PREVALENCE OF HYPERTENSION IN UNIVERSITY STUDENTS AND ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE (VAL158MET) AND DOPAMINE D2 RECEPTOR (TAQ1A) POLYMORPHISM WITH HYPERTENSION

By

GAN KELVIN

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ABSTRACT

PREVALENCE OF HYPERTENSION IN UNIVERSITY STUDENTS AND ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE (VAL158MET) AND DOPAMINE D2 RECEPTOR (TAQ1A) POLYMORPHISM WITH HYPERTENSION

GAN KELVIN

Hypertension is a common public health condition that impacts nearly one billion people globally. It is a risk factor for coronary artery disease (CAD). The prevalence and rate of diagnosis of hypertension in young adults appear to be increasing. Yet, the crucial roles of environmental and genetic factors in the pathophysiology of hypertension are still widely debated. So, the primary aim of this study was to determine the prevalence of hypertension among university students. A total of 145 students (40% males and 60% females; 21.35 ± 2.149 years) from Universiti Tunku Abdul Rahman (UTAR), Perak campus were recruited. From the responses, 54 participants (37.2%) were found to be prehypertensive and 2.1% hypertensive. Males accounted for the larger percentage and statistical significance was established (63.2% vs 36.8%; $p < 0.05$). The study described herein also sought to explore the association between hypertension and various environmental parameters. Parameters such as health history, physical activity, dietary factor, and stress were included in the questionnaire. The magnitude of correlation was determined by Chi-square

test performed in SPSS software version 20.0 for Windows. No significant association was found between students' blood pressure and consumption of fried foods, physical activity, or stress. This study also identified the prevalence of *COMT* Val158Met and *DRD2 Taq1A* polymorphisms by using molecular genetic techniques. The *COMT* wild type genotype held the highest frequency at 49%; whereas the predominant genotype for *DRD2* gene was heterozygous (67.2%). No significant association was found between hypertension and *COMT* Val158Met polymorphism and *DRD2 Taq1A* polymorphism ($p > 0.05$). In conclusion, the data of this study has rejected the hypothesis that *COMT* Val158Met (rs4680) and *DRD2 Taq1A* (rs1800497) polymorphisms act as a genetic susceptibility factor for hypertension. Both genetic variants were not independently associated with the prevalence of hypertension.

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DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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(GAN KELVIN)

APPROVAL SHEET

This project entitled **"PREVALENCE OF HYPERTENSION IN UNIVERSITY STUDENTS AND ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE (VAL158MET) AND DOPAMINE D2 RECEPTOR (TAQ1A) POLYMORPHISM WITH HYPERTENSION"** was prepared by GAN KELVIN and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Assistant Professor Dr. Phoon Lee Quen) Date: ……...

Supervisor

Department of Biomedical Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: …….

PERMISSION SHEET

It is hereby certified that **GAN KELVIN** (ID No: **12ADB05788**) has completed this final year project entitled "**PREVALENCE OF HYPERTENSION IN UNIVERSITY STUDENTS AND ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE (VAL158MET) AND DOPAMINE D2 RECEPTOR (TAQ1A) POLYMORPHISM WITH HYPERTENSION**" under the supervision of Assistant Professor Dr. Phoon Lee Quen, from the Department of Biomedical Science, Faculty of Science and Ms. Kavitha Subramania from the Department of Physical and Mathematical Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project thesis in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

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(GAN KELVIN)

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LIST OF ABBREVATIONS

ANKK1	Ankyrin repeat and kinase domain containing-1
BMI	Body mass index
COMT	Catechol-O-methyltransferase
DRD2	Dopamine D2 receptor
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
HWE	Hardy-Weinberg equilibrium
NaCl	Sodium chloride
PCI	Phenol-chloroform-isoamyl alcohol
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNase A	Ribonuclease A
SDS	Sodium dodecyl sulphate
SNP	Single nucleotide polymorphism
TE	Tris-EDTA
UV	Ultra-violet

CHAPTER 1

INTRODUCTION

Hypertension, also called high blood pressure, is a common condition characterized by persistent elevated blood pressure in the arteries. Blood pressure is expressed in systolic and diastolic pressures which measured in millimeter mercury (mmHg). According to World Health Organization (WHO) criteria, a systolic blood pressure equal to or above 140 mmHg or diastolic blood pressure equal to or above 90 mmHg is considered to be hypertension. Hypertension is classified into two types: primary and secondary hypertension. About 90-95% of all hypertension cases are categorized as primary hypertension (Tanira and Balushi, 2005). In this form of hypertension, there is no underlying cause and is believed to be linked with other environmental factors such as obesity, smoking, diet, stress, and family history. Meanwhile, secondary hypertension comprises the remaining 5-10% of cases. It normally results from an identifiable cause such as chronic kidney disease, renal artery stenosis, and other endocrine disorders.

Overall, the worldwide prevalence of hypertension in adults aged 25 and above was approximately 40% (World Health Organization, 2013). Meanwhile, in Malaysia, about 32.7% of Malaysians aged 18 years and above are hypertensive due to a primary cause of salt overconsumption (Ministry of Health Malaysia, 2014). Nonetheless, public remain largely unaware of the

current prevalence of hypertension and tend to underestimate its consequences. This is because hypertension is a silent killer that rarely causes symptoms. It is given more attention only when it leads to a more serious cardiovascular disease such as stroke. In fact, hypertension acts as a warning sign for people, indicating that it is time for them to change their unhealthy lifestyle.

More relevant to this study, is the fact that hypertension showed an increasing trend in university students along the past few years. Soliman, et al. (2014) reported that among 954 university students in Fayoum University (Egypt), the prevalence of hypertension and prehypertension was 5.7% and 47.4% respectively. This study was a two phases study with first cross-sectional descriptive study and second case control study. The study revealed that the proportions of prehypertension and hypertension increased significantly by age. Other from that, their study also showed that the proportions of prehypertension and hypertension in males were higher than in females.

A recent study on undergraduate medical students in Odisha (India) found that the prehypertension and hypertension percentage was 67% among 200 study subjects (Patnaik and Choudhury, 2015). This study was particularly detailed, which included the relevant data on risk factors for hypertension like family history of hypertension, consumption of alcohol and cigarette smoking, physical activity level, body mass index, and dietary habits. The authors reported that besides family history of hypertension, sedentary lifestyle, high salt intake, BMI $\geq$ 25 had significantly associated with prehypertension and

hypertension status. Furthermore, Patnaik and Choudhury (2015) also stated that out of 112 male subjects, 80 were prehypertensive and 4 were hypertensive whereas out of 88 female subjects, 48 were prehypertensive and 2 were hypertensive. This was consistent with findings of Soliman, et al. (2014), who reported that the prevalence differed in gender.

Furthermore, numerous epidemiological studies have previously reported comparatively high prevalence of prehypertension or hypertension among university students in Hungary (Antal, et al., 2006), Libya (Tayem, et al., 2012), Nigeria (Familoni and Familoni, 2011), and Malaysia (Lee, et al., 2010). This is because university students are always being exposed to unhealthy environment condition such as sedentary lifestyle, cigarette smoking, bad eating habit, etc (Girotto, et al., 1996).

Hypertension is a multifactorial disorder that results from a complex interaction between genetic and environmental factors. It is believed that 30 to 50% of blood pressure variation is contributed by the genetic element (Tanira and Balushi, 2005). The catechol-O-methyltransferase (*COMT*) gene, a well-studied gene, has been reported by increasing scientific literature to show association to hypertension (Annerbrink, et al., 2008; Stewart, et al., 2009; Yeh, et al., 2010). On the other hand, there is limited research focused on the association between Dopamine D2 receptor (*DRD2*) gene and hypertension. Despite that, the findings from Rosmond, et al. (2001) suggested the possible involvement of variation at *DRD2* gene in the regulation of blood pressure.

1.1 Objectives of the Study

Increasing public awareness is the key to counter this striking disorder worldwide. Research that focuses on identifying genetic variations that are responsible for hypertension may not only provide better understanding on the pathophysiology of the disease but also may reveal the biochemical and physiological pathways that link various risk factors in hypertension (Tanira and Balushi, 2005). Yet, there is lack of robust evidence on molecular genetics study of hypertension due to the complex nature of the regulatory mechanisms involved in regulating the blood pressure. The need of public awareness regarding hypertension is as essential as the need for more research effort to show and characterize the clear relationship between genetic variation and hypertension. All these efforts may shift the focus of management from treatment towards prevention.

Therefore, the objectives of this study are as follow:

i. To study the prevalence of hypertension among students from Universiti Tunku Abdul Rahman (UTAR), Perak campus.
ii. To identify the prevalence of *COMT* (Val158Met) and *DRD2* rs1800497 (*Taq1A*) polymorphisms among a cohort of UTAR students.
iii. To assess the association between consumption of fried foods, physical activities, stress, and risk of hypertension.
iv. To investigate the association between *COMT* (Val158Met) and *DRD2* rs1800497 (*Taq1A*) polymorphisms and hypertension.

CHAPTER 2

LITERATURE REVIEW

2.1 Hypertension

Hypertension or high blood pressure is a worldwide public health issue that affects approximately 40% of adults aged 25 and above (World Health Organization, 2011). Hypertension is known as a silent killer as it rarely causes symptoms. Due to this reason, many people go undiagnosed even though the diagnosis is simple. Hypertension is one of the major risk factors for cerebrovascular disease and coronary heart disease. Apart from that, uncontrolled blood pressure causes peripheral vascular disease, heart failure, retinopathy, and renal impairment (Danaei, et al., 2011). Furthermore, hypertension is also associated with many chronic complications that affect vital body functions such as atherosclerosis, stoke, insulin resistance, obesity, and hyperuricacidemia (Chen, et al., 2007).

Various antihypertensive drugs such as β-blockers, calcium channel antagonist, angiotensin converting enzyme (ACE) inhibitors, and diuretics are usually used to manage hypertension and to alleviate the symptoms of accompanying conditions, for instance, cardiovascular diseases (The Japanese Society of Hypertension, 2014). The antihypertensive drugs from different categories are frequently combined upon prescribed by doctors so as to obtain the optimal

results. This is because the efficacy of these drugs is not 100%, they normally only exert up to 60% efficacy (Vasant, et al., 2012).

2.1.1 Essential and Secondary Hypertension

Essential, primary or idiopathic hypertension is defined as a form of high blood pressure with unknown cause. It is the most common type of hypertension, which accounts for 95% of all hypertension cases (Carretero and Oparil, 2000). Although the exact causes of essential hypertension are still unknown, it has frequently been considered as a result of combined action between many genetic, behavioral, and environmental factors (Bolivar, 2013).

Markedly different from essential hypertension, secondary hypertension refers to a form of high blood pressure with a specific etiology. Although secondary hypertension affects only minority of the general hypertensive population, it may be cured by identifying its etiology and giving appropriate treatment (Ogihara, et al., 2009). Secondary hypertension can be caused by a variety of medical conditions which include Cushing's syndrome, hyperthyroidism, renal parenchymal disease, renal artery stenosis, and sleep apnea syndrome (Ogihara, et al., 2009).

2.2 Prevalence of Hypertension

The prevalence of hypertension differs globally, with the lowest 5.1% prevalence reported in rural India to the highest 70.7% prevalence reported in Poland (Kearney, et al., 2004). Meanwhile, according to WHO statistics on hypertension, the prevalence was highest in the African region while lowest in the America, with 46% and 35% respectively (World Health Organization, 2011). As far as we know, the latest statistical records for hypertension prevalence among the Malaysian adults can be adopted from the report of Ministry of Health Malaysia (2014), which reported a prevalence of 32.7%. The distribution of worldwide prevalence of hypertension is illustrated in Figure 2.1.

