GENETIC ASSOCIATION OF *ACE* I/D AND *ACTN3* R577X POLYMORPHISMS WITH SPORTS PERFORMANCE AMONG MALAY MALE SECONDARY SCHOOL STUDENTS

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by

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TABLE OF CONTENTS

Acknowledgements		ii
List of contents		iv
List of appendices		xi
List of tables		xiii
List figures		xvi
List of abbreviations		xix
Abstrak		xxi
Abstract		xxiii
CHAPTER 1 : INTRODUCTION		
1.1 Introduction		1
1.2 Literature Review		3
1.2.1 *α-actinin 3 (ACTN3)* gene		3
1.2.1.(a)	α-actinins in skeletal muscle	3
1.2.1.(b)	Studies in *ACTN3* knock-out mouse and deficiency of α-actinin-3 on muscle performance	7
1.2.1.(c)	The *ACTN3* gene	8
1.2.1.(d)	The *ACTN3* R577X Polymorphism (rs1815739)	10
1.2.1.(e)	Association of *ACTN3* gene and sport of performance	12
1.2.1.(f)	The *ACTN3* gene and indicator of performance	14
1.2.1.(g)	Adaptations to training	15

1.2.2 *Angiotensin Converting Enzyme (ACE)* gene 17

1.2.2.(a) The Angiotensin Converting Enzyme (*ACE*) gene 17

1.2.2.(b) The RAAS (renin–angiotensin-aldosteron system) and blood pressure regulation 19

1.2.2.(c) The *ACE* gene polymorphism 22

1.2.2.(d) The *ACE* gene and maximum oxygen uptake (VO_{2max}) 24

1.2.2.(e) Association of *ACE* gene and endurance performance 26

1.2.2.(f) Association of *ACE* gene and strength or power performance 28

1.2.2.(g) The *ACE* I/D polymorphism and potential physiological mechanisms involved 28

1.3 Justification 30

1.4 Aims of the study 31

1.4.1 General objective 31

1.4.2 Specific objectives 31

CHAPTER 2 : MATERIALS AND METHODS

2.1 Study design, ethics approval and flowchart of the study 32

2.2 Sample size calculation 35

2.3 Subject Recruitment 37

2.3.1 Consent taking 39

2.3.2 International physical Activity Questionnaire (IPAQ) 39

2.3.3 Structured questionnaire 39

2.4 Plasticware 40

2.5 Instruments 40

2.5.1 Pipettes 40

2.5.2 Centrifuge 40

2.5.3 NanoQuant Infinite M200 40

2.5.4 Waterbath 41

2.5.5 SuperCycler Thermal Cycler SC300T 41

2.6 Physical paramenters 41

2.6.1 Mass 41

2.6.2 Height 41

2.6.3 Body Mass Index 42

2.6.4 Blood pressure 42

2.7 Physical fitness tests 42

2.7.1 20 m Shuttle Run Test 42

2.7.2 30 m Sprint Test 45

2.8 Materials for Polymorphisms Analyses 47

2.8.1 Specimen collection (saliva) 47

2.8.2 prepIT^{TM}.C2D Genomic DNA MiniPrep Kit 47

2.8.3 Polymerase Chain Reaction (PCR) Master Mix 48

2.8.4 *Dde1* restriction enzyme 48

2.8.5 Loading Buffer 49

2.8.6 DNA marker/ Ladder 49

2.8.7 10X TBE and 1X TBE buffer solution 49

2.8.8 Staining material 50

2.9 Methods for DNA Analysis 50

2.9.1 Genomic DNA extraction from saliva sample 50

2.9.2 Determination of DNA concentration 51

2.9.3 Agarose gel electrophoresis 51

2.9.4 Detection of *ACTN3* R577X Polymorphisms 53

2.9.4.(a) *ACTN3* R577X polymorphism genotyping 53

2.9.4.(b) Restriction enzyme digestion of *ACTN3* gene polymorphism 54

2.9.5 Detection of *ACE* I/D Polymorphism 60

2.9.5.(a) *ACE* I/D polymorphism genotyping 60

2.9.6 Direct DNA Sequencing 65

2.10 Data analysis 66

CHAPTER 3 : RESULTS

3.1 Demography characteristics of the subjects 67

3.2 Results of the physical parameters 68

3.3 Results of the physical fitness test 70

3.3.1 30 m sprint test 70

3.3.2 20 m shuttle run test 71

3.4 Results of the genetic polymorphism analysis 72

3.4.1 Isolation and amplification of DNA from saliva 72

3.4.2 Genotyping *ACTN3* R577X Polymorpshisms 74

3.4.2.(a) Visualisation of PCR amplification and digested PCR product of *ACTN3* R577X polymorphism 74

3.4.2.(b) Validation of *ACTN3* genotype: Sequencing result 77

3.4.3 Genotyping *ACE* I/D Polymorphisms 81

3.4.3.(a) Visualisation of PCR product of *ACE* I/D polymorphisms 81

3.4.3.(b) Validation of *ACE* genotype: Sequencing reuslt 83

3.4.4 Genotype and allele frequencies of *ACTN3* R577X and *ACE* I/D polymorphisms 88

3.4.4.(a) Genotype and allele frequencies comparisons of *ACTN3* R577X polymorphism in sprint-type subjects, endurance-type subjects and sedentary subjects 88

3.4.4.(b) Genotypes and alleles frequencies comparisons of *ACE* I/D polymorphism in sprint-type subjects, endurance-type subjects, and sedentary subjects 93

3.4.5 Association between genotypes and physical parameters 98

3.4.5.(a) Association between *ACTN3* R577X polymorphism and physical parameters 98

3.4.5.(b) Association between *ACE* I/D polymorphism and physical parameters 102

3.4.6 Association between genotypes and type of sports 106

performance

3.4.6.(a) Association between *ACTN3* R577X polymorphism and sports performance 106

3.4.6.(b) Association between *ACE* I/D polymorphism and sports performance 110

3.4.7 Association of combination of two genotypes with sports performance**.** 114

3.4.7 (a) Association of *ACE* I/D and *ACTN3* R577X combination genotypes with s ports performance between sprint-type subjects and sedentary subjects 114

3.4.7.(b) Association of *ACE* I/D and *ACTN3* R577X combination genotypes with sports performance between endurance-type subjects and sedentary subjects 116

CHAPTER 4 : DISCUSSION

4.1 Demographic features 118

4.2 Physical parameter 119

4.3 Genotyping techniques 120

4.4 Genotype frequencies of the polymorphisms 121

4.4.1 Genotype and allele frequencies of *ACTN3* gene 121

4.4.2 Genotype and allele frequencies of *ACE* gene 124

4.5 *ACTN3* R577X and *ACE* I/D polymorphisms and their 127

association with physical parameters

4.5.1 Association between *ACTN3* polymorphism and physical parameters 127

4.5.2 Association between *ACE* polymorphism and physical parameters 129

4.6 *ACTN3* R577X and *ACE* I/D polymorphisms and their association with sports performance 131

4.6.1 Association between *ACTN3* polymorphism and sports performance 131

4.6.2 Association between *ACE* I/D polymorphism and sports performance 134

CHAPTER 5 : SUMMARY AND CONCLUSIONS

5.1 The Findings of the Present Study 138

5.2 Conclusions 138

5.3 Limitations of Study 139

5.4 Future Implications 139

REFERENCES 141

APPENDICES

LIST OF PUBLICATIONS AND PRESENTATIONS

LIST OF APPENDICES

Appendix A USM ethical approval (A study on the association among *ACTN3* R577X polymorphism, sprint achievement and academic performance in Malay male school children)

Appendix B USM ethical approval (Influence of *ACE* I/D polymorphism on the performance of Malay male school children in endurance type sport)

Appendix C Surat kelulusan untuk menjalan kajian di sekolah dari bahagian perancangan dan penyelidikan dasar pendididkan, kementerian pelajaran malaysia (A study on the association among *ACTN3* R577X polymorphism, sprint achievement and academic performance in Malay male school children)

Appendix D Surat kelulusan untuk menjalan kajian di sekolah dari bahagian perancangan dan penyelidikan dasar pendididkan, kementerian pelajaran malaysia (Influence of *ACE* I/D polymorphism on the performance of Malay male school children in endurance type sport)

Appendix E Surat kebenaran menjalankan penyelidikan dari Jabatan Pelajaran Negeri Kelantan (A study on the association among *ACTN3* R577X polymorphism, sprint achievement and academic performance in Malay male school children)

Appendix F Surat kebenaran menjalankan penyelidikan dari Jabatan Pelajaran Negeri Kelantan (Influence of *ACE* I/D polymorphism on the performance of Malay male school children in endurance type sport)

Appendix G Borang maklumat dan keizinan pesakit

Appendix H Borang pemeriksaan kesihatan dan BMI

Appendix I Soal selidik aktiviti fizikal antarabangsa

Appendix J Borang ujian larian ulang-alik 20 meter secara berperingkat

Appendix K Sequence alignment using BLAST showing that the sequence electrophoregram as shown above is part of the human alpha-actinin 3 (*ACTN3*)

Appendix L Sequence alignment using BLAST showing that the sequence identity of the *Alu*-element in I allele as obtained in this study was 96 % in agreement with the I allele of the intron 16 of human *ACE* gene. The box (red colour) indicates the complete sequence of the *Alu* element insertion. The blue box indicates that C and G nucleotides were substituted with G and C nucleotides respectively while the green box showed insertion of G nucleotide, indicating the 4 % differences as compared to reference sequence.

