00	25.8	104.5
58	Male	Futsal	23	167.5	65.0	23.2	18.2	560.00	33.5	105.7
59	Male	Futsal	23	170.0	63.4	21.9	12.9	520.00	37.3	106.2
60	Male	Futsal	20	176.5	68.5	22.0	13.3	440.00	46.4	130.8
61	Male	Futsal	23	175.0	77.2	25.2	23.0	320.00	42.6	133.5
62	Male	Futsal	26	185.5	87.9	25.5	22.6	120.00	45.6	151.5
63	Male	Hockey	21	174.0	64.9	21.4	15.2	360.00	36.5	46.5
64	Female	Hockey	19	161.0	63.9	24.7	29.2	80.00	31.5	71.7
65	Female	Hockey	19	156.5	55.9	22.8	29.4	80.00	48.1	72.0
66	Female	Hockey	19	162.0	108.6	41.4	42.1	80.00	36.7	81.2
67	Male	Hockey	18	168.5	62.4	22.0	17.9	320.00	28.6	91.0
68	Male	Hockey	22	163.0	52.6	19.8	9.9	400.00	44.5	107.5
69	Male	Hockey	20	180.0	78.7	24.3	20.4	320.00	53.7	110.3
70	Male	Hockey	20	184.0	72.5	21.4	19.3	240.00	32.9	113.0
71	Male	Hockey	20	174.0	49.1	16.2	11.5	320.00	52.7	157.2

72	Male	Rugby	22	164.5	85.7	31.7	25.6	200.00	43.7	94.7
73	Male	Rugby	20	145.5	62.9	29.7	17.5	280.00	42.4	104.8
74	Male	Rugby	21	169.5	60.5	21.1	13.2	400.00	42.7	114.7
75	Male	Rugby	21	178.5	78.3	24.6	17.4	240.00	58.5	121.5
76	Male	Rugby	25	171.0	91.0	31.1	30.0	200.00	47.5	137.7
77	Male	Rugby	21	177.0	102.8	32.8	33.2	120.00	46.4	142.7
78	Male	Rugby	20	165.0	66.5	24.4	13.8	640.00	48.6	143.3
79	Male	Rugby	22	169.5	103.6	36.1	28.6	200.00	46.1	143.7
80	Male	Rugby	20	173.0	75.9	25.4	18.5	240.00	59.6	146.8
81	Male	Rugby	22	172.5	124.8	41.9	36.2	120.00	47.8	171.5
82	Male	Rugby	22	166.0	83.7	30.4	26.4	240.00	47.0	222.2
83	Female	Sepak takraw	21	160.5	57.8	22.4	24.0	200.00	26.7	36.8
84	Female	Sepak takraw	19	154.0	46.9	19.8	20.9	200.00	25.4	45.7
85	Male	Sepak takraw	18	160.0	53.4	20.9	6.5	440.00	36.8	74.5
86	Male	Sepak takraw	18	178.0	95.7	30.2	22.6	280.00	54.2	79.8
87	Male	Sepak takraw	18	170.5	64.3	22.1	15.1	440.00	35.6	82.7
88	Male	Sepak takraw	19	166.0	57.7	20.9	14.3	240.00	24.9	92.7
89	Male	Sepak takraw	18	172.0	67.7	22.9	22.8	360.00	35.2	98.0
90	Male	Sepak takraw	22	170.0	67.5	23.4	12.4	640.00	52.6	102.7
91	Male	Sepak takraw	21	174.0	76.1	25.1	20.6	240.00	37.5	112.7
92	Male	Sepak takraw	20	170.0	57.5	19.9	11.6	480.00	44.9	113.0
93	Male	Sepak takraw	18	168.0	70.5	25.0	22.1	280.00	41.7	113.2
94	Male	Sepak takraw	23	170.5	55.1	19.0	18.5	240.00	35.0	117.5
95	Male	Sepak takraw	19	175.5	78.6	25.5	17.7	240.00	39.1	119.5
96	Female	Squash	22	161.5	47.5	18.2	25.3	482.00	22.7	64.5

97	Male	Volleyball	20	174.0	67.9	22.4	13.4	320.00	49.2	126.0
98	Male	Volleyball	22	180.0	83.6	25.8	23.1	240.00	49.2	126.7
99	Male	Volleyball	20	185.5	81.5	23.7	16.7	200.00	44.4	137.0
100	Male	Volleyball	21	189.0	93.0	26.0	18.8	320.00	44.1	145.3
101	Male	Volleyball	20	170.0	73.6	25.5	19.5	320.00	41.0	153.0
102	Male	Volleyball	22	180.0	95.7	29.5	23.8	200.00	54.9	162.7
103	Male	Volleyball	23	181.5	73.7	22.4	17.0	320.00	58.0	176.7
104	Male	Volleyball	25	178.0	78.3	24.7	18.3	240.00	57.2	177.7
105	Male	Volleyball	19	184.5	71.6	21.0	10.6	280.00	59.6	193.0
		Mean ± SD	21.0 ± 1.8	170.8 ± 7.9	70.0 ± 13.4	24.0 ± 4.3	18.7 ± 6.4	340.8 ± 148.7	40.8 ± 9.2	112.3 ± 33.5

LIST OF THESIS RELEVANT PUBLICATIONS AND CONFERENCE PRESENTATIONS

ARTICLE

Ahmad Yusof H, Singh R, Zainuddin Z, Rooney K. & Che Muhammed A. (2015) The angiotensin I-converting enzyme I/D gene polymorphism in well-trained Malaysian athletes. *Sport Sciences for Health.* 11**,** 187-193.

Dr. Ahmad Munir Che Muhamed, **Hazwani Yusof,** Prof. Rabindarjeet Singh dan Prof. Madya Dr. Zafarina Zainuddin. (2014) Pengaruh Genetik ke atas Prestasi Sukan. *Arena IPT (Edisi Jan-Mac 2014)*. 34-35.

CONFERENCE PRESENTATION (ORAL)

Hazwani Ahmad Yusof, Rabindarjeet Singh, Zafarina Zainuddin, Kieron ROONEY and Ahmad Munir Che Muhamed. (2014) Association of Angiotensin Converting Enzyme (ACE) with physical performance among Malaysian Varsity Athletes. *International Conference of Physical Education and Sports*. Sultan Azlan Shah Campus, Sultan Idris Education University, Perak, Malaysia. October 28-29.

Hazwani Ahmad Yusof, Rabindarjeet Singh, Zafarina Zainuddin, Kieron Rooney and Ahmad Munir Che Muhamed. (2014) The presence of R allele of the Alpha-Actinin-3 (ACTN3) R/X polymorphism among strength and power athletes in Malaysia. *Movement, Health & Exercise (MoHE) Conference*. Kuantan, Pahang, Malaysia. September 2-3.

CONFERENCE PRESENTATION (POSTER)

Hazwani AY, Singh R, Zafarina Z, & Ahmad Munir CM. (2012) Association of Angiotensin Converting Enzyme (ACE) and Alpha-Actinin-3 (ACTN3) Polymorphisms with physical performance among Malaysian Varsity Athletes. *10th Asia Pacific Conference on Human Genetics.* Crown Plaza Hotel Kuala Lumpur, Malaysia. December 5-8.