Turning the focus to university students, numerous epidemiological studies have shown an increasing trend of prehypertension or hypertension among university students. In Brazil, Martins and colleagues (2010) reported that among 605 university students participated in their study, the prevalence of hypertension was 9.7% with predominantly in males. At the same time, a cross sectional study among the undergraduate medical students in Kolkata, India also revealed a relatively high prevalence of hypertension. Out of the 850 subjects, 19.18% of them were prehypertensive while 13.88% were hypertensive (Chattopadhyay, et al., 2014). In Malaysia, the prevalence of prehypertension and hypertension among 237 Universiti Malaysia Sarawak (UNIMAS) undergraduate students was reported as 42.9% and 12.8% respectively (Lee, et al., 2010).

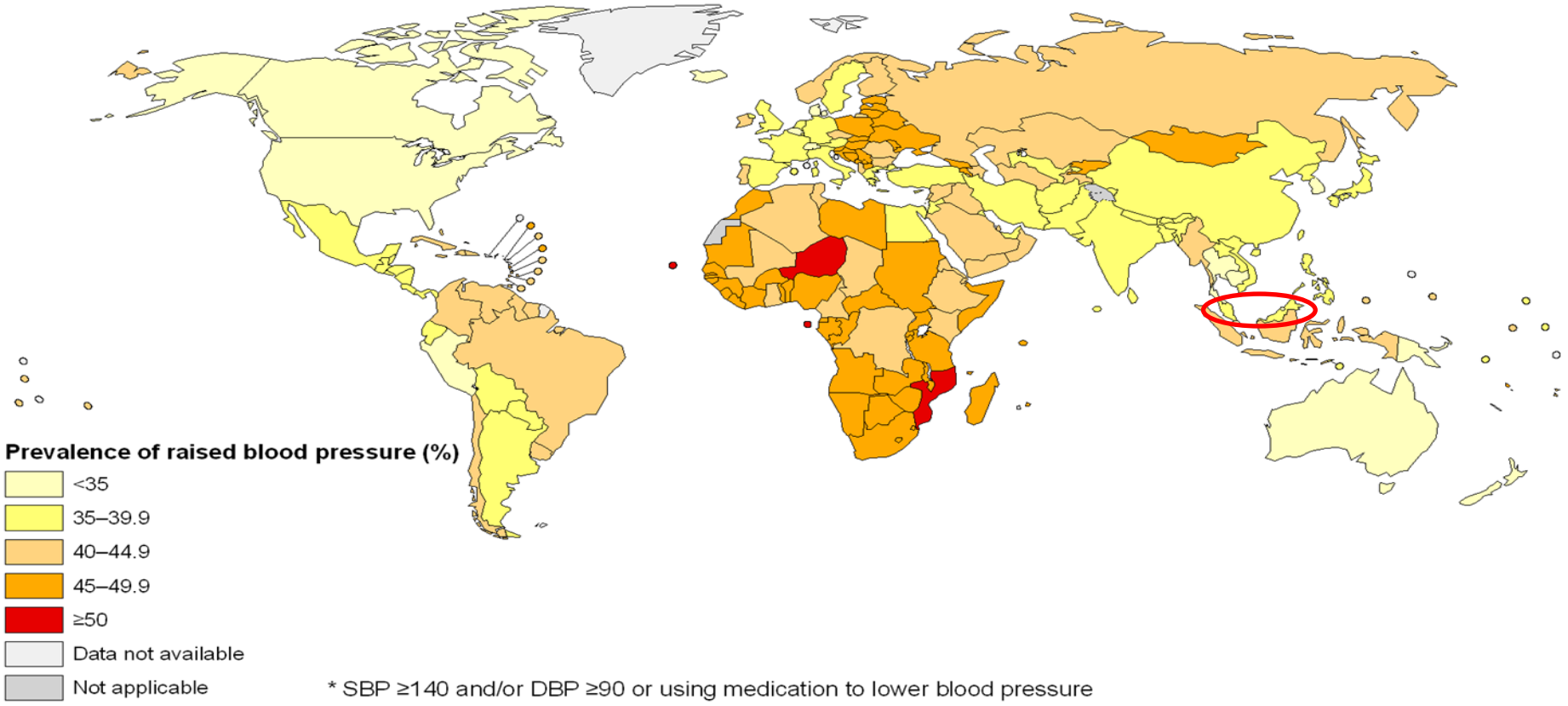


Figure 2.1: Worldwide prevalence of hypertension in adults aged 25 and above (World Health Organization, 2013).

2.3 Risk Factors for Development of Hypertension

At the present day, most hypertension is thought to result from a combined action between genetics, behavioral, and environmental factors (Bolivar, 2013). Numerous studies have shown that blood pressure correlates with various behavioral and environmental factors, including age, sex, obesity, physical inactivity, smoking, overconsumption of salt, excessive alcohol intake, and stress.

2.3.1 BMI and Obesity

Many population studies have shown that hypertension is associated with high body mass index (BMI) and obesity. In the Framingham Offspring Study, 70% of the new hypertension cases were associated with obesity (Garrison, et al., 1987). Furthermore, findings from Kotsis, et al. (2005) also suggested that the incidence of increased ambulatory blood pressure was more significant in obese patients and they had higher prevalence of isolated office hypertension. Moreover, in a cross sectional study conducted by Todkar, Gujarathi and Tapare (2009), 34% of 94 obese patients were hypertensive while only 5.15% of 1203 normal BMI patients were hypertensive. Similar association has also been reported by Suwarna, Vaishali and Sudeepa (2012). These findings further affirmed the correlation between obesity and hypertension.

2.3.2 Excess Salt Intake and Alcohol

Many researchers claimed that excessive salt and alcohol intake has been the exclusive dietary factor for hypertension. This conclusion is supported by plenty of reported evidence and is generally recognized worldwide. A case control study conducted in Tasgaon, India discovered that among the dietary factors, high salt intake was significantly associated with hypertension ($p = 0.0003$) (Sagare, Rajderkar and Girigosavi, 2011). Besides that, people who consume more than 5 grams of salt per day are twice as likely to develop hypertension as other people (Wang, Tiwari and Wang, 2014). On the other hand, the association between hypertension and alcohol consumption is controversial. Current research suggests that light to moderate alcohol consumption can decrease the risk of hypertension and is advantageous to the cardiovascular system (Son, 2011; Worm, Belz and Stein-Hammer, 2013). However, an epidemiological study conducted in Greece demonstrated that chronic alcohol consumption (more than 3 drinks per day) was statistically associated with hypertension (Skliros, et al., 2012). Furthermore, Briasoulis, Agarwal and Messerli (2012) also reported that alcohol was associated with the increased incidence of hypertension, but only in a condition of heavy drinking. Therefore, it is commonly accepted that only excessive alcohol intake is associated with the risk of developing hypertension (Hiromi, et al., 2014).

2.3.3 Fried Foods

Frequent consumption of fried foods has been reported to be statistically associated with a higher incidence of obesity (Guallar-Castillon, et al., 2007; Sayon-Orea, et al., 2013). This brings about an idea that the fried food consumption might also be associated with an increased risk for hypertension. However, evidence from related epidemiological studies is scarce. A cross-sectional study from Spain found that consumption of fried foods was associated with a higher prevalence of arterial hypertension, particularly when reused oils were used for frying (Soriguer, et al., 2003). Furthermore, in a Mediterranean cohort of university graduates, a positive association between frequent consumption of fried foods and increased risk for hypertension was reported (Sayon-Orea, et al., 2014). These findings correspond to the results in a study performed by Wang, et al. (2010), which demonstrated that dietary intake of trans-fatty acids was associated with higher risk of hypertension. Thus, hypertension might be associated with frequent consumption of fried food as the amount of trans-fatty acids in food increased greatly during the frying process (Gadiraju, et al., 2015).

2.3.4 Physical Inactivity

As for other exogenous factors, multiple studies also showed the relationship between physical inactivity and hypertension. In one related study, leisure time physical inactivity was found to be statistically significant associated with hypertension, with p-value = 0.009 (Sagare, Rajderkar and Girigosavi, 2011). Additionally, the authors also reported that subjects with no leisure

time physical activity have about twice the risk of developing hypertension as somebody with regular leisure time physical activity. As support to that discovery, findings of Kokkinos, Narayan and Papademetriou (2001) showed that blood pressure can be effectively reduced with moderate-intensity aerobic exercise. This study found that long term aerobic exercise had beneficial effects towards hypertensive patients and the response did not appear to be limited by gender or age.

2.3.5 Stress

Large amount of vasoconstricting hormones that raise blood pressure can be induced by stress and cause hypertension through duplicate blood pressure elevations (Kulkarni, et al., 1998). A related study carried out in Birmingham, England tested on the reactivity of blood pressure towards psychological stress and from that predicts the incidence of hypertension (Matthews, et al., 2004). They then came to a conclusion that young adults whose blood pressure responds greatly to psychological stress may have high chance of developing hypertension. Besides that, a cross-sectional study conducted by Hu, et al. (2015) demonstrated that psychological stress was accounted for approximately 9.1% to the risk for hypertension. This was consistent with findings of Perez, et al. (2001), who reported that psychological stress was associated with a greater risk of hypertension.

2.3.6 Genetic Risk

Since hypertension is a multifactorial disorder, the genetic elements are believed to play a part in its onset as well. Numerous genetic association studies have reported that the risk for hypertension was increased in certain genetic variants (Izawa, et al., 2003). Yet, no clear conclusions can be drawn regarding their association due to the complexity and inconsistency in the results obtained. Therefore, it is generally acknowledged that risk for hypertension is affected by interactions between multiple genes (Williams, et al., 2000).

2.4 Catechol-O-methyltransferase (*COMT*) Gene

2.4.1 *COMT* Gene and its Polymorphism

The catechol-O-methyltransferase (*COMT*) gene is located on the q-arm of chromosome 22, in region eleven and band twenty one (22q11.21) (Lajin and Alachkar, 2011). This gene consists of six exons and is responsible for the production of both soluble (*S-COMT*) and membrane bound (*MB-COMT*) proteins (Mannisto and Kaakkola, 1999). In human, *S-COMT* comprises 221 amino acids while *MB-COMT* contains 271 amino acids (Mannisto and Kaakkola, 1999). The expression of both *S-COMT* and *MB-COMT* transcripts was found in most human tissues. However, *MB-COMT* is highly expressed in the brain while other tissues including liver, kidneys, and gastrointestinal tract are dominated by *S-COMT* (Hong, et al., 1998). Generally, both *S-COMT* and *MB-COMT* share a similar function, which involves in the catecholamines

inactivation by transferring a methyl group from S-adenosylmethionine (Tenhunen and Ulmanen, 1993).

A common single nucleotide polymorphism (SNP) in the fourth exon, codon 158 of the human *COMT* gene has been widely employed for association studies. It consists of a G-to-A transition in the coding sequence of the *COMT* gene (rs4680), resulting a valine (Val) to methionine (Met) amino acid change in the protein (Lajin and Alachkar, 2011). This variation has been shown to decrease the enzymatic activity by 67-75% (Lotta, et al., 1995; Lachman, et al., 1996). The *COMT* alleles are codominant as heterozygous carriers have intermediate enzyme activity between wild type individuals (Val/Val) and homozygous mutant individuals (Met/Met) (Weinshilboum, Otterness and Szumlanski, 1999). In the meantime, Egan, et al. (2001) also reported that the Met/Met genotype displayed up to four-fold reduction in the enzymatic activity of catecholamine degradation compared with the Val/Val genotype, while intermediate activity was observed in the Val/Met genotype.