Appendix M Sequence alignment using BLAST showing that the sequence identity of the PCR product with D allele as obtained in this study was 100 % in agreement with the D allele of the intron 16 of human *ACE* gene. The arrow indicates the breakpoint where the *Alu* element would appear in I allele

LIST OF TABLES

		Page
Table 2.1	References for sample calculation	36
Table 2.2	Inclusion and exclusion criterias	38
Table 2.3	Normative data (Heywood, 1998) for male of 20 m Shuttle Run Test (values in ml/kg/min)	44
Table 2.4	Normative data for 16 to 19 year olds of 30 meters sprint test (Davis, 2000)	46
Table 2.5	Primer sequence used for the amplification of *ACTN3* gene (Massidda, *et al*., 2015)	55
Table 2.6	Final concentration and total volume of reagents used in the amplification of *ACTN3* gene	56
Table 2.7	PCR condition for *ACTN3* polymorphism	57
Table 2.8	Reagents for the restriction enzyme digestion of *ACTN3* gene	58
Table 2.9	Primer sequence used for the amplification of *ACE* gene (Sasongko, *et al*., 2005)	62
Table 2.10	Final concentration and total volume of reagents used in the amplification of *ACE* gene	63
Table 2.11	PCR condition for *ACE* I/D polymorphism	64
Table 3.1	Descriptive statistics of the subjects (n = 382)	69
Table 3.2	Descriptive statistic in 30 m sprint test between sprint-type and sedentary subjects	70
Table 3.3	Descriptive statistic in 20 m shuttle run test between endurance-type and sedentary subjects	71
Table 3.4	Genotype and allele frequencies of *ACTN3* R577X	90

	polymorphism in sprint-type subjects, endurance –type subjects and sedentary subjects	
Table 3.5	Comparison of frequencies genotype of the *ACTN3* R577X polymorphism by country	91
Table 3.6	Genotypes and alleles frequencies comparisons of *ACE* I/D polymorphism in sprint-type subjects, endurance-type subjects, and sedentary subjects.	95
Table 3.7	Comparison of frequencies genotype of the *ACE* polymorphism by country	96
Table 3.8	Comparisons of physical parameters according to *ACTN3* R577X genotypes between sprint-type subjects and sedentary subjects	99
Table 3.9	Comparisons of physical parameters according to *ACTN3* R577X genotypes between endurance-type subjects and sedentary subjects	101
Table 3.10	Comparisons of physical parameters according to *ACE* I/D genotypes between sprint-type subjects and sedentary subjects	103
Table 3.11	Comparisons of physical parameters according to *ACE I/D* genotypes between endurance-type subjects and sedentary subjects	105
Table 3.12	Association between *ACTN3* R577X polymorphism and sprint performance	107
Table 3.13	Association between *ACTN3* R577X polymorphism and endurance performance.	109

Table 3.14	Association between *ACE* I/D polymorphism and sprint performance.	111
Table 3.15	Association between *ACE* I/D polymorphism and endurance performance.	113
Table 3.16	Association of *ACE* I/D and *ACTN3* R577X combination genotypes with sports performance between sprint-type subjects and sedentary subjects.	115
Table 3.17	Association of *ACE* I/D and *ACTN3* R577X combination genotypes with sports performance between endurance-type subjects and sedentary subjects.	117

LIST OF FIGURES

		Page
Figure 1.1	Structure of the sarcomeric α–actinins	6
Figure 1.2	The genomic position of *ACTN3* gene in the long arm (q) of chromosome 11 band 13.1. The gene spans 16,407 bases of genomic DNA. *Picture was taken from Yusof (2016)	9
Figure 1.3	Nonsense mutation of *ACTN3* R577X. Figure was modified from http://https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations 11	11
Figure 1.4	Location of the *ACE* gene on the long arm (q) of chromosome 17 on band 23.3. (National Center for Biotechnology Information, 2008)	18
Figure 1.5	Diagram of human renin–angiotensin-aldosteron system (RAAS) (Lubel et al., 2008).	21
Figure 1.6	Insertion or deletion of a 287-bp *Alu* sequence at intron 16 of the *ACE* I/D polymorphism	23
Figure 2.1	Flowchart of the study	34
Figure 2.2	Diagram showing the restriction sites of *Dde1* enzyme in the amplified region of *ACTN3* gene. Different conformation of fragmented products within the gel electrophoresis shows different genotypes of the polymorphism	59
Figure 3.1	Gel figure showing electrophoresis of genomic DNA. Electrophoresis was performed on 0.5% agarose gel for 90 minutes at 70 V. (Lane 1 – 4 represents genomic DNAs.	73
Figure 3.2	Gel electrophoresis figure showing PCR amplicons of	75

ACTN3 gene. Electrophoresis was performed on 2 % agarose gel for 45 minutes at 90 V

Figure 3.3 Gel electrophoresis showing different banding patterns of *ACTN3* R577X polymorphism genotypes where the sequence electropherograms were described in Figures 3.4, 3.5 and 3.6. Electrophoresis was performed on 3 % agarose gel for 60 minutes at 90 V. 76

Figure 3.4 Electropherogram showing the sequence of homozygous major (subject 2) with 577RR (as reflected by a homozygous C allele) genotype with banding pattern as shown in Figure 3.3. Lane 2 (Forward sequence). 78

Figure 3.5 Electropherogram showing the sequence of homozygous minor (subject 3) with 577XX (as reflected by a homozygous T allele) genotype with banding pattern as shown in Figure 3.3. Lane 3 (Reverse sequence). 79

Figure 3.6 Electropherogram showing the sequence of heterozygous (subject 1) with 577RX (as reflected by a CT peak) genotype with banding pattern as shown in Figure 3.3. Lane 1 (Forward sequence). 80

Figure 3.7 Gel electrophoresis figure showing different genotypes of *ACE* I/D polymorphism. Electrophoresis was performed on 2 % agarose gel for 45 minutes at 90 V. 82

.Figure 3.8 Sequence electropherogram showing the sequencing result of the PCR product with DD genotype (Forward sequence). The arrow showed the breakpoint where the I allele positioned. 84

Figure 3.9 Sequence electropherogram showing the sequencing 85

result of the PCR product with II genotype. (Reverse sequence). The blue highlighted part showed the I allele with the complete sequence of the *Alu* element insertion.

Figure 3.10A Sequence electropherogram showing the sequencing result of the PCR product with ID genotype for I allele (Reverse complement sequence) after purification of gel. The arrow showed the breakpoint of the *Alu* insertion. 86

Figure 3.10B Sequence electropherogram showing the sequencing result of the PCR product with ID genotype for D allele (Forward sequence) after purification of gel. The arrow showed the breakpoint where the I allele (*Alu* insertion) should have been positioned. 87

Figure 3.11 Distribution genotypes of *ACTN3* 92

Figure 3.12 Distribution genotypes of *ACE* I/D polymorphism. 97

Figure 4.1 *ACE* I/D alleles distribution map from the West to East Populations. Picture (map) was taken from http://mapssite.blogspot.my/2008/06/world-map template.html. 126

LIST OF ABBREVIATIONS

%	: Percentage
^{o}C	: Degree celcius
α	: Alpha
µl	: Microliter
µg	: Microgram
µM	: Micromolar
etc	: Et cetera
VO_2 max	: Maksimum oxygen update
ACE	: Angiotensin-converting enzyme
ACTN	: Alpha-actinin-3
DNA	: Deoxyribonucleic acid
m	: Meter
kg	: Kilogram
cm	: Centimeter
I	: Insertion
D	: Deletion
CI	: Confidence interval
SD	: Standard deviation
df	: Degrees of freedom
MPQ	: Muscle Power Quality
bp	: Base pair
RAAS	: Renin-angiotensin-aldosteron system
BP	: Blood pressure
AT1	: Angiotensin-type1 receptor

AT2	: Angiotensin-type2 receptor
IPAQ	: International Physical Activity Questionnaire
Min	: Minute
s	: Second
BMI	: Body mass index
PCR	: Polymerase chain reaction
mmHG	: Millimeter mercury
ng	: Nanogram
mM	: Milimolar
ddH_2O	: Deionized distilled water
PCR-RFLP	: Polymerase chain-reaction-Restriction fragment length polymorphism
TBE	: Tris/ Boric acid/ EDTA buffer
UV	: Ultra-violet
dNTPs	: Dinucleotide triphosphates
SYBR Green 1	: SYBR Green 1 Nucleic Acid gel stain
$MgCl_2$	: Magnesium chloride
Taq	: *Thermuphilus aquaticus*
SPSS	: Statistical Packages for the Social Sciences
OR	: Odd ratio
EDTA	: Ethylenediamine tetraacetic acid
ACEi	: Angiotensin Converting Enzyme Inhibitor