2.4.2 *COMT* Polymorphism may be implicated with Hypertension

Scientific literature has provided inconsistent results for the connection between the functional variant (Val158met) of the *COMT* gene and blood pressure. In a cohort study from Sweden reported that subjects homozygous for the low-activity allele (Met) were associated with higher systolic blood pressure ($p = 0.003$) and diastolic blood pressure ($p = 0.03$) (Annerbrink, et al.,

2008). Furthermore, study from Yeh, et al. (2010) also suggested the possible involvement of *COMT* Val158Met polymorphism in the blood pressure regulation. Nevertheless, a population-based cohort study by Hagen, et al. (2007) noted that high systolic blood pressure presented more frequently in Val/Val carriers rather than Met/Met carriers, which pointing to an opposite conclusion. A possible explanation for these divergent findings is due to the differences in enrolled subjects' genetic background, age, sample size, and sex ratio (Annerbrink, et al., 2008).

In a Japanese cohort study which targeted on the influence of Val158Met variant on blood pressure, the Met allele was found to be significantly associated with hypertension (Htun, et al., 2011). In addition to that, they also proposed that this genetic effect may have an interaction with dietary energy intake. In another similar study of alcohol dependent individuals conducted by Stewart, et al. (2009), they concurred that the Met/Met genotype possesses greater risk of high blood pressure than Val/Val genotype.

However, in an association study in China, there was no distinct association between *COMT* Val158Met polymorphism and blood pressure (Bian, et al., 2015). Although *COMT* 158-Met allele presented more frequently in the study population, the *COMT* Val158Met was correlated negatively with blood pressure. Meanwhile, this conclusion agreed with finding reported by Jordan, et al. (2002), where inhibition of *COMT* enzyme with entacapone did not show a significant increase in blood pressure. This suggested that the blood pressure

is not associated with *COMT* Val158Met polymorphism even though individuals with Met/Met genotype were reported to have a reduced enzyme activity.

2.4.3 Pathogenesis of Hypertension in relation to *COMT* Polymorphism

There is no clear consensus on the implication of *COMT* gene variants in pathogenesis of hypertension. Nonetheless, the pathogenesis of hypertension in relation to *COMT* polymorphism has normally been postulated to involve a simple pathway that affects the levels of active catecholamine. A common functional polymorphism (Val158Met) in the *COMT* gene has been shown to account for a three- to four-fold reduction in enzyme activity and dopamine catabolism, lead to increased level of dopamine in the blood plasma (Egan, et al., 2001). Dopamine plays a crucial role in the blood pressure regulation through a complex process. It acts on the β_1-adrenergic and dopaminergic receptors to induce a series of cardiostimulatory effects, including vasoconstriction, increased heart rate, and increased myocardial contractility (Goldberg, 1972). All these actions generate higher blood pressure. Therefore, some aberrations in dopamine regulation which are genetically caused will probably contribute to hypertension (Zeng, et al., 2007).

COMT Val158Met has not only been related to decreased enzyme activity and elevation of dopamine concentration, but also linked to hypertension due to a high uric acid. Clinical evidence has indicated that hyperuricemia is associated

significantly with hypertension in children and adults (Jones, et al., 2008). Thus, Yeh, et al. (2010) speculated that there might be a possibility for *COMT* Val158Met modulates blood pressure by affecting the plasma uric acid levels.

2.5 Dopamine D2 Receptor (*DRD2*) Gene

2.5.1 *DRD2* Gene and its Polymorphism

The dopamine D2 receptor (*DRD2*) gene is located on the q-arm of chromosome 11, from position 22 to 23 (11q22-23). This gene comprises eight exons and encodes the D2 subtype of the dopamine receptor (Grandy, et al., 1989). In human, *DRD2* is expressed in two molecular distinct isoforms, namely D2S (shorter form) and D2L (longer form). D2S acts primarily at presynaptic sites while D2L at postsynaptic sites (Usiello, et al., 2000). Both D2S and D2L receptors are found in dopamine neurons and serve as somatodendritic autoreceptors (Khan, et al., 1998).

Over the last few decades, many polymorphisms have been identified in *DRD2* gene, for instance *Taq1A* (rs1800497), *-141C Ins/Del*, and *957C>T* (rs6277). Among all these, *Taq1A* is the most commonly studied polymorphism. It was found within an adjacent gene named the ankyrin repeat and kinase domain containing-1 (*ANKK1*) gene, which is located downstream of the *DRD2* gene (Voisey, 2009). It is caused by a C (A2 allele) to T (A1 allele) substitution, leading to a non-conservative amino acid change (Glu713Lys). Association between the T allele and reduced number of

dopamine D2 receptor in the brain was reported by Pohjalainen, et al. (1998). Apart from that, the T allele is also associated with a decreased dopaminergic function in the brain (Noble, 2003).

2.5.2 *DRD2* Polymorphism may be implicated with Hypertension

To date, there is no a definite or clear explanation for the functional status of the *Taq1A* variant in humans, but scientific evidence has suggested that the T allele is associated with a higher risk of developing Parkinson's disease (Noble, 2003). Studies concerning the relationship between *Taq1A* polymorphism and hypertension are limited. Instead, this variant has been frequently tested for its role in certain neuropsychiatric disorders such as schizophrenia, migraine, and posttraumatic stress (Voisey, et al., 2009).

The *DRD2* gene has previously been described to be involved in the pathogenesis of hypertension in animal model, but studies in humans are limited (Buuse, Versteeg and Jong, 1984; Linthorst, et al., 1991). Nonetheless, findings from Rosmond, et al. (2001) and Fang, et al. (2005) demonstrated that *Taq1A* polymorphism in humans is associated with an elevated blood pressure. In their study, the homozygous mutant TT genotype (A1/A1) showed an association with elevated systolic and diastolic blood pressure. Furthermore, the frequency of TT genotype was significantly higher among subjects with stage 1 hypertension compared to normal subjects (Rosmond, et al., 2001). Hence, the authors postulated that the *DRD2* represses release of

catecholamine at the sympathetic nerve terminals. Thus, a deficient *DRD2* function in *Taq1A* variant might increase the catecholamine release and contribute to the onset of primary hypertension.

However, in another study conducted by Thomas, Tomlinson, and Critchley (2000), the *Taq1A* C allele was associated with elevated blood pressure, instead of T allele reported by Rosmond, et al. (2001). The authors were then further their investigation to assess the relationship between *Taq1A* polymorphism and blood pressure in both normoglycemic and hyperglycemic subjects (Thomas, et al., 2001). Interestingly, a significant linear relationship between the polymorphism and blood pressure was obtained. Increased proportions of the C allele come along with higher mean arterial pressure (Thomas, et al., 2001). Therefore, there is no clear consensus on the association between *DRD2 Taq1A* polymorphism (rs1800497) and hypertension to date.

2.5.3 Pathogenesis of Hypertension in relation to *DRD2* Polymorphism

The *DRD2* is located along the dopaminergic nerves. D2-like receptors can mediate vasoconstriction or vasodilation in response to blood pressure changes. However, the exact role of *DRD2* in hypertension remains unclear because of the deficient in selective D2 receptor agonists and antagonists (Zeng, et al., 2007).

As part of the baroreceptor reflex pathway, the *DRD2* in the brain can be involved in blood pressure regulation (Cornish and Buuse, 1995). The sympathetic nervous system is usually hyperactive in hypertension. This hyperactivity is controlled by negative feedback regulation through the action of presynaptic α_2-adrenergic and *DRD2* (Mannelli, et al., 1997). Yet, the T allele has been previously reported to be associated with reduced *DRD2* binding in the human brain (Thompson, et al., 1997). So, the lower *DRD2* expression may reduce the dopamine binding and hence impair the dopamine ability to inhibit the release of norepinephrine, leading to increased norepinephrine-mediated vasoconstriction (Thomas, Tomlinson, and Critchley, 2000).

In addition to the central dopamine receptor system, dopamine also influences blood pressure regulation through peripheral dopaminergic receptors (Fang, et al., 2005). Gordon, et al. (1989) found out that abnormal dopaminergic regulation of aldosterone secretion mediated by *D2DR* might also play role in the pathophysiology of hypertension. The *DRD2* is involved in the dopaminergic inhibition of aldosterone secretion. Disruption of the *DRD2* in *Taq1A* variant may impair the dopamine action, leading to hyperaldosteronism and hypertension (Jose, Eisner and Felder, 1998). Moreover, the counter-regulatory system which is responsible for the increased levels of the renal dopamine to counteract the elevated tubular sodium reabsorption may also subject to impaired signal transduction due to the disruption of *DRD2* (Fang, et al., 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Specimen and Sample Population

The specimen used for Single Nucleotide Polymorphism (SNP) genotyping in this study is extracted from buccal cells. This was collected from consenting participants through a simple mouth wash to extract the genomic DNA from cells. The list of chemicals and materials used in the genomic DNA extraction, Polymerase Chain Reaction (PCR), as well as the subsequent Restriction Fragment Length Polymorphism (RFLP) procedure were summarized in Table 3.1 and Table 3.2 respectively.

The population assembled in this research consists of students from Universiti Tunku Abdul Rahman (UTAR), Perak campus. The study duration was from October 2015 to December 2015. The total sample size was 145 and the study subjects were selected by random sampling method. Informed consent was obtained from each subject prior to participate in this research. The sample population comprised of 58 males (40%) and 87 females (60%). Majority of the population is from the Chinese ethnic group, which accounts for 123 students (84.8%), while the remaining 22 students (15.2%) are from Indian ethnic group. The figure for the sample population was acquired by calculation,

and 10% dropout rate (139 samples) was allocated to prevent uncertainty. The final number of individuals participated in this study was 145.

The margin of error, E was calculated as follow:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

$$n = \frac{(1.96)^2 (0.09)(0.91)}{(0.05)^2}$$

$$\therefore n = 126$$

≈ 139 samples with 10% margin of error

n = Sample size

Z = Z statistic for a level of confidence

d = Precision (in proportion of one)

P = Expected prevalence or proportion (in proportion of one)

** Proportion of COMT and DRD2 homozygous mutant genotype control group based on previous study were **0.08** and **0.09** respectively (Yeh, et al., 2010; Rosmond, et al., 2001).*

Table 3.1: List of chemicals used.

Chemicals	**Manufacturer**
Chloroform	MERCK, New Jersey
Chloroform isoamyl alcohol	Biotech, Malaysia
SDS solution	Prepared in-house
RNase A	PureLink, Malaysia
Proteinase K	Novagen, Germany
Saline solution (0.9% NaCl)	Pharmsafe, Malaysia
Cold absolute ethanol	MERCK, New Jersey
Tris-base boric acid EDTA	Norgen-Biotek, Canada
Agarose powder	Choice-Care, Malaysia
PCR green master mix	Promega, Malaysia
Pair wise primers	Integrated DNA Technologies, Malaysia
6× loading dye	Thermo Scientific, Malaysia
Ethidium bromide	Bio Basic, Canada
Lambda DNA/*Hind*III ladder	Vivantis, Malaysia
50 bp DNA ladder	Norgen, Malaysia
100 bp DNA ladder	Thermo Scientific, Malaysia
*Nla*III enzyme and buffer	Thermo Scientific, Malaysia
*Taq*I enzyme and buffer	Thermo Scientific, Malaysia

Table 3.2: List of consumables used.

Consumables / Instruments	**Manufacturer**
Conical flask, 50 mL	Favorit, Malaysia
PCR tubes	Axygen Scientific, USA
Pipettes	Watson Nexty, Japan
Pipette tips	Axygen Scientific, USA
Measuring cylinder (25, 50, 100 mL)	Favorit, Malaysia
Schott bottle, 1000 mL	Duran, Germany
Falcon tubes	Axygen Scientific, USA
Water bath	Memmert, Germany
Electrophoresis sets	Major Science, Taiwan
Fume hood	Esco, USA
4 °Freezer	Pensonic, Malaysia
Microwave	Sharp, Malaysia
Weighing machine	Adventurer, New Jersey, USA
PCR machine	Eppendorf, Germany
MutliDoc-It, bench-top UV-Transilluminator	VWR International LLC, USA

3.2 Overview of Methodology

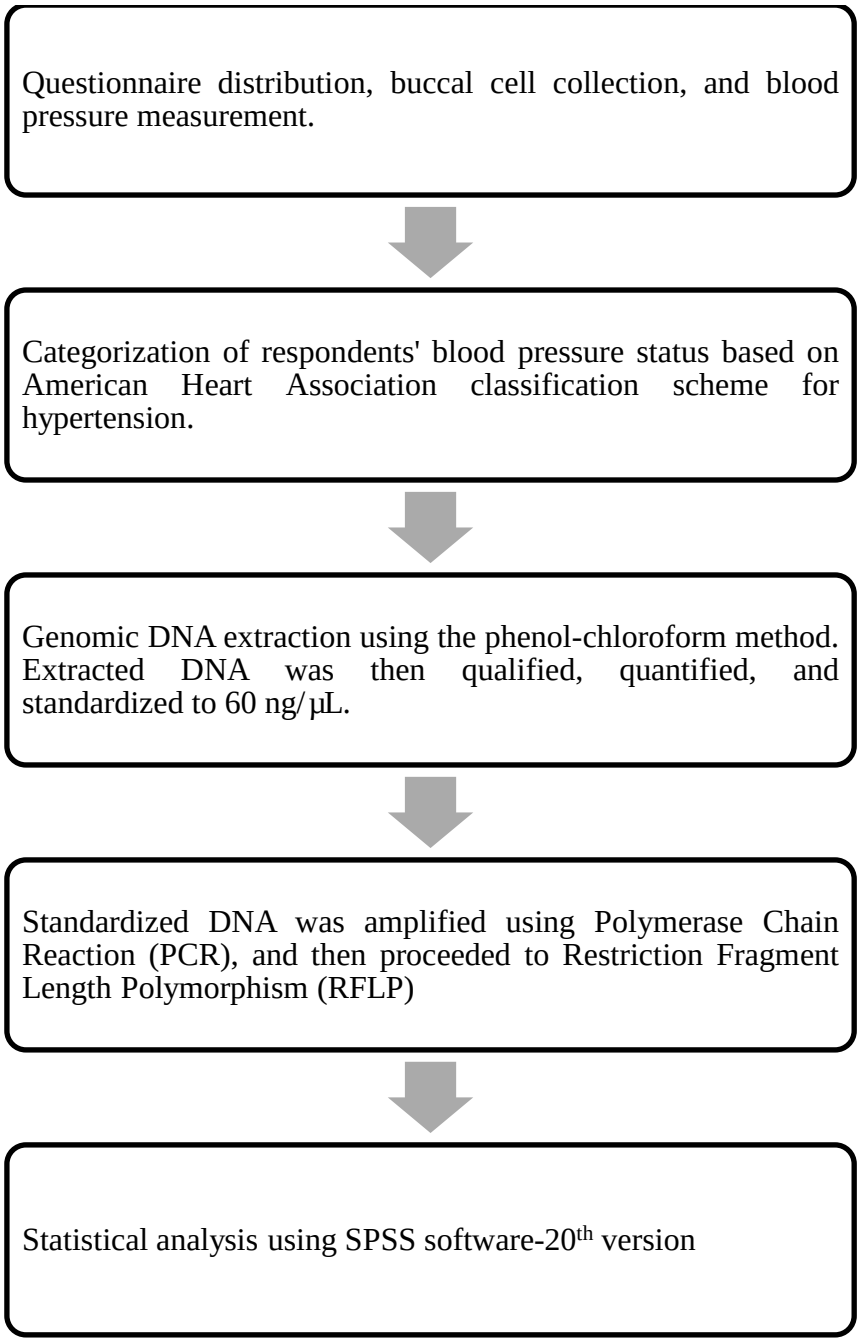


Figure 3.1: Overview of methodology.

3.3 Methodology

3.3.1 Consent Form and Anthropometric Survey

Prior to enrolling in this study, eligible subjects were given a consent form to enlighten them about the study, the potential risks and benefits of their participation, and their confidentiality rights. Upon reaching an agreement, a signature was obtained to represent that the subject is aware of the risks and is consenting to donate buccal cells for the genomic DNA extraction and further genotyping.

The questionnaire was attached together with the consent form. A questionnaire sample was attached in Appendix C. Subjects were required to fill up the questionnaire for data collection. Besides the normal parameters like age, gender, race, and education level, the questionnaire also sought to identify the health history, physical activity level, dietary behavior and nutrition intake, as well as stress management of each subject.

3.3.2 Blood Pressure Measurement

Apart from anthropometric parameters, all the subjects also underwent clinical assessments, which included the measurement of heart rate, systolic blood pressure (BP), and diastolic BP. BP was measured based on a standardized procedure by referring to the American Heart Association guidelines. The measurement was performed in the left arm using a digital sphygmomanometer after the subject had rested for 15 minutes in the sitting

position. All the measurements were taken thrice and the average value was calculated. BP was categorized based on the American Heart Association blood pressure classification reference (American Heart Association, 2016).

Table 3.3: Classification reference of blood pressure based on American Heart Association criteria.

Blood Pressure Category	Systolic (mm Hg)	Diastolic (mm Hg)
Normal	Less than 120	Less than 80
Prehypertension	120-139	80-89
Hypertension Stage 1	140-159	90-99
Hypertension Stage 2	160 or higher	100 or higher
Hypertensive Crisis	Higher than 180	Higher than 110

3.3.3 Buccal Cell Collection

Buccal cell collection poses minimal risk and discomfort to donor as it is a non-invasive method to collect sample from donor. Donors were advised not to consume any food or drink 30 min prior to sample collection. Upon consent, a Falcon tube which contained 15 mL of sterile saline (0.90% NaCl) solution was given to the donors. Firstly, donors were required to rinse the mouth with water to cleanse the mouth from food particles. After a brief cleaning, donors were advised to contain and rinse again with the saline solution provided for at least 2 min before expectorated the solution back to the tube. A mild massage of the cheeks against the tongue simultaneously during the mouthwash was

recommended to increase the yield of buccal cells. The sample contained the buccal cells was then used for genomic extraction for subsequent genotyping work.

3.3.4 Genomic Deoxyribonucleic Acid (DNA) Extraction

Genomic DNA was extracted from the buccal cells by employing simple phenol-chloroform method. The sample was then subjected to centrifugation at 10,000 rpm for 20 min and the resulted supernatant was discarded. The pellet was resuspended in 460 µL of Tris-EDTA (TE) buffer, 30 µL of 10% SDS, and 10 µL of Proteinase K (4 mg/mL). The mixture was mixed well and vortexed before it was transferred to a new microcentrifuge tube. The sample was incubated for 1 hour at 37^{o}C. After incubation, a volume of 500 µL Phenol-chloroform-isoamyl alcohol (PCI) in the ratios of 25:24:1 was added into the tube, and it was mixed well before centrifuged at 14,000 rpm for 10 min. After which, the supernatant was carefully removed to a new microcentrifuge tube without disturbed the tissue debris at the interface.

Subsequently, 20 µL of RNAse A (4 mg/mL) was added into the tube to remove other cellular contaminants. The tube was incubated at 37^{o}C for 5 min prior to centrifugation at 13,000 rpm for 10 min, and again the resulted supernatant was transferred to a new microcentrifuge tube. Then, a volume of 500 µL chloroform was added into the new tube and centrifuged again at 14,000 rpm for 10 min. After that, the top layer formed was transferred into a

new microcentrifuge tube followed by the addition of 2× volume of cold absolute ethanol and 40 µL of NaCl (5M) into the tube. The tube was mixed well and centrifuged at 14,000 rpm for 15 min. After the resulted supernatant was discarded, the pellet was washed with 500 µL of 70% ethanol. The pellet was then air-dried at room temperature for approximately 20 minutes. Lastly, the DNA pellet was dissolved in 50 µL of TE buffer and was stored at -20^{o}C refrigerator.

3.3.5 Quantification of Genomic DNA

The quantification of the extracted genomic DNA was conducted with the use of NanoPhotometer (Implen). The concentration and the purity of DNA were evaluated by measuring the absorbance of genomic DNA samples at the wavelength of 260 nm and 280 nm respectively. At the beginning, the NanoPhotometer was calibrated to ensure the accuracy and reliability of the measurements. Next, a Submicroliter Cell was inserted into the cell holder with the cell windows facing to the direction of the light beam passage. After everything was set, a volume of 2 µL genomic DNA sample was pipetted and transferred to the measurement window carefully. The window was then covered with a 1 mm path length lid prior to initiating the measurement. The absorbance readings at OD260 nm, OD280 nm, the ratio, as well as the genomic DNA concentration were recorded. Then, the measurement window and the lid were wiped with a Kimwipe paper before continue for the next genomic DNA sample.

The concentration of the genomic DNA was calculated based on the formula:

$$Genomic\ DNA\ concentration = OD260\ nm \times 50 \times 10 \times Dilution\ factor$$

OD260 nm: Absorbance reading at 260 nm

50 & 10: Substance specific factor for double-stranded DNA; Lid factor

Dilution factor: Set at 1.0 for genomic DNA

This formula was also used for the calculation of DNA concentration at the path length of 280 nm. On the other hand, the purity for each of the genomic DNA samples was examined through the ratio between OD260 nm and OD280 nm. In the end, all genomic DNA samples were standardized to a final concentration of 60 ng/μL. The standardized genomic DNA was used as the template for the subsequent Polymerase Chain Reaction (PCR) amplification.

3.3.6 Qualification of Genomic DNA by Gel Electrophoresis

Before the start of PCR amplification, the standardized genomic DNA was qualified by gel electrophoresis, with the utilization of 1× Tris-Boric acid-EDTA (TBE) buffer and 1% agarose gel. The 1× TBE buffer was prepared by mixing 10 mL of 100× TBE buffer with 90 mL of sterile distilled water in a conical flask.

In order to prepare 1% agarose gel that to be run in a big casting tray, 0.30 g of agarose powder was added into 30 mL of 1× TBE buffer. The mixture was boiled in a microwave to fully dissolve the agarose powder. The agarose mixture was then cooled down to approximately 50-60^{o}C before being poured into the gel casting tray. A 0.75 mm comb was inserted immediately into the casting tray and the agarose gel was allowed to solidify at room temperature for approximately 30 min. Once the gel was completely solidified, the comb was carefully removed and the agarose gel together with the casting tray was placed into a gel electrophoresis tank. The tank was then filled up with 1× TBE buffer until around 5 mm above the agarose gel was covered.

For each gel, the first lane was exclusively loaded with 2 µL of Lambda DNA/*Hind*III ladder. Prior to loading 2 µL of the genomic DNA samples into the wells, they were mixed with 1 µL of 6× loading dye on a piece of clean parafilm. Once everything was loaded into the wells, gel electrophoresis was executed at 100 V for 30 minutes. Upon completion, the agarsoe gel was stained with Ethidium Bromide for about 10 minutes and de-stained with distilled water for few seconds. The duration of staining and de-stain was largely depend on how fresh the Ethidium Bromide was. Ultimately, the gel image was viewed and captured under a UV-Transilluminator.

3.3.7 *COMT* Val158Met Genotyping

The forward primer sequence used was 5'-CGA GGC TCA TCA CCA TCG AGA TC-3' while the reverse primer was 5'-CTG ACA ACG GGT CAG GAA TGCA-3' (Lajin and Alachkar, 2011). Each PCR tube in a reaction contained 1 unit of *Taq* Master Mix, 0.4 µM of forward primer, 0.4 µM of reverse primer, DNA template, and sterile distilled water. The PCR components are summarized in Table 3.4 and Table 3.5.