PERKAITAN POLIMORFISMA GENETIK *ACE* I/D DAN *ACTN3* R577X DENGAN PRESTASI SUKAN DALAM KALANGAN PELAJAR LELAKI MELAYU SEKOLAH MENENGAH

ABSTRAK

Prestasi sukan dipengaruhi oleh interaksi antara faktor alam sekitar dan poligenik (*polygenic*) dan mencirikan kepelbagaian antara individu. Dianggarkan bahawa perbezaan lebih kurang 66 % dalam kemampuan sukan dipengaruhi oleh faktor keturunan, sementara 34 % perbezaan adalah disebabkan faktor persekitaran. Oleh itu, penerokaan variasi genetik individu terhadap prestasi sukan telah menjadi tumpuan penyelidikan. Tujuan kajian ini adalah untuk menentukan hubungan antara polimorfisma *ACTN3* R577X dan *ACE* I/D terhadap prestasi sukan dalam pelajar lelaki Melayu sekolah menengah. Kajian ini dibahagikan kepada 2 fasa. Dalam fasa 1, aktiviti fizikal dalam kalangan peserta adalah berdasarkan cadangan guru dan International Physical Activity Questionnaire (IPAQ). Subjek tidak aktif dipilih apabila skor IPAQ kurang daripada 600 MET-min per minggu, sementara subjek jenis pecut dan ketahanan dipilih apabila skor IPAQ melebihi 600 MET-min per minggu. Ujian kecergasan fizikal (ujian pecut 30 m dan ujian ulang alik 20 m) telah dijalankan untuk mengesahkan status pecut, ketahanan dan tidak aktif di kalangan peserta. Dalam fasa 2, genotip dilakukan untuk polimorfisma *ACTN3* R577X dan *ACE* I/D dengan menggunakan kaedah PCR dan RFLP. Perbezaan frequensi genotip dan alel untuk gen *ACTN3* dan *ACE* antara peserta jenis pecut, peserta jenis ketahanan dan peserta jenis tidak aktif dianalisis dengan menggunakan ujian *one-way ANOVA*. Perkaitan antara genotip *ACTN3* R577X dan *ACE* I/D, secara tunggal dan gabungan kedua-dua genotip,

dan prestasi fizikal telah dianalisis dengan menggunakan regresi logistik bersyarat dan nisbah odds (OR) dan 95 % Ci (*confidence interval*). Sebanyak 382 peserta lelaki Melayu berusia 16-17 tahun terlibat dalam kajian ini (n = 120 untuk peserta jenis pecut, n = 131 untuk peserta jenis ketahanan dan n = 131 untuk peserta jenis tidak aktif). Tiada perbezaan signifikan untuk frequensi genotip dan alel *ACTN3* R577X di kalangan peserta jenis pecut, peserta jenis ketahanan dan peserta jenis tidak aktif. Terdapat perbezaan yang signifikan diperhatikan untuk frequensi genotip DD untuk gen *ACE* di kalangan peserta jenis pecut, peserta jenis ketahanan dan peserta jenis tidak aktif. Terdapat perkaitan yang penting diperhatikan antara genotip II (OR: 5.277, 95 % CI: 1.845 - 15.090, $p = 0.002$), ID genotip (OR: 3.312, 95 % CI: 1.165 - 9.423, $p = 0.025$) dan genotip ID (OR: 4.144, 95 % CI: 1.503 - 11.425, $p = 0.006$) dan prestasi pecut. Adalah dicadangkan bahawa peserta yang ada I alel untuk *ACE* gen lebih cenderung dikaitkan dengan prestasi pecut. Walaubagaimanapun, tiada perkaitan signifikan diperhatikan antara genotip *ACTN3* R577X dan prestasi sukan. Data dari kajian ini boleh digunakan untuk memberi gambaran yang mendalam bagi memahami perubahan fisiologi kepada prestasi sukan. Walau bagaimanapun, lebih banyak kerja diperlukan untuk menerokai penemuan ini menggunakan sampel yang lebih besar dari negeri-negeri yang berlainan di Malaysia dan menganalisis lebih banyak polimorfisma untuk lebih memahami hubungan antara polimorfisma dan prestasi sukan.

GENETIC ASSOCIATION OF *ACE* I/D AND *ACTN3* R577X POLYMORPHISMS WITH SPORTS PERFORMANCE AMONG MALAY MALE SECONDARY SCHOOL STUDENTS

ABSTRACT

Sports performance is influenced by the interaction of environmental and *polygenic* factors, characterising inter-individual variability. Sixty six percentage of the differences in athletic ability is influenced by hereditary factors, while the 34 % differences are due to individual environmental factors. Therefore, exploration of the individual genetic variation on the sports performance has been an area of research interest. The aim of this study was to determine the association between *ACTN3* R577X and *ACE* I/D polymorphisms with sports performance in Malay male secondary school students. This study was divided into 2 phases. In phase 1, physical activity of the participants were recorded based on teacher's recommendation and the International Physical Activity Questionnaire (IPAQ). Sedentary subjects were recruited when their IPAQ scores were less than 600 MET-min per week while sprint-type and endurance-type subjects were recruited when their scores were more than 600 MET-min per week. Physical fitness tests (30 (meter) m sprint test and 20 m shuttle run test) were conducted to confirm subject's sprint, endurance and sedentary status. In phase 2, genotyping was performed for *ACTN3* R577X and *ACE* I/D polymorphisms by using PCR and RFLP methods. Differences in the frequencies of genotypes and alleles of the *ACTN3* and *ACE* genes between sprint, endurance and sedentary subjects were analysed using one-way ANOVA test. The associations between the *ACTN3* R577X and *ACE* I/D genotype, singly and in combination were

analysed using the conditional logistic regression by deriving odds ratio (OR) and 95 % (confidence interval) CIs. There were 382 Malay males aged 16-17 years recruited as participants in this study (n = 120 for sprint-type subjects, n = 131 for endurance-type subjects and n = 131 for sedentary subjects). No significant difference in the frequencies of *ACTN3* R577X genotypes and alleles was found among sprint-type subjects, endurance-type subjects and sedentary subjects. Significant difference was observed for the frequencies of DD genotype of *ACE* gene among sprint-type subjects, endurance-type subjects and sedentary subjects. Significant association was observed between II genotype (OR: 5.277, 95 % CI: 1.845 – 15.090, p = 0.002), ID genotype (OR: 3.312, 95 % CI: 1.165 – 9.423, p = 0.025) as well as combination of II and ID genotypes (OR: 4.144, 95 % CI: 1.503 – 11.425, p = 0.006) and sprint performance. It is suggested that subjects with I allele of *ACE* gene is more likely to be associated with sprint performance. However, no significant association was observed between *ACTN3* R577X genotypes and sports performance. The data from this study can provide further insights into understanding the physiological changes to sports performance. However, more work is needed to explore these findings using larger samples from different states of Malaysia and analyses on a greater number of polymorphisms for better understanding of the relationship between the polymorphisms and sports performance.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Heritability is the degree of difference in a phenotype between individuals in a population owing to individual's genetic variability (Visscher *et al*., 2008). Currently, science is able to quantify the significance of heredity in relation to the effects of genetic on athletic ability (Collins, 2009). Athletic performance is a result of the combined action of a number of variables including external variables (training, environmental and nutritional) and internal variables (such as hormones, metabolic rate and pulmonary function) (An *et al*., 2000; Hong *et al*., 2001; An *et al*., 2001). It is estimated that approximately 66 % of the differences in athletic ability is under the influence of heredity factors, while the remaining 34 % differences are due to individual environmental factors (De Moor *et al*., 2007). It is interesting to know the effect of different genetic variants on performance.

A heritability of near to 0 % indicates that genetic factors do not have any impact on differences in the phenotype and was primarily due to the variations in the environmental factors. A heritability near to 100 % indicated that the phenotype variations mainly depends on the genetic factors (Anderson, 2011). Bouchard *et al.* (1998) demonstrated a significant association between responsiveness of maximal oxygen uptake (VO_2 max) and trainability in sedentary individuals where 50 % is attributed to the heritability factors. Besides that, other traits like cardiac output, muscle fiber type composition and muscle strength showed 42-46 %, 40-50 %, and 67 % genetic contribution respectively (MacArthur *et al*., 2005).

Currently more than 200 have shown some association with athletic performance (both endurance and power) and about 20 polymorphisms were correlated with elite athletes (Bray *et al*., 2009). Among genetic factors that have been associated with athletic performance are *ACE* I/D and *ACTN3* R577X polymorphisms. Both genes were selected from the Human Gene Map for Physical Performance Phenotypes (Rankinen *et al*., 2004). Both genes were selected for this study as they yield contrasting effects in the human body where *ACE* I/D polymorphism is linked to the endurance performance, whereas *ACTN3* R577X polymorphism is linked to the power performance. Besides that, the genes selected were widely investigated by worldwide scientists for its association with athletic ability (Yang *et al.*, 2017; Li *et al.*, 2017; Wang *et al*., 2012, Kim *et al*., 2015) and the genotyping methodologies are also well established. However, there have been inconsistent outcomes on the association between both polymorphisms and athletic performance (Ma *et al*., 2013).