The conditions used for PCR amplification was summarized in Table 3.6. The PCR amplification began with an initial pre-denaturation step at 94^{o}C for 5 min followed by 32 cycles of denaturation, annealing, and elongation at 94^{o}C for 30 sec, 62^{o}C for 30 sec, and 72^{o}C for 10 sec respectively. The final extension step was carried out at 72^{o}C for 5 min. The PCR products were then loaded on a 2% agarose gel and electrophoresis was performed at 90 V for approximately 35 min to confirm the presence of the desired amplicon size (108 base pairs).

Table 3.4: Primers for the *COMT* Val158Met.

Val158Met primers	Primer sequence (5'−3')	PCR amplicon (bp)
Forward	CGA GGC TCA TCA CCA TCG AGA TC	108
Reverse	CTG ACA ACG GGT CAG GAA TGCA	

Table 3.5: Volume and concentration of the PCR components used for PCR amplification.

PCR components	Stock concentration	Final concentration	Volume (µL)
2× *Taq* Master Mix	2×	1×	9.00
Forward primer	10 µM	0.4 µM	0.72
Reverse primer	10 µM	0.4 µM	0.72
DNA template	-	60 ng/µL	1.00
Sterile dH_2O	-	-	6.56
Total			18.00

Table 3.6: Conditions for PCR amplification in *COMT* Val158Met.

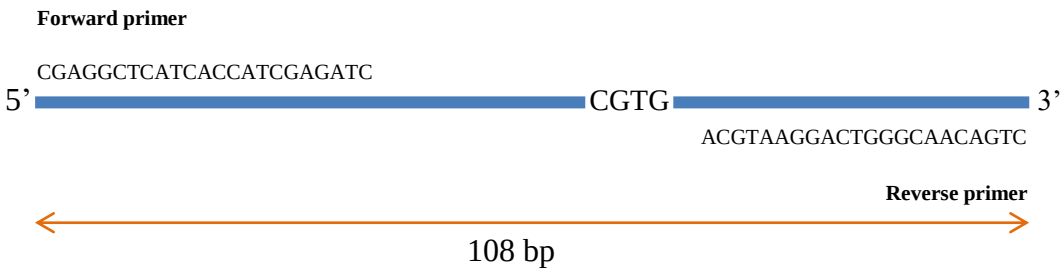
Stage	Temperature (^oC)	Duration	Cycle
Pre-denaturation	94	5 min	1
Denaturation	94	30 sec	32
Annealing	62	30 sec	
Elongation	72	10 sec	
Final extension	72	5 min	1
Hold	10	∞	-

Once the presence of amplicon at desired size was confirmed, the PCR products were digested with *Nla*III (*Neisseria lactamica*) restriction enzyme, which was commercially purchased. The recognition sequences and cutting sites of *Nla*III have been illustrated in Figure 3.2. Meanwhile, the components of restriction enzyme reaction were summarized in Table 3.7. After all the components were added and mixed evenly, the mixtures were incubated in a 37^oC water bath for a minimum of 1 hour. After that, the samples were loaded on 3% agarose gel and electrophoresed at 90 V for about 35 minutes in order to determine the genotype. The homozygous wild type (Val/Val) genotype showed only one band at 108 bp. Three bands were seen at 108 bp, 72 bp, and 36 bp for the heterozygous (Val/Met) genotype. The homozygous mutant (Met/Met) genotype showed two bands at 72 bp and 36 bp.

Table 3.7: Components of restriction enzyme reaction.

RFLP components	Volume (µL)
dH_2O	8.2
10 × RE buffer	1.5
PCR product	5.0
Restriction enzyme	0.3
Total	15.0

Wild type (Val allele)



Mutant (Met allele)

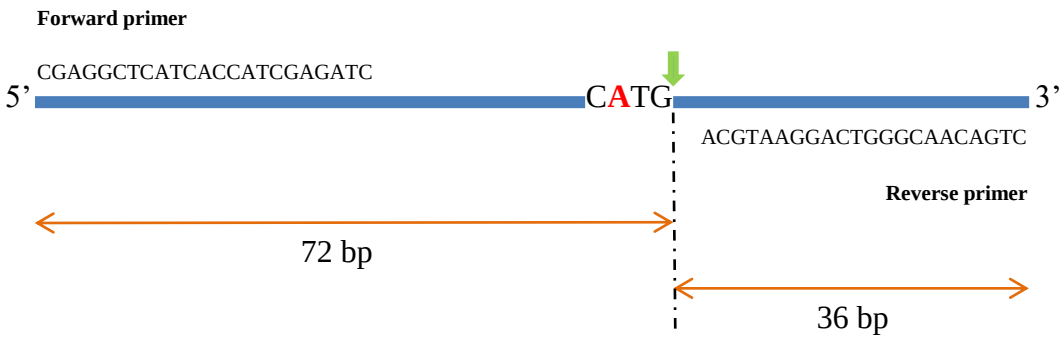


Figure 3.2: A schematic illustration of the expected fragment size showing the location of *Nla*III recognition sequence (CATG) and its cutting site. Fragment is not drawn at scale. The red bold letter represents the SNP for the *COMT* Met allele. The downward arrow "↓" indicates the cutting site of the *Nla*III.

3.3.8 *DRD2 Taq1A* Genotyping

The forward primer sequence used was 5'-GCA CGT GCC ACC ATA CCC-3' while the reverse primer was 5'-TGC AGA GCA GTC AGG CTG-3' (Lawford, et al., 2012). The PCR components used for PCR amplification in *DRD2 Taq1A* were same as the *COMT* Val158Met, which had been summarized in Table 3.5. The thermal cycling parameters used in this amplification started with 1 cycle of pre-denaturation at 94^{o}C for 4 min. Consecutively, 35 cycles of 94^{o}C for 30 sec, 68^{o}C for 30 sec, 72^{o}C for 30 sec, and a final extension step at 72^{o}C for 3 min (Table 3.9). The PCR products were then loaded on a 2% agarose gel and electrophoresis was performed at 90 V for approximately 35 min to qualify the presence of the desired amplicon size (501 base pairs).

Table 3.8: Primers for the *DRD2 Taq1A*.

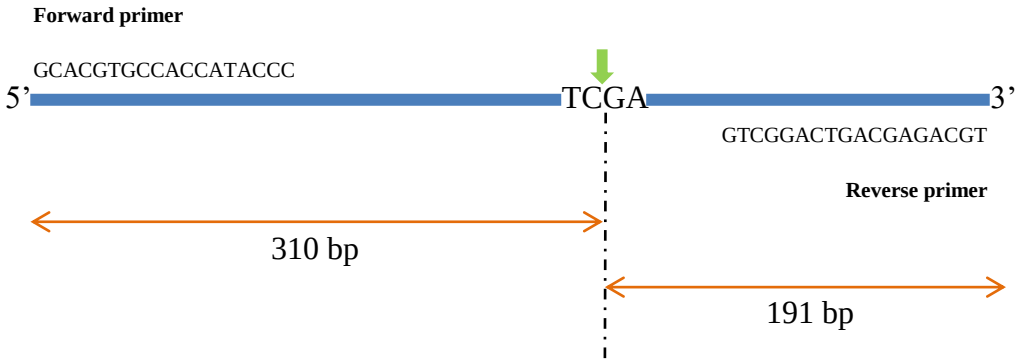
Taq1A primers	Primer sequence (5'−3')	PCR amplicon (bp)
Forward	GCA CGT GCC ACC ATA CCC	501
Reverse	TGC AGA GCA GTC AGG CTG	

Table 3.9: Conditions for PCR amplification in *DRD2 Taq1A*.

Stage	Temperature (^{o}C)	Duration	Cycle
Pre-denaturation	94	4 min	1
Denaturation	94	30 sec	35
Annealing	68	30 sec	
Elongation	72	30 sec	
Final extension	72	3 min	1
Hold	10	∞	-

After PCR amplification, the PCR products were digested with *Taq*I restriction enzyme (*Thermus aquaticus* YTI), which was commercially purchased. The recognition sequences and cutting sites of *Taq*I have been illustrated in Figure 3.3. The components of restriction enzyme reaction were similar to the previous gene, which had been summarized in Table 3.7. After all the components were added and mixed evenly, digestion was ensured to be completed following incubation at 37^{o}C water bath for a minimum of 4 hours. After that, the samples were loaded on 3% agarose gel and electrophoresed at 90 V for about 35 minutes in order to determine the genotype. The homozygous wild type (C/C) genotype showed two bands at 310 bp and 191 bp whereas the homozygous mutant (T/T) genotype showed only one band at 501 bp. Three bands were seen at 501 bp, 310 bp, and 191 bp for the heterozygous (C/T) genotype.

Wild type (C allele)



Mutant (T allele)

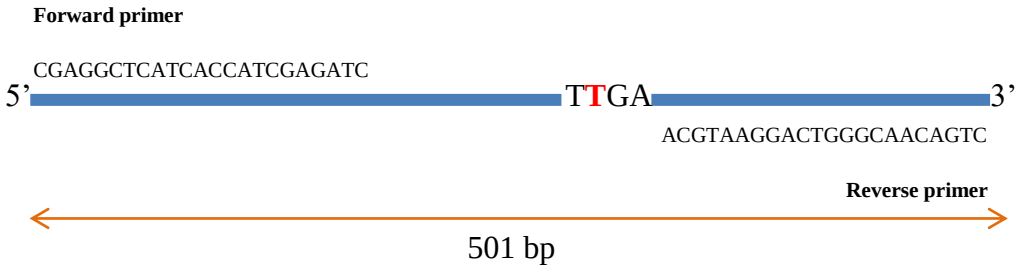


Figure 3.3: A schematic illustration of the expected fragment size showing the location of *Taq*I recognition sequence (TCGA) and its cutting site. Fragment is not drawn at scale. The red bold letter represents the SNP for the *DRD2* T allele. The downward arrow "↓" indicates the cutting site of the *Taq*I.

3.3.9 Statistical Analysis

Analysis was performed separately for each polymorphism. Prevalence of hypertension, frequency of each blood pressure category as well as other demographic data obtained from the survey was expressed in percentages together with 95% confidence intervals. For each polymorphism, the allelic frequencies and genotypic frequencies were calculated. The hypothesis that the mutant allele is associated with increased risk of hypertension in the positive group was tested by employing the Hardy-Weinberg equilibrium. Besides that, the crosstab functions and the Pearson's chi-square test were utilized to identify whether BP was related to gender, family history of hypertension, stress, dietary factors, and sedentary behavior. All analyses were conducted using SPSS software version 20.0. A p-value < 0.05 was considered statistically significant.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Epidemiology of Hypertension among UTAR Kampar Students

In this study, a total of 145 study subjects were recruited. Statistical analysis of the sample population revealed that it was comprised of 58 males (40%) and 87 females (60%). Of these, the population is predominantly Chinese ethnic group, which accounts for 123 students (84.8%), while Indians make up the remaining portion with only 22 students (15.2%). The overwhelming number of Chinese participants is basically attributed to the fact that the vast majority of students in UTAR Kampar campus are Chinese. The basic demographic information for the UTAR Kampar students participated in this study was summarized in Table 4.1.

The study population was assembled from various faculties, which includes Center for Foundation Studies (CFS), Faculty of Arts and Social Science (FAS), Faculty of Business and Finance (FBF), Faculty of Information, Communication and Technology (FICT), Faculty of Engineering and Green Technology (FEGT), and Faculty of Science (FSc). Majority of the recruited subjects were undergraduate students (71.7%) while some of them were only in their foundation years (20.7%). In addition to that, 7.6% of the study subjects were seeking master's degrees. Furthermore, 89% of the study population was students with Science background whereas the remaining 11%

comprised of students with Arts background. The age group of the study population ranged from 18 to 27 years. Since the target population is university students, most of the participants were under the age group of 21-23, with the average age of 21.35 $\pm$ 2.149.