The objective of this study is to determine the association between sports performance and gene variants: *ACE* I/D and *ACTN3* R577X which are correlated with endurance and power athletic status in Malay male adolescent population of physically-active students compared to sedentary school students. In schools, the choice of sport mainly depends on the interest of the students. Depending on the progress of their training, some students continue to develop their sport skills and make achievements; others leave it as their hobby while the rest do not even like to exercise. Questions remain on why achievements varied, as far as environmetal factors concerned. Very few attentions have been put on the genetic make-up in making choice of what type of sports may best suit the talent of a student. Genetic make-up may help to determine the type of sport a student can best progress.

In the past, few studies have been carried out on elite athletes to investigate performance gene frequencies; but this only show a small portion from the whole population (Yang *et al*., 2007; Ahmetov II *et al*., 2010; Kikuchi *et al*., 2014; Orysiak *et al*., 2014). Hopefully my current study will encourage more research to explore this aspect of sports performance genetics.

Previous studies suggested that *ACTN3* R577X and *ACE* I/D polymorphisms may have some effect on sprint-type achievements and endurance-type among elite athletes. However, its specific influence on the sprint-type and endurance-type achievements among school children, which can be used to recommend specific type of sports, has yet to be explored.

1.2 LITERATURE REVIEW

1.2.1 α-actinin-3 (*ACTN3) g*ene

1.2.1.(a) α -actinins in skeletal muscle

The main function of cytoskeleton is to maintain the shape and mechanical stability besides the provision of intracellular transport (Sjoblom *et al*., 2008). It is composed of different protein structures: polymers actin, tubulin or intermediate filament proteins (Sjoblom *et al*., 2008). In skeletal muscle, actin is a protein that belongs to the contractile apparatus (Sjoblom *et al*., 2008). Proteins that have binding sites with actin are important for the formation and customized functioning of the cytoskeleton organization (Sjoblom *et al*., 2008). In mammals, there are four genes (*ACTN1, 2, 3, 4*) that encode these proteins (Beggs *et al*., 1992). Each of these genes has a specific expression pattern in different tissues (Beggs *et al*., 1992; Honda *et al*., 1998).

Myofibrils consist of long tubes structure of muscle fiber called filaments. Each of muscle fiber is formed by filaments. Filaments are classified into two types:

actin which is a thin filament whereas myosin is a thick filament. Both filaments formed a parallel structure in the sarcomere alpha-actinin and slide each other during muscle contraction. Alpha-actinins are also known as actin-binding protein which provides stabilization of actin filaments. Alpha-actinin is classified into two categories: *ACTN2* and *ACTN3* are only found in skeletal muscle, whereas *ACTN1* and *ACTN4* are found in non-skeletal muscle (Blanchard *et al*., 1989).

The skeletal muscle isoforms are called sarcomere alpha-actinin (Figure 1.1) which is the smallest muscle structure and are the key structural components of the contractile apparatus, and are situated on the line Z-sarcomeric (Squire, 1997). *ACTN2* is found in all types of skeletal and cardiac muscle fiber while secretion of *ACTN3* is restricted to the muscle fibers to fast-twitch and less expression in the brain (Mills *et al*., 1992). Although there was different pattern of expression between *ACTN2* and *ACTN3*, they have 91 % similarity in amino acid sequences (Beggs *et al*., 1992).

The z-lines, found at the alpha-actinin, are key components because they bind to actin filaments of adjacent sarcomeres to form a stable structure of muscle tissue during contraction (MacArthur and North, 2004). Due to the structural organisation of the sarcomeres, alpha-sarcomere actinin also function to coordinate and maintain the order of the contractile process as well as determination of fiber type specification (MacArthur and North, 2004; Semsarian *et al*., 1999). Such diversity of function has been attributed to the large multivalent platform of the α-actinins, in particular isoform 3, for protein-protein interactions (Djinovic-Carugo *et al*., 2002).

Besides the above characteristics, the sarcomeric alpha-actinin is also involved in the metabolic pathway. For example, glycogen phosphorylase (Chowrashi *et al*., 2002), fructose-1,6-bisphosphatase (Gizak *et al*., 2003), and the calsarcins that bind to the calcineurin – which regulate the metabolism, size and muscle fiber type

specification (Djinovic-Carugo *et al*., 2002). *ACTN3* encodes alpha-actinin. Deficiency of alpha-actinin 3 is associated with low level of glycogen phosphorylase activity in muscle. The capacity of glycogen phosphorylase to hydrolyse glycogen to glucose would be affected, leading to disadvantage among sprint athletes as glucose is a very important substrate to produce fast energy for contraction of muscles. Hence, this shift to muscle metabolism towards aerobic metabolism which is advantageous to endurance athletes (Berman & North *et al*., 2010; Lee *et al*., 2016). Similiarly, sarcomeric alpha-actinin binds to the fructose 1, 6 bisphosphate which induce low levels of glucose in skeletal muscle (Yang *et al*., 2003). On the other hands, it has been suggested that interactions of between calsarcins and calcineurin promote formation of fast twitch fibers which is associated with sprint performance (Yang *et al*., 2003). Thus, deficiency in alpha-actinin protein was associated with increased in calcineurin signaling activity which facilitated adapative response to endurance training (Seto *et al*., 2013).

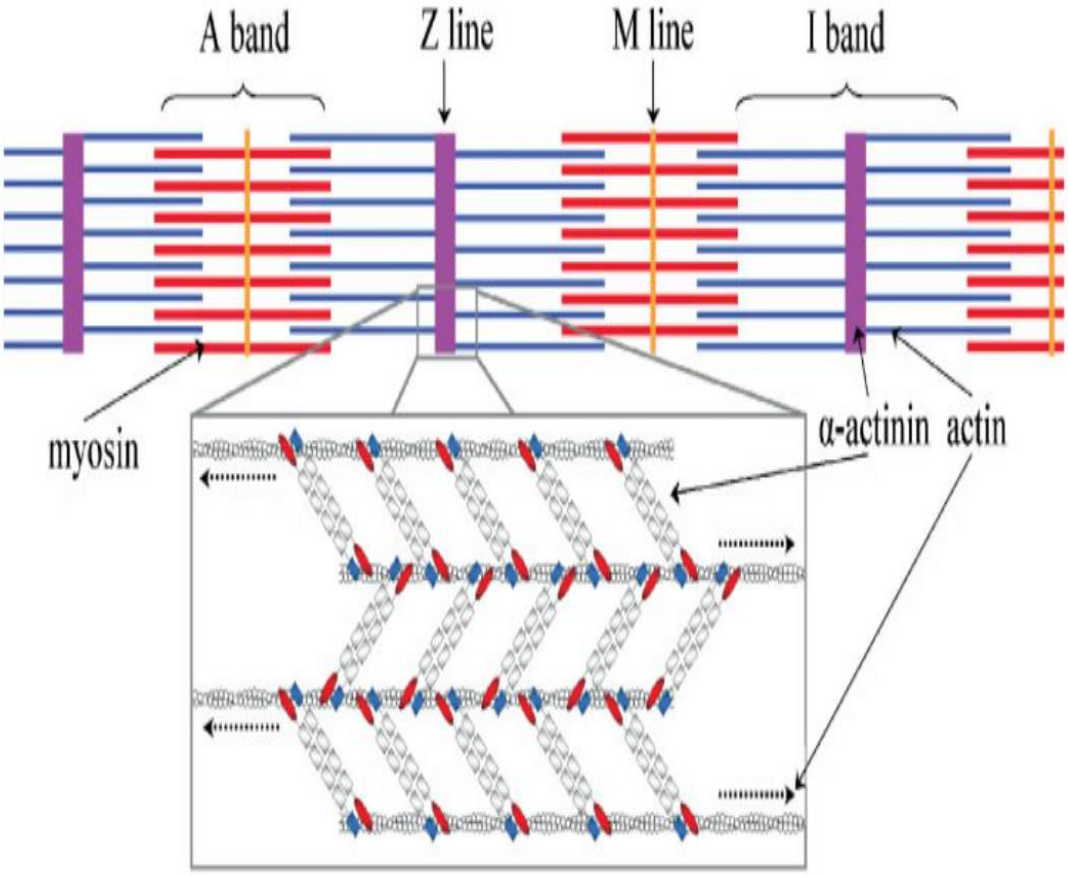

Figure 1.1: Structure of the sarcomeric α–actinins (MacArthur and North, 2004).

1.2.1.(b) Studies in *ACTN3* knock-out mice and deficiency of α-actinin-3 on muscle performance

In order to have further insight of how the structure of muscle and physiological properties changed due to the deficiency of alpha-actinin-3 in human with *ACTN3* XX genotype, a mouse knocked-out (KO) model (a genetically modified mouse in which an existing gene have been turned off) with deficiency in the expression of alpha-actinin-3 was created by MacArthur *et al*. (2007) and compared to the wild type (WT) mice with normal patterns of expression of this protein. Deficiency of alpha-actinin-3 (as produced by *ACTN3* XX genotype) was due to premature stop codon where Arginine (Arg) was substituted (North *et al.*, 1999). Results showed that in the knockout mice, alpha actinin-2 protein is expressed in all fiber types. This expression pattern is similar to the human muscle with the *ACTN3* XX genotype. In the WT mice, alpha actinin-2 is not found in all type of fast fibers where this protein is normally expressed in humans (MacArthur *et al*., 2007).