Table 4.1: Demographic characteristics of the 145 recruited subjects.

Variable	**Subjects (n = 145)**	
	Males	Females
Age		
18-20	16 (27.6%)	29 (33.3%)
21-23	35 (60.3%)	43 (49.4%)
24-27	7 (12.1%)	15 (17.3%)
Stream of study		
Science	50 (86.2%)	79 (90.8%)
Art	8 (13.8%)	8 (9.2%)
Education level		
Foundation	9 (15.5%)	21 (24.1%)
Undergraduate	43 (74.1%)	61 (70.1%)
Postgraduate	6 (10.4%)	5 (5.8%)
Race		
Chinese	52 (89.7%)	71 (81.6%)
Indian	6 (10.3%)	16 (18.4%)
BMI*		
Obese ($\geq$ 30)	6 (10.4%)	1 (1.2%)
Overweight (25-29.9)	12 (20.7%)	13 (14.9%)
Healthy range (18.5-24.9)	30 (51.7%)	52 (59.8%)
Underweight ($\leq$ 18.5)	10 (17.2%)	21 (24.1%)

*Cut-off points used were based on the standard that has been set by the World Health Organization.

4.1.1 Prevalence of Hypertension among UTAR Kampar Students

In this study, among the total 145 participants, 54 (37.2%) were prehypertensive and 3 (2.1%) were suffering stage 1 hypertension. Due to fewer numbers of respondents belong to the hypertension category, the prehypertension category and hypertension category were integrated during statistical analysis. Therefore, an overall prevalence of prehypertension and hypertension was calculated to be 39.3% in this cohort (Figure 4.1). This finding was slightly higher compared to the 32.7% reported by Ministry of Health Malaysia (2014). Besides that, in a study conducted in Penang, the prevalence of hypertension was reported as 32.2% in general population (Tee, et al., 2010). Thus, the prevalence was lower in previous studies as they covered the general population, whereas our study was done among university students. As a support for this, a related study in Universiti Malaysia Sarawak (UNIMAS) also reported a prevalence of prehypertension and hypertension as 42.9% and 12.8% respectively out of 237 undergraduate students, which was closer to our finding (Lee, et al., 2010).

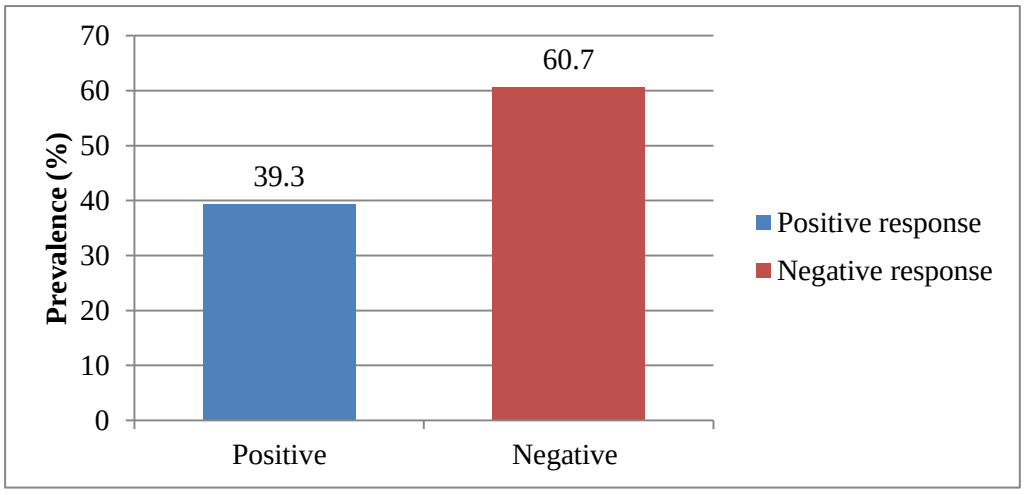


Figure 4.1: Responses of participants to hypertension: Positive response (prehypertension or hypertension); negative response (normal blood pressure).

4.1.2 Evaluating Gender and BMI Differences in Hypertension

Both gender and BMI differences in hypertension were assessed. When comes to the gender distribution in the positive responses, it was found that males accounted for 63.2%, while 36.8% were females (Figure 4.2). Besides that, the analysis also showed a statistical significance that can correlate gender to hypertension ($p < 0.05$). This finding is consistent with the results from a study conducted by Soliman, et al. (2014), where higher proportion of males with hypertension was noted. Similarly, Patnaik and Choudhury (2015) also demonstrated that male subjects had higher prevalence of hypertension than female. The age of participants for both studies was between 17−22 years and 18−21 years, respectively. Moreover, according to American Heart Association (2014), it has been commonly accepted that hypertension is much more prominent in men until age 45. Up to now, the underlying mechanisms for the gender-associated differences in blood pressure still remain unknown. However, substantial evidences have supported that androgens might be one of the contributing factors for it (Reckelhoff, 2001).

As for the prevalence of excessive weight gain, the analysis of body mass index (BMI) showed that 4.8% of the individuals were obese, and 17.2% were overweight (Table 4.1). Our data demonstrated that a large proportion of students had a healthy range of body weight, and there was no statistical significance between BMI and hypertension ($p = 0.137$). The distribution of BMI for normotensive and hypertensive individuals is shown in Figure 4.3. In this case, our findings were contradict with published suggestions of

significant positive correlation between BMI and hypertension (Suwarna, Vaishali and Sudeepa, 2012; Dua, et al., 2014). Nonetheless, the implication of relative body weight on pathophysiology of hypertension has never been clearly elucidated. BMI does not reflect linearly with the changes in body composition and body fat amount. In addition to that, to be more detailed, the body composition and fat distribution between males and females are different. BMI should thereby be considered only as an indicator for other causal factors; either it is hormonal changes, diet or other factors that link excessive weight gain to hypertension (Kaufman, et al., 1997).

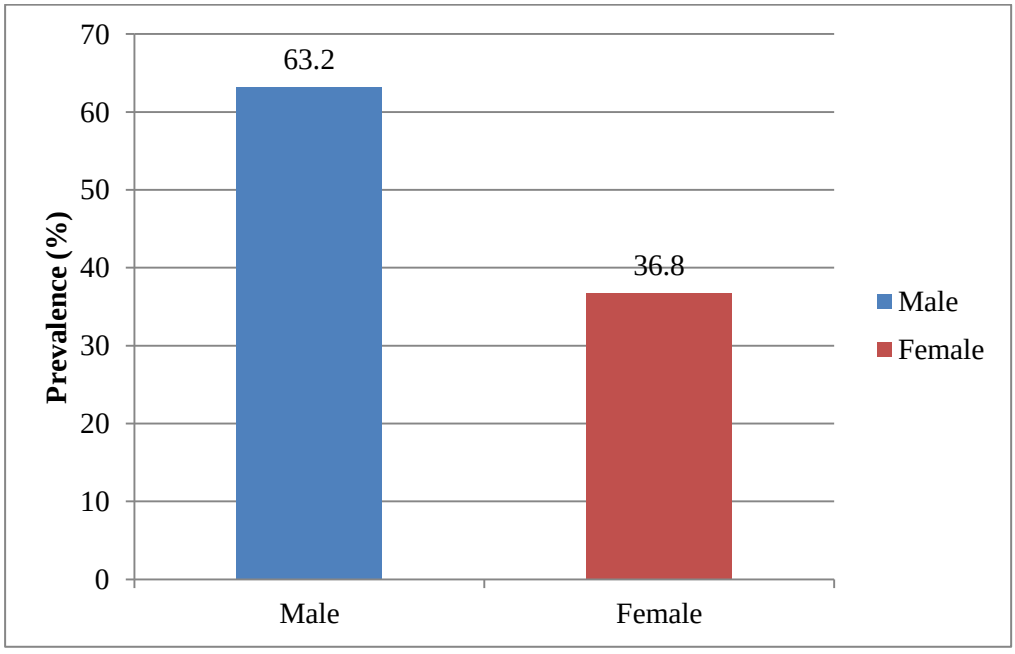


Figure 4.2: Prevalence of hypertension in male and female.

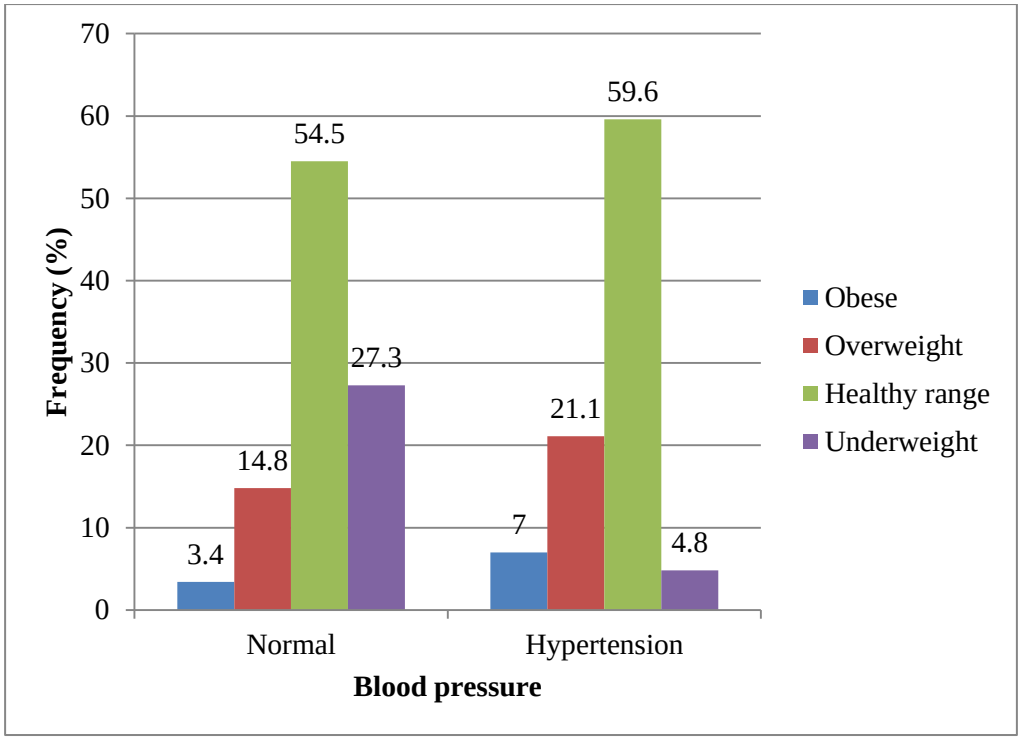


Figure 4.3: BMI distribution for normotensive and hypertensive individuals.

4.2 Socio-demographic Analysis

Apart from the demographic factors, the questionnaire distributed also contained components for sociological factors, which could help in understanding the probable exogenous risk factors for hypertension. These components seek to know the health history of the participants that may related to hypertension, and the way they manage their blood pressure. In addition to that, through the questionnaire also, the level of physical activity, dietary behavior and nutrition intake, and stress management of the participants were assessed. All the findings are summarized in Table 4.2 alongside with 95% confidence intervals (CI).

Table 4.2: Socio-demographic characteristics of the 145 recruited subjects.