In fiber contractile properties, knock-out mice displayed a faster recovery from fatigued compared with wild-type mice. Therefore, it is observed that *ACTN3* knock-out mice have an advantage in longer distance compared to the wild-type mice (MacArthur *et al*., 2007).

In terms of muscle strength and power, *ACTN3* knock-out mice showed 7.4 % lower compared with the wild-type mice (MacArthur *et al*., 2008). These data are in accordance to Moran *et al*. (2007) in the non-athlete adolescent Greek populations, individuals with XX genotype took a longer time (mean: XX = 6.13 s) in 40-m sprint test compared with individuals with RR genotype (Arginine was not substituted) (mean: RR = 5.92 s). A plausible explanation is that the presence of α-actinin-3 in the

fast fibers in individuals with RR genotype made them performed better than individuals with XX genotype.

1.2.1.(c) *ACTN3* gene

ACTN3 is one of the sports genes extensively studied by worldwide scientists. *ACTN3* gene is located in chromosome 11q13.1 as shown in Figure 1.2 which consisted of 21 exons. Its size encompasses about 16,407 bases of nucleotide. In 1999, North and her colleagues found a common polymorphism of *ACTN3* gene known as R577X. This polymorphism will modify the alpha-actinin 3 protein. Since each person has two copies of the *ACTN3* gene, there are three different genotypes (RR, RX and XX genotypes) possible which are associated with different types of performance. This gene encodes for alpha-actinin 3 plays important role in helping to generate powerful muscle contraction (Blanchard *et al*., 1989), thus increases sprint capacity (Papadimitriou *et al*., 2007; Kikuchi *et al*., 2013). People with XX genotype are completely deficiency in alpha-actinin 3. Therefore, people with XX genotype simply cannot produce this protein, and in return produce more alpha-actinin-2, which increases endurance capacity (Shang *et al*., 2010; Yang *et al*., 2003).

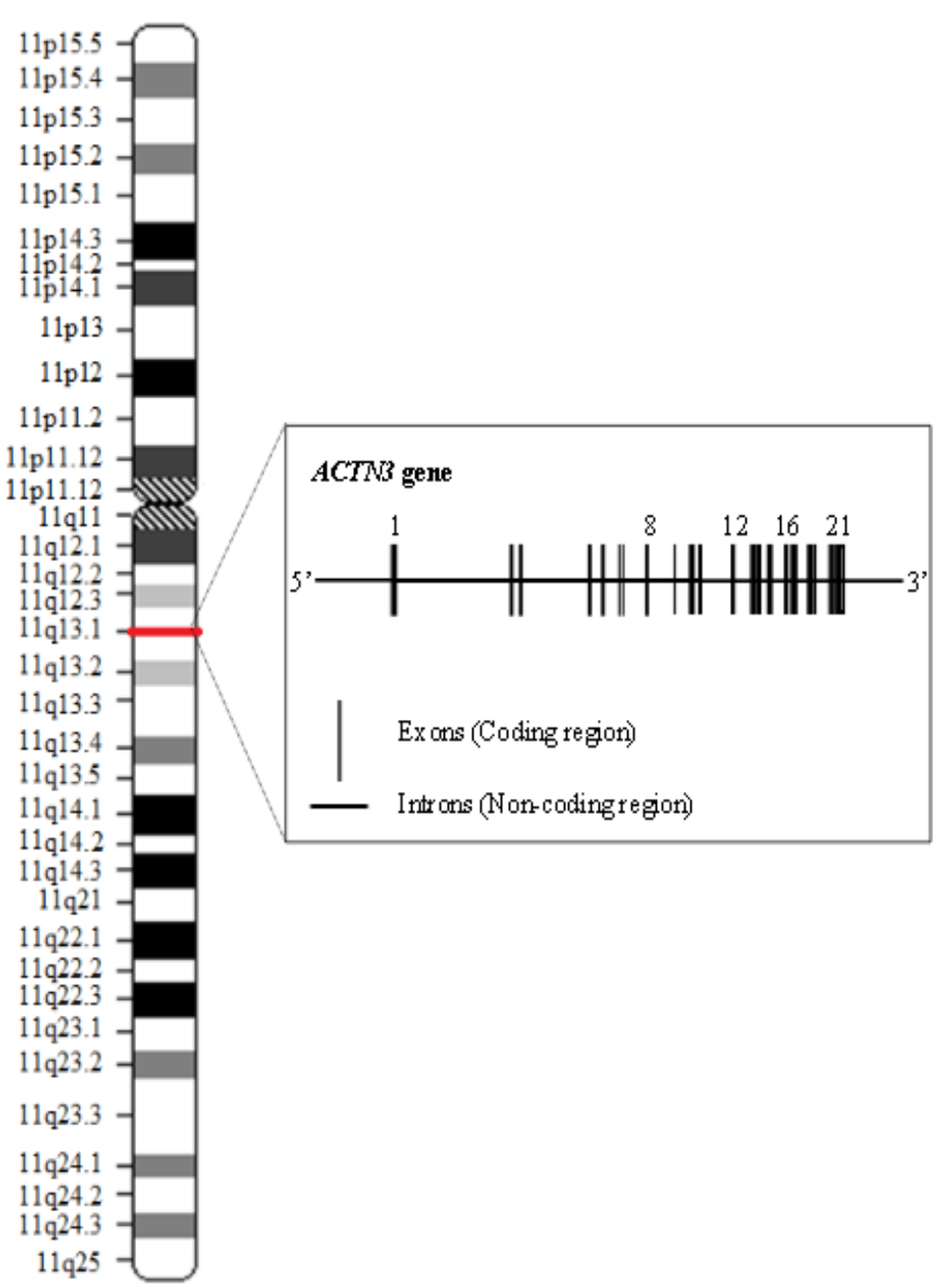

Figure 1.2: The genomic position of *ACTN3* gene in the long arm (q) of chromosome 11 band 13.1. The gene spans 16,407 bases of genomic DNA. *Picture was taken from Yusof (2016)

1.2.1.(d) The *ACTN3* R/X Polymorphism (rs1815739)

A common polymorphism R577X (Arg) in the *ACTN3* of the human population was identified. This polymorphism produces a premature stop codon where arginine (R) at position 577 was converted to stop codon (X), resulting in a shorter and non-functional protein as shown in Figure 1.3 (North *et al*., 1999). This variation results in two types of alleles of *ACTN3* gene in human where R-allele is longer and functional and X-allele is shorter and encoded for a truncated alpha-actinin 3 protein, thus cause the absence of the fully functional protein (North *et al*., 1999).

Individuals that have XX genotype demonstrated complete absence of alpha–actinin-3. Meanwhile, since RX genotype is a heterozygote, it is lack of functional allele (allele R), thus the amount of alpha–actinin-3 synthesised in skeletal muscle is lower. According to North *et al*. (1999), the frequency of X allele of *ACTN3* in worldwide population varies ranged from 22 - 52 % among populations of Americas, Africa, Europe and Asia. Besides that, there are study which showed that approximately more than one billion or 18 % of world population who has XX genotype, predicting a possibility of compensation for the non-functional alpha–actinin-3 by a closely related protein, alpha-actinin 2, for its loss in muscle (Mills *et al*., 2001). Patients with history of muscular dystrophy are hardly found with functional α-actinin-3 in their muscle biopsies, which initially prompted the researchers toward possible correlation (North *et al*., 1996). However, this possibility was rejected by surprising evidence on the absence of alpha-actinin3 within healthy individuals. In fact, people with XX genotype have no disturbances in building muscle (North *et al*., 1999; Surninaga *et al*., 2000) and the only fast-twitch fiber contraction was affected. Absence of the functional protein resulted by the XX genotype is a non-pathological condition or a mere phenotype variation in humans.