Variable	Subjects (n = 145)			Confidence interval
	Yes	No	Unsure	
Part A: Health history				
High blood pressure	3 (2.1%)	116 (80.0%)	26 (17.9%)	-
Vascular disorders	1 (0.7%)	134 (92.4%)	10 (6.9%)	-
Neurological disease	-	142 (97.9%)	3 (2.1%)	-
Homeostatic disorders	2 (1.4%)	137 (94.5%)	6 (4.1%)	-
Glaucoma disease	-	141 (97.2%)	4 (2.8%)	-
High cholesterol	6 (4.1%)	131 (90.3%)	8 (5.5%)	0.9-7.3
Psychiatric disorders	6 (4.1%)	128 (88.3%)	11 (7.6%)	0.9-7.3
Family hypertension	63 (43.4%)	61 (42.1%)	21 (14.5%)	35.3-51.5
Family diabetes mellitus	60 (41.4%)	66 (45.5%)	19 (13.1%)	33.4-49.4
Family heart disease	38 (26.2%)	88 (60.7%)	19 (13.1%)	19.0-33.4
Family neurological disease	1 (0.7%)	127 (87.6%)	17 (11.7%)	-
Alcohol drinking	29 (20.0%)	116 (80%)	NA	13.5-26.5
Smoking	-	145 (100%)	NA	-
Part B: Physical activity				
Regular exercise	103 (71.0%)	42 (29.0%)	NA	63.6-78.4
Part C: Dietary behavior and nutrition intake				
Eat salty				
• Very salty	2 (2.9%)			
• A little salty	9 (13.2%)			
• Modestly	42 (61.8%)			
• Little salty	14 (20.6%)			
• Not salty	1 (1.5%)			
Caffeine intake				
• Low	124 (85.5%)			
• High	21 (14.5%)			

Table 4.2: (Continued)

Variable	Subjects (n = 145)			Confidence interval
	Yes	No	Unsure	
Frequency of fried food				
• > 1 a day	10 (14.7%)			
• 3-6 a week	19 (27.9%)			
• 1-2 a week	20 (29.4%)			
• 2-3 a month	16 (23.5%)			
• < 1 a month	3 (4.4%)			
Part D: Stress				
Stress level				
• None	3 (2.1%)			
• Mild	35 (24.1%)			
• Moderate	77 (53.1%)			
• High	27 (18.6%)			
• Very high	3 (2.1%)			
Manage stress well	103 (71.0%)	42 (29.0%)	NA	63.6-78.4
Had time of depression	59 (40.7%)	86 (59.3%)	NA	32.7-48.7
Had mood swings	67 (46.2%)	78 (53.8%)	NA	38.1-54.3
Worry excessively	59 (40.7%)	86 (59.3%)	NA	32.7-48.7
Preoccupied with food and body image	40 (27.6%)	105 (72.4%)	NA	20.3-34.9
Concerned about academic performance	93 (64.1%)	52 (35.9%)	NA	56.3-71.9

4.2.1 Evaluating Health History of Participants

From the socio-demographic analysis (Table 4.2), it was found that only minority of the participants claimed to have history of hypertension (2.1%), vascular disorders (0.7%), homeostatic disorders (1.4%), or high cholesterol level (4.1%). This part of the questionnaire sought to find out that whether the participants have any history of hypertension, and to identify the underlying causes of secondary hypertension, if present. Besides that, it was also used to

determine whether hypertension has caused any complications at the kidneys, heart, or eyes.

Among all the 145 participants in this study, about 43.4% of them claimed that at least one of their family members or first-degree relatives was suffering from hypertension. Nonetheless, out of the 63 participants who claimed that they have a family history of hypertension, only 24 of them (38.1%) were hypertensive. Statistical analysis also revealed that there was no significant association between family history of hypertension and increased risk of developing hypertension in the present cohort ($p = 0.178$). This finding contradicts with extant research, which has reported correlation between prevalence of hypertension and family history of hypertension (Ranasinghe, et al., 2015). In their study, a significantly higher blood pressure was noted in those subjects with a family history of hypertension than those without a family history ($p < 0.001$). Furthermore, their finding was in keeping with previous study conducted by Masuo, et al. (1998). This implies that hypertension is subjected to genetic susceptibility. For instance, the British Genetics of Hypertension (BRIGHT) study had found a principle locus on chromosome 6q related to hypertension (Caulfield, et al., 2003). This study involved a group of severely affected sibling pairs, which were selected from families with history of hypertension. This might imply that onset of hypertension is under the influence of genetics.

So, the opposing results obtained in this study might be caused by some study limitations. The major limitation of this study was the fact that family history was evaluated through a simple questionnaire. This could create a recall bias for family history. Moreover, previous studies have also shown that individuals with a positive family history tend to be missed out by a simple enquiry as compared with a detailed questionnaire (Wijdenes-Pijl, et al., 2011).

4.2.2 Relating Physical Activity to Hypertension

When considering the engagement in physical activity for the recruited subjects, our data demonstrated that majority of the students got involved in regular physical activity. There were up to 103 students (71.0%) answered "yes" for having regular physical activity while 42 students (29.0%) answered "no" (Table 4.2). When assessing physical activity between the two genders, it was found that more females (22.1%) were physical inactive as compared to males (6.9%), with a statistical significance ($p = 0.011$). Al-Hazzaa, et al. (2012) reported a similar result in Saudi youngsters, in which sedentary lifestyle was more commonly seen among females. This part of results has been summarized in Table 4.3. As for the prevalence of regular physical activity among the response group, the analysis showed that there was no significant difference between the positive cases and control group ($p = 0.571$). Surprisingly, there were up to 73.7% of hypertensive subjects in this cohort asserted of having regular physical activity (Table 4.3). In addition, no association was detected between duration of exercise and hypertension ($p = 0.438$). The relative data is illustrated in Figure 4.4.

Table 4.3: Physical activity engagement with respect to gender and response group in the population.

Variable	Regular physical activity (n = 145)		p-value
	Yes	No	
Gender			
Male	48 (82.8%)	10 (17.2%)	0.011*
Female	55 (63.2%)	32 (36.8%)	
Response group			
Positive	42 (73.7%)	15 (26.3%)	0.571*
Control	61 (69.3%)	27 (30.7%)	

*p < 0.05 shows significant difference

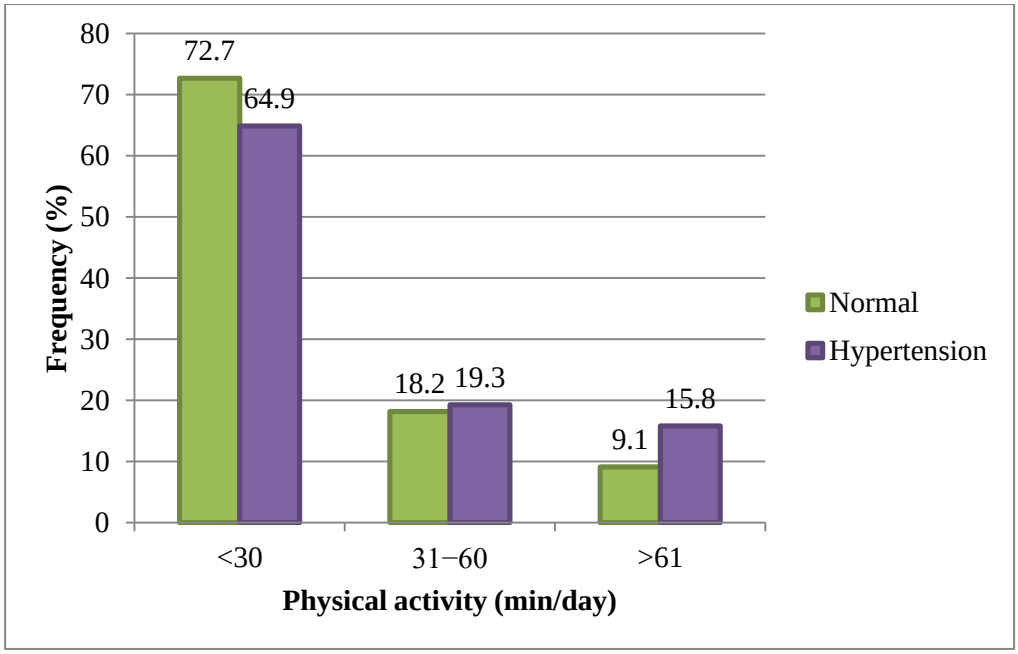


Figure 4.4: Frequency distribution of physical activity duration between positive cases and control group.

Numerous studies have previously demonstrated that physical activity is associated with hypertension (Ishikawa and Ohta, 2000; Staffileno, et al., 2007). Besides that, in their case-control study, Sagare, Rajderkar and Girigosavi (2011) reported that there was a linear trend between leisure time

physical inactivity and higher blood pressure. The underlying mechanisms responsible for the positive impact of physical activity on hypertension remain uncertain. Despite that, decrease in plasma norepinephrine levels and renin activity alongside with reduced vascular resistance have been previously reported as the primary factors for the reduction in blood pressure after exercise (Cornelissen and Fagard, 2005). So, it is generally accepted that engagement in physical activity of appropriate duration and intensity comes with a lower risk of developing hypertension.

On the contrary, Yoon and So (2013) recently reported that physical activity had little or no association with hypertension in a population of Korean adults. Moreover, Mkhonto, Labadarios and Mabaso (2012) also reported physical activity was not associated with hypertension status in South African adults. The results of our study concur with the findings of these studies in that frequency and duration of physical activity have no association with hypertension. However, these differences could be due to the study limitation. Our study did not directly assess or measure the frequency, duration, intensity, and the type of exercise that each subject involved. Hence, since all data were self-reported, they might subject to potential biases.

4.2.3 Relating Consumption of Fried Foods to Hypertension

The main characteristics of the participants' dietary behavior and nutrition intake are given in Table 4.2. The list of fried foods that commonly consumed by Malaysians is demonstrated in Appendix A. In terms of fried food consumption, highest response was seen in the category of 1-2 times/week while lowest response in the category of less than 1/month. The interaction between fried food consumption and gender was not statistically significant ($p = 0.090$). Additionally, the Pearson's chi-square test between the frequency of fried food consumption and blood pressure status also showed that they were no associated ($p = 0.632$). This indicates that high frequency of fried food intake did not come with a higher risk of developing hypertension. The results have been tabulated in Table 4.4.

Table 4.4: Frequency of fried food consumption according to gender and response group in the population.

Variable	**Frequency of fried food (n = 68)**					**p-value**
	>1/day	3-6/week	1-2/week	2-3/month	<1/month	
Gender						
Male	6 (19.4%)	12 (38.7%)	9 (29.0%)	3 (9.7%)	1 (3.2%)	0.090
Female	4 (10.8%)	7 (18.9%)	11 (29.7%)	13 (35.2%)	2 (5.4%)	
Response group						
Positive	3 (15.0%)	5 (25.0%)	8 (40.0%)	4 (20.0%)	0 (0%)	0.632
Control	7 (14.6%)	14 (29.2%)	12 (25.0%)	12 (25.0%)	3 (6.2%)	

No association was found between consumption of fried foods and incidence of hypertension in the cohort studied. Several studies have shown the risk of hypertension was associated with the consumption of fried foods (Soriguer, et al., 2003; Sayon-Orea, et al., 2014). The increased amount of trans-fatty acids in fried foods has been reported as the predisposing factor for higher risk of hypertension following frequent fried food consumption (Wang, et al., 2010). However, results were not always consistent as contradicting findings have been reported. A recent study conducted in Korea has reported that there was no distinct association between fried food consumption and hypertension in men but association was found in Korean women (Kang and Kim, 2016). Hence, the relationship between intake of fried food and hypertension remains controversial.

The plausible mechanism for these differences might be explained by the various factors involved during the frying process. For example, the frying technique (pan frying or deep frying), the type of food and oil used, and the extent of oil degradation. All these factors will affect the amount of trans-fatty acids generated (Boskou, et al., 2006). Thus, this mechanism may account for the differences in the present study as Wang, et al. (2010) have found a positive correlation between higher trans-fatty acids intake and increased risk of hypertension. Another possible reason could be due to the social desirability bias because the participants might perceive fried food as unhealthy and under-report the frequency.