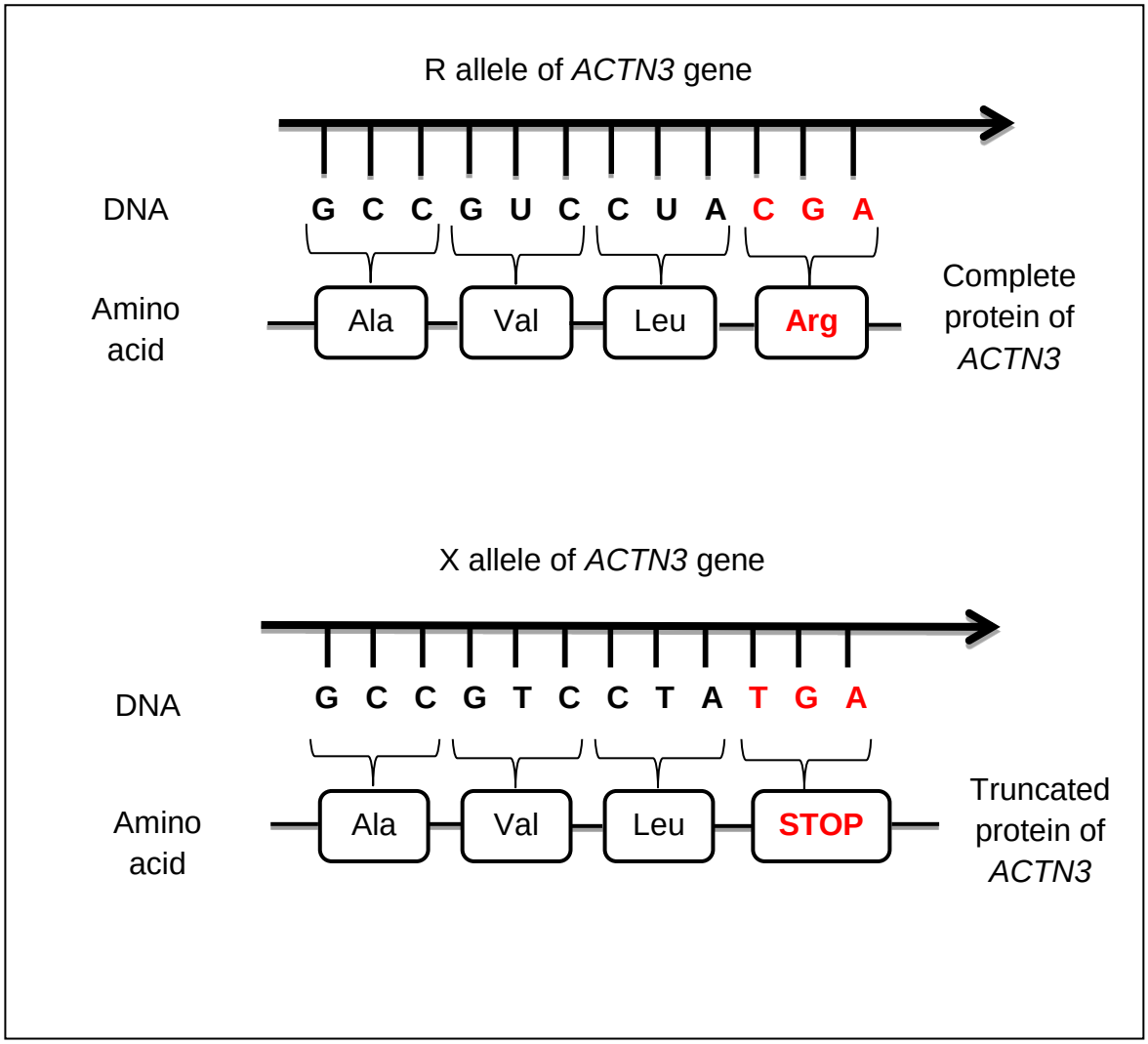

Figure 1.3: Nonsense mutation of *ACTN3* R577X. Figure was modified from http://https://ghr.nlm.nih.gov/primer/mutationsanddisorders/possiblemutations.

1.2.1.(e) Association of *ACTN3* gene and the sports performance

ACTN3 polymorphism is the most extensively polymorphism studied so far associated with sports performance as worldwide scientist believed this polymorphism might able to predict the athletic talent or performance (Yang *et al.*, 2003; MacArthur and North, 2004; Griffiths *et al*., 2013). The first study on the association of the *ACTN3* R577X polymorphism with sports performance was described by Yang *et al*. (2003). In Yang *et al.* (2003) study, 301 elite Australian Caucasian athletes and 436 healthy Caucasian controls were genotyped for *ACTN3* R577X polymorphism. It was concluded that there were significant differences in frequency of RR, RX and XX genotypes between sprint and control subjects ($p < 0.001$). However, there were no significant difference in *ACTN3* genotypes frequencies between overall athlete (combined sprint and endurance subjects) compared to controls. The control population had an increase frequency of XX genotype (18 %) and lower frequency of RR genotype (30 %) as compared to the sprint/ power athletes where the frequency of XX genotype is 5 % and frequency of RR genotype is 50 %. From this study, it is suggested that there was positive relationship of R577 allele with sprint performance among Australian Caucasian athletes.

In the following years, several studies have indicated that there was positive relationship between the RR genotype and sprint/ power athletic capacity in different ethnic groups (Cieszczyk *et al*., 2011; Druzhevskaya *et al*., 2008; Papadimitriou *et al*., 2007; Roth *et al*., 2008). In a Polish study (Cieszczyk *et al.*, 2011), 158 athletes from different sports disciplines, including track and field, swimming and weighting showed statistically significant difference ($p = 0.005$) in the frequency of the *ACTN3* R577X alleles as compared to 254 controls. On the other hand, larger sample size was recruited from Russian cohort study where 486 Russian national professional athletes

specialized in power-oriented sports were involved in a wide range of multidiscipline sports and 1197 controls were involved in this study. There were significant difference in terms of *ACTN3* genotypes between power-athletes and controls ($p < 0.0001$). Besides that, the researchers also found that frequency of XX genotype in the athletes was significantly lower compared than the controls. This supports the findings that most of the athletes with X alleles use less strength and power (Yang *et al.*, 2003; Eynon *et al*., 2009; Niemi *et al*., 2005).

In a study on Greek population (Papadimitriou *et al*., 2007), the frequency of RR genotype (48 %) was found to be elevated in athletes who are oriented with sprint/ power sports relative to control (26 %), and there was also significant difference in the frequency of RR genotypes ($p = 0.016$) and R alleles ($p = 0.017$) of *ACTN3* between the athletes and control population. Several studies to date also showed similar association that *ACTN3* RR allele is over represented among power athletes (Eynon *et al*., 2009; Chiu *et al*., 2011; Erskine *et al*., 2013; Pimenta *et al*., 2013; Mikami *et al*., 2014).

In a study on Finnish athletes from track and field category, the authors observed a significant reduction in the frequency of XX genotype among the sprinters and increase of the genotype among endurance athletes. These results showed that XX genotype of *ACTN3* gene could be directly linked to the endurance type sports (Niemi *et al*., 2005). This hypothesis is further supported by another study with similar results (Yang *et al*., 2003).

A study in Japanese population, 627 sprint athletes from track and field disciplines were involved. Result showed there was significant difference ($p = 0.003$) compared to the 810 controls in the frequency of *ACTN3* R577X allele. The author concluded that there was a positive relationship between RR/RX genotype with the

level of athletic status in sprint athletes where the RR and RX genotype are in increasing trend in regional, national and international level respectively (Kikuchi *et al*., 2015).

Collectively, the results of these studies show a negative association between the X allele and performance in strength or power. However, the relationship between the *ACTN3* gene and the endurance performance seems less clear. Taken together, these data support the hypothesis that R allele is associated with performance in sprint sports. Nevertheless, there are studies which showed conflicting findings or no association was found.

For example, in a study on Asian populations, a group of 856 elementary school students (126 athletes and 730 non-athletes) in Korean population was evaluated, there was no significant difference of *ACTN3* genotypes between athletes and non-athletes. The frequency of *ACTN3* genotypes was quite similar between athletes (RR = 30.7 %, RX = 49.6 %, XX = 19.7 %) and non-athletes (RR = 30.7 %, RX = 48.5%, XX = 20.9 %) (Kim *et al*., 2015).

1.2.1.(f) The *ACTN3* gene and indicator of performance

Moran *et al*. (2007) evaluated 992 adolescent Greeks of both genders by analyzing the association between the *ACTN3* R577X polymorphism with body composition and power/strength-related indicators like hand grip strength, vertical jump, 40 m sprint, agility run and aerobic capacity. Association was only noted in the 40 m sprints in males where RR individuals (mean time: 5.92 s) showed higher values of mean time in 40 m sprint when compared to other genotypes (mean time: RX = 6.00 s, XX = 6.13 s).

In a study by Santiago *et al*. (2009), a total of 284 non-athlete young adults were evaluated. The researchers determined the association among *ACTN3* R577X polymorphism and jump tests (squat jump and counter movement jump) and 30 m sprint times. The results showed that absence of the *ACTN3* gene did not negatively affect the performance of power tests even after potential cofounders like sex, age, height and weight were adjusted.

In a study on Asian populations, a group of 452 young Chinese male soldiers were evaluated for association between the genotypes and handgrip strength. There is significant differences (p = 0.025) in the handgrips where individuals with XX genotype (46.5 kg) showed a weaker handgrip strength when compared to RR genotypes (52.9 kg) (Shang *et al*., 2010).

Yet, in another study, a group of 856 elementary school students in Korean population were evaluated on association between *ACTN3* R577X polymorphism and physical fitness indicators like handgrip, 30 m sprint test, sit-up, 100 m dash, vertical jump, long jump and medicine ball throw. Again, there was no significant difference between the genotypes and the physical fitness indicators (Kim *et al*., 2015).

1.2.1.(g) Adaptations to training

Clarkson *et al*. (2005) suggested that *ACTN3* may affect muscle performance and response to resistance training. The growth rate of muscle by strength training in women in non-athletes cohort study was compared and it was reported that individuals with minor allele (577XX) showed higher gain of muscle, followed by heterozygous (R577RX), and homozygous major (577RR) after resistance training. The researchers proposed that the lack of α-actinin-3 could disrupt the structure of the sarcomere resulting in more susceptible to the damage and consequently improve adaptive

response to the resistance training of the skeletal muscle. This is because the damage of muscle is seen as an important stimulus to increase the adaptation of muscle and thereby produce greater strength gain.

Additionally, Delmonico *et al*. (2007) revealed that in the strength exercise, there were more prominent differences in Muscle Power Quality (MPQ) among *ACTN3* genotypes in men and women aged 50 – 85 years. This highlighted that *ACTN3* R577X polymorphism influenced the differences in strength training among population of older men and women.

Besides, another study conducted by Vincent *et al*. (2007) revealed that the fast-twitch fibers were more prominent in the homozygous major (RR) than the minor allele (XX) individuals. Study by Vincent *et al*. (2007) was paralleled to the fact that α-actinin 3 enhances the formation of fast-twitch fibers in individuals with R allele which provides an advantage for sprint activities (Yang *et al*., 2003).

In a study by Gentil *et al*. (2011), 141 young men were evaluated for the effect of 11 weeks of strength training. It was observed that muscle strength gains after training period does not depend on *ACTN3*. However, it was observed that only the R allele carriers of *ACTN3* showed significant increase in thickness of muscle which was measured by ultrasound.

1.2.2 *Angiotensin Converting Enzyme* (*ACE*) gene

1.2.2.(a) The *Angiotensin Converting Enzyme* (*ACE*) gene

In humans, *ACE* is a gene located in chromosome 17q23.3 as shown in Figure 1.4 which consists of 26 exons and 25 introns (Coates, 2003). Its size encompasses about 21,000 bp of genomic DNA and encodes a protein of 1306 amino acids (Coates, 2003). *ACE* has a polymorphism insertion/deletion (I/D), which is due to the presence (insertion) or absence (deletion) of a 287-bp genomic segment in intron 16 of *Alu* repetive sequence (Rigat *et al*., 1990; Stroth *et al*., 1999; Hubert *et al*., 1991). This *ACE* I/ D polymorphism make up approximately 50 % of the interindividual variability in the plasma concentration of ACE (Rigat *et al*., 1990). The D allele is correlated with increased enzyme ACE activity whereas the I alelle is correlated with decreased activity (Rigat *et al*., 1990). This means that individual with homozygous DD genotype have increased circulating ACE levels, twice those found in individual with homozygous II genotype. The individuals with heterozygous ID genotype have intermediate levels of ACE.

Angiotensin Converting enzyme (ACE) is a circulating serum which is part of the renin-angiotensin-aldosterone system (RAAS). ACE plays a very significant role in regulating blood pressure, blood volume and balance electrolytes. In the RAAS system, pulmonary and renal endotherlial cells are responsible for the secretion of ACE enzyme and production of angiotensin II (a potent vasoconstrictor) from conversion of angiotensin I (Erdos *et al*., 1987).

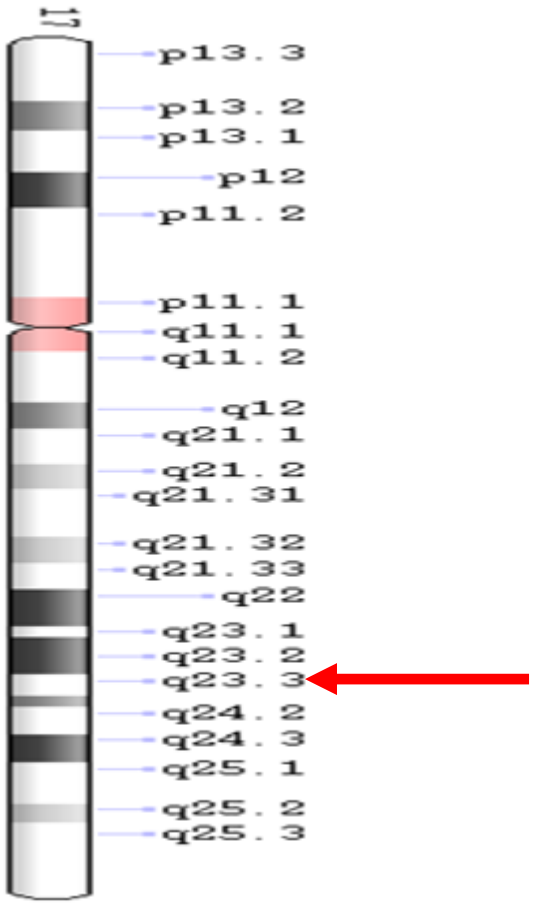

Figure 1.4: Location of the *ACE* gene on the long arm (q) of chromosome 17 on band 23.3 (National Center for Biotechnology Information, 2008)

1.2.2.(b) The RAAS (renin–angiotensin-aldosteron system) and blood pressure regulation

Controlling blood pressure (BP) (Van Berlo *et al*., 2003), sodium balance (Van Berlo *et al*., 2003), tissue growth and inflammation process (Dzau, 1989) are part of pivotal role in the renin-angiotensin-aldosteron system (RAAS) as illustrated in Figure 1.5 (Lubel *et al*., 2008). This system is determined by renin which is predominantly produced and secreted by the kidney. It involves a series of biochemical reactions which will cleave the single substrate angiotensinogen (synthesized in the liver) to produce angiotensin I, subsequently by ACE to yield angiotensin II (Joans & Woods, 2003).

Two subtypes of angiotensin II receptors have been identified which are AT1 and AT2, of which the AT2 is not involved in cardiovascular homeostasis (Nouet and Nahmias, 2000), while AT1 produce vasoconstriction effect (Matsubara, 1998). Angiotensin II is an enzyme in the renin-angiotensin system which has vasoactive effect to regulate the blood pressure and artery pathophysiology of hypertension and chronic heart failure (Sayed *et al*., 2003; Cambien *et al*., 1992). Angiotensin II does not only function as vasoconstrictor, but also activates adrenal glands to secrete aldosterone (Jones *et al*., 2002). Aldosterone is a hormone which, in turn, stimulates the kidney to re-absorb water and sodium in the renal tubules (Jones *et al*., 2002). ACE enzyme is able to inactivate bradykinin, a vasodilator, in regulating blood pressure by the constriction and dilation of blood vessels (Jones *et al*., 2002). In cases where RAAS was abnormally active, angiotensinogen is converted to Angiotensin I by renin. Then, ACE converts the Angiotensin I to Angiotensin II, leading increased production of Angiotensin II, thus create an increased vasoconstriction, secretion of aldosterone, retention of sodium and water, resulting in higher blood pressure (Sayed *et al*., 2006).

The *ACE* polymorphism has insertion/ deletion of 287 bp in intron 16, resulting in 3 genotypes including DD homozygote, II homozygote and ID heterozygote (Villard & Soubrierr, 1996). ACE is one of the important component in the RAAS system (Holdys *et al*., 2011). Individuals with D allele is associated with higher ACE activity (Rigat *et al*., 1990). Therefore, *ACE* polymorphism also plays important role in exerting ACE effect at cellular level. This was because *ACE* polymorphism have downstream impact in terms of production of angiotensin II and breakdown of bradykinin (Mizuiri *et al*., 2001). For example, the ACE enzyme may also elevate the concentration of angiotensin II (Rigat *et al*., 1990) and also involved in the regulation of oxygenation of tissues and efficiency of skeletal muscle in functional performance (Jones *et al*., 2002).

Renin–angiotensin-aldosterone system (RAAS) is not only located in endocrine system but also in many tissues such as liver, heart, skeletal muscle and fat tissues to provide multifunctions (Jones *et al*., 2002).

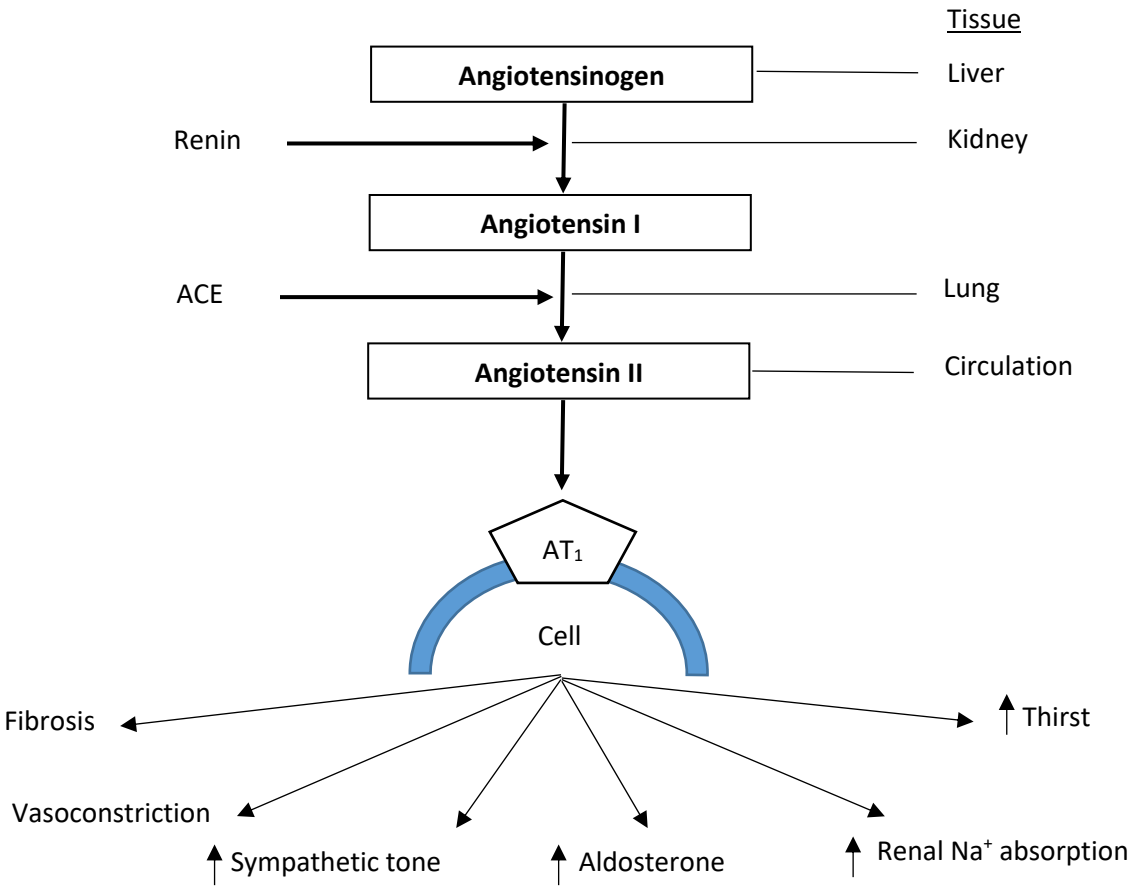

Figure 1.5: Diagram of human renin–angiotensin-aldosteron system (RAAS) (Lubel *et al*., 2008).

1.2.2.(c) The *ACE* gene polymorphism

Polymorphism is defined as an alteration of DNA sequence with the frequency of more than 1 % in the population (Ferreira *et al.,* 2005). Montgomery *et al*. (1998) were the first to show that a relationship exists between the *ACE* I/D polymorphism and human physical performance in a cohort of British Army recruits. Following his study, many association studies on the genes and human physical performance were started.

ACE I/D polymorphism denotes the presence (insertion, I allele) or the absence (deletion, D allele) of a 287 bp fragment within intron 16 as illustrated in Figure 1.6 (Yang *et al.,* 2007). Scientists thought that any changes would have been non-functional as this polymorphism take place in an intron (Sayed *et al*., 2006; Jones *et al*., 2002). However, it was reported that, among Caucasians, *ACE* DD genotype showed the highest frequency, followed by ID genotype and then II genotype (Collins, 2009). Therefore, it was believed that this polymorphism act as consistent biomarker in the population of Caucasians for ACE activity. This polymorphism is not only resposible for 50 % the differences in serum ACE but also have linkage to ACE activity in local tissues such as kidney, heart, and fat tissue (Jones *et al.,* 2002). The decreased activity of ACE and elevated half-life of bradykinin correlated with II genotype possibly will change substrate metabolism (Jones *et al*., 2002). As a result, this will increase and improve mitochondrial respiration and, also contractile process in both cardiac and skeletal muscles (Jones *et al*., 2002).

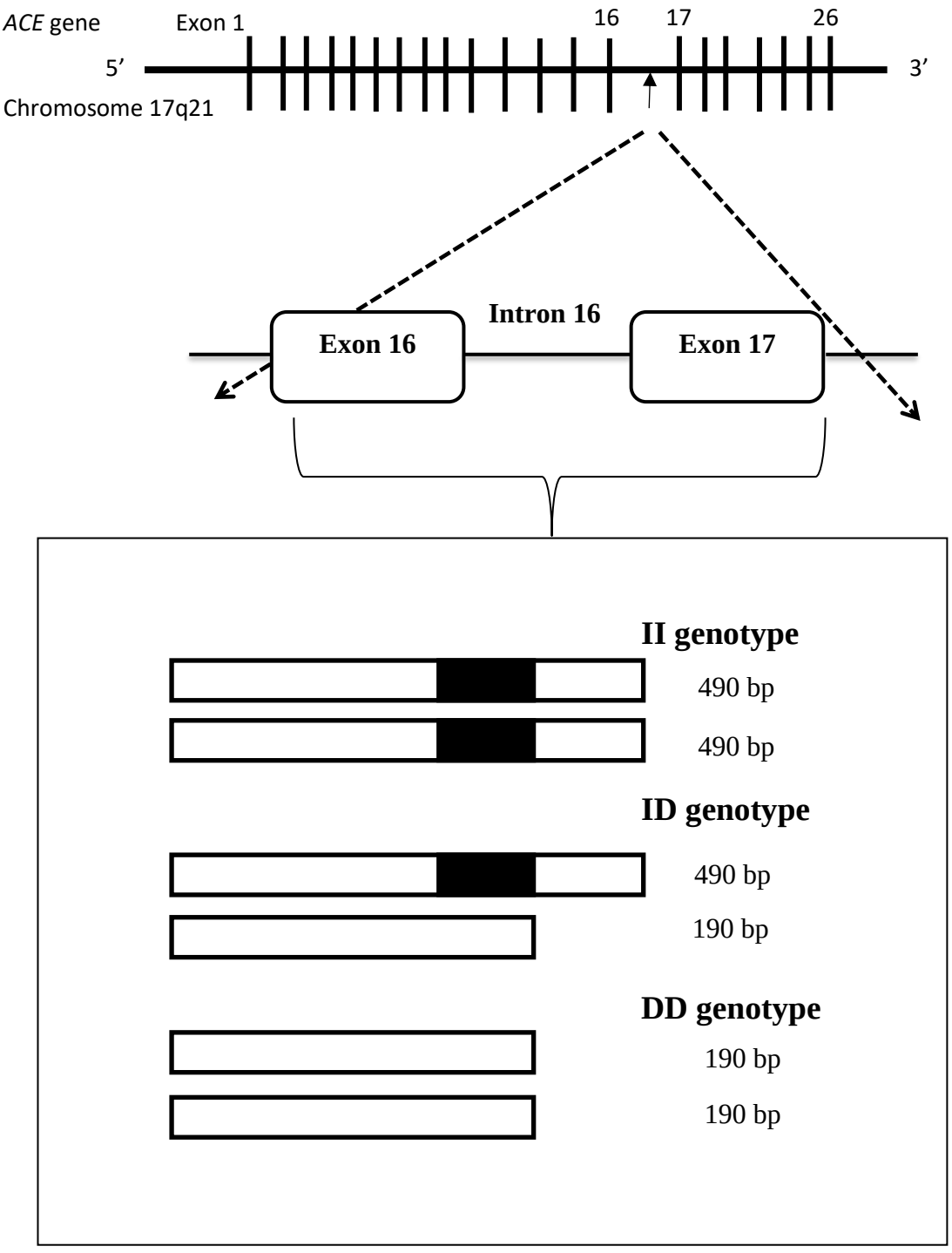

Figure 1.6: Insertion or deletion of a 287 bp *Alu* sequence at intron 16 of the *ACE* I/D polymorphism.

1.2.2.(d) *ACE* and Maximum Oxygen Uptake (VO_2 max)

Maximum oxygen uptake (VO_2 max) is one of three physiological factors and is an important determinant of physical ability in endurance capacity (Basset and Howley, 2000) and also associated with endurance-based sports (Hagerman, 1984; Hagerman. *et al*., 1978; Secher *et al*., 1982). Maximum oxygen uptake is an important index, which reflects the ability of the respiratory system, the heart, the circulatory system and muscle function during maximal muscular effort. In a study conducted by Tsianos *et al*. (2004), 35 elite endurance swimmers were tested in a swimming event. The results showed I allele was over-represented in longer distance races while D allele was over-represented in shorter distance races.

Although the above studies have shown an association of the *ACE* I/D polymorphism with improved physical activity, there are studies showing contrasting results (Karjalainen *et al*., 1999; Taylor *et al*., 1999; Rankinen *et al*., 2000; Sonna *et al*., 2001; Hagberg *et al*., 1998). In a study conducted by Taylor and colleagues (1999) where 120 white Caucasian Australian national athletes from various sport disciplines were randomly recruited. Their research revealed that there was no linkage between athlete and control group in the *ACE* allele and genotype frequency.

Hagberg *et al*. (2008) studied 58 physically active at Latvia in the postmenopausal period where the group hypothesized that VO_2 max could be influenced by *ACE* I/D polymorphism. After genotyping the subjects, it was observed that differences in allele frequency distributions were not significant. The frequency of the II genotype was 21 % (n = 12), ID genotype was 57 % (n = 33) and DD genotype was 22 % (n = 13). Among the genotypes group, the II genotype presented VO_2 max 23 % higher than DD genotype and 10 % higher than ID genotype. In conclusion, the