4.2.4 Relating Stress Level to Hypertension

In order to evaluate the association between stress and hypertension, a self-assessment of stress level was included in the questionnaire. Our data showed that about 53.1% of the participants experienced moderate level of stress while 18.6% of them had high level of stress (Table 4.2). The interaction between gender and stress was tested. It was found that more females (13.8%) experienced high level of stress than males (4.8%), and the association was statistically significant ($p = 0.036$). These results were presented in Table 4.5. It has been well-established that females are more likely to experience emotional stress than males (American Psychological Association, 2010). Gender differences in response to stress have been reported previously. Women were reported to have greater sadness and anxiety than men after stress induction (Chaplin, et al., 2008). However, in our study, only the stress variable of "had time of depression" came with a good degree of statistical significance ($p = 0.013$) in gender differences (Table 4.6). Although the specific causes for the gender differences in emotional responses to stress are not clear, it has been suggested that extra workload in females such as household chores and child care might be a possible explanation (Lundberg, 2005).

Table 4.5: The results of stress analysis across gender and response group.

Variable	Stress level (n = 145)					p-value
	None	Mild	Moderate	High	Very high	
Gender						
Male	1 (1.7%)	21 (36.2%)	29 (50.0%)	7 (12.1%)	0 (0%)	0.036
Female	2 (2.3%)	14 (16.1%)	48 (55.2%)	20 (23.0%)	3 (3.4%)	
Response group						
Positive	1 (1.7%)	18 (31.6%)	28 (49.1%)	10 (17.6%)	0 (0%)	0.345
Control	2 (2.3%)	17 (19.3%)	49 (55.7%)	17 (19.3%)	3 (3.4%)	

Table 4.6: Stress variables with respect to gender.

Variable*	Gender		p-value
	Male (n = 58)	Female (n = 87)	
Had time of depression	14 (24.1%)	45 (51.7%)	0.013
Less interested in doing things	18 (31.0%)	19 (21.8%)	0.177
Had mood swings and irritability	21 (36.2%)	46 (52.9%)	0.142
Worry excessively	20 (34.5%)	39 (44.8%)	0.445
Nervous or anxious	13 (22.4%)	22 (25.3%)	0.376
Preoccupied with food or body image	16 (27.6%)	24 (27.6%)	0.906
Concern about academic performance	35 (60.3%)	58 (66.7%)	0.175

*Participants were allowed to pick more than one answer.

Previous studies have reported that psychological stress was associated with elevated blood pressure and increased risk of hypertension (Tobe, et al., 2007; Hu, et al., 2015). It has been hypothesized that sympathetic hyperactivity during prolonged stress could directly increase the cardiovascular reactivity and lead to hypertension (Gasperin, et al., 2009). Meanwhile, stress is also believed to have indirect effects on hypertension. For example, stress has been found to be associated with other risk factors of hypertension such as alcohol abuse, smoking, and obesity (Schuler and O'Brien, 1997). Yet, contradicting results were obtained by Fauvel and his colleagues (2003). Our results demonstrated that no significant association was found between psychological stress and hypertension. In a study conducted by Chaplin, et al. (2008), although females had shown to have greater emotional arousal following stress, their systolic blood pressure responses were similar with males' and lower diastolic blood pressure was actually noted in females. This suggests that there may not be a direct link between stress and hypertension. So, the relationship between stress and hypertension still remains debated and inconclusive.

Nevertheless, there were several limitations as well. First of all, this study was cross-sectional in design; hence, the causal factors cannot be inferred in this case. Secondly, another potential limitation of the present study was the involvement of only university students, because it is not a representative sample. The effect of psychological stress on blood pressure may vary across ages. So, this might have affected the generalizability of our findings.

4.3 *COMT* Val158Met Polymorphism in Hypertension

4.3.1 Evaluating Genomic DNA

Genomic DNA was extracted from buccal cells. The reason to choose buccal cell mouthwash sampling is simply because it is non-invasive, cost-effective, and most importantly it has been proven suitable for SNP study (Lum and Le Marchand, 1998). In the present study, the purity of genomic DNA extracted from the buccal cells was good (Mean = 1.7734 ± 0.1139 ng/μL). Generally, a "pure" DNA has a ratio of between 1.7−2.0. A ratio of less than 1.8 may indicate existing phenol or protein contamination in the samples. Meanwhile, a value larger than this range may represent the RNA contamination (Thermo Scientific, 2008).

The genomic DNA was qualified before the PCR amplification and restriction enzyme digestion steps were performed. All DNA samples were electrophoresed on a 1% agarose gel for approximately 30 minutes at 90V. An example of gel image is presented in Figure 4.5. The presence of bands on the gel indicates the successful extraction of genomic DNA. The DNA samples were then standardized to 60 ng/μL and electrophoresed on a 1% agarose gel again. The standardized genomic DNA appears to be more uniformly distributed in all the wells as illustrated in Figure 4.6.

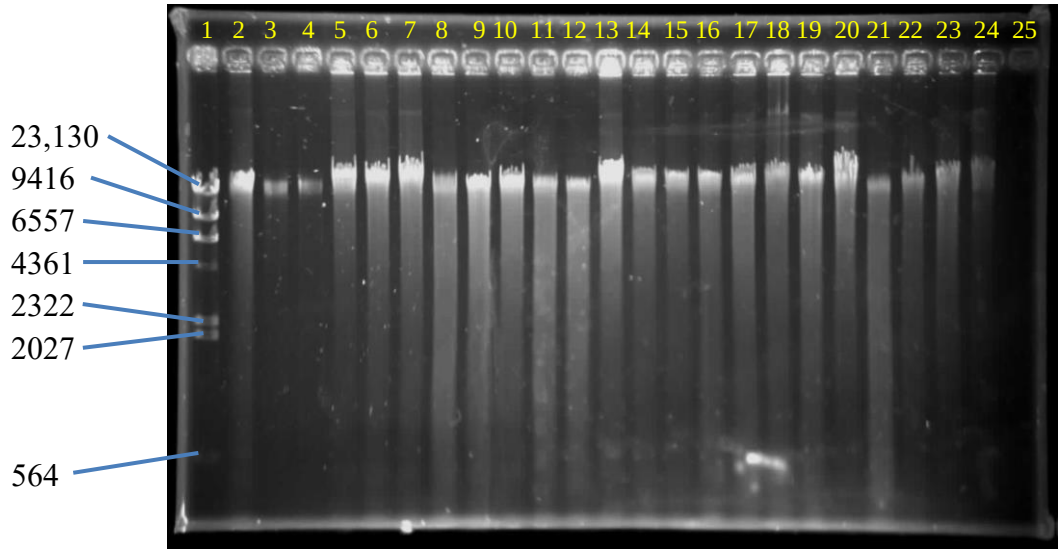


Figure 4.5: Gel image of unstandardized genomic DNA extracted from buccal cells (1% agarose gel). The Lambda DNA/*Hind*III ladder is loaded into lane 1. Subsequent lanes are loaded with genomic DNA samples. Lane 25 is loaded with distilled water as negative control.

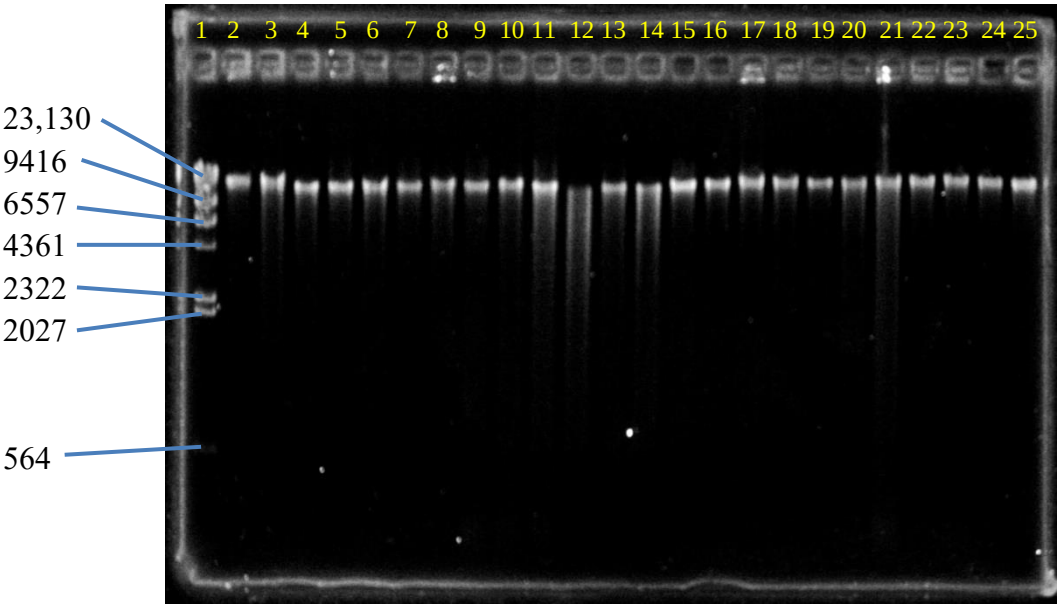


Figure 4.6: Gel image of 60 ng/µL standardized genomic DNA (1% agarose gel). The Lambda DNA/*Hind*III ladder is loaded into lane 1. Subsequent lanes are loaded with genomic DNA samples.

4.3.2 *COMT* Val158Met (rs4680) Genotyping

In this study, PCR was used to amplify a specific region of the *COMT* Val158Met for the subsequent RFLP analysis. The target DNA sequence is the 3' translated region of exon 4 of the *COMT* gene, with a total length of 108 base pairs. The PCR amplicon was electrophoresed on a 2% agarose gel (Figure 4.7).

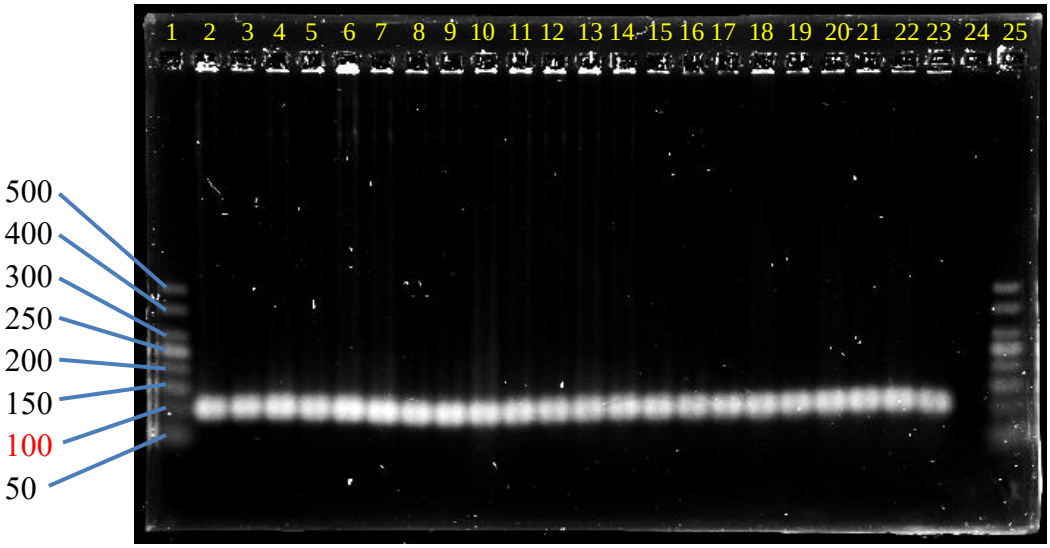


Figure 4.7: The PCR amplicons of targeted *COMT* gene fragment run on a 2% agarose gel. The 50 bp DNA ladder is loaded into lane 1 and lane 25. The subsequent lanes 2-23 are loaded with PCR products. Lane 24 is loaded with negative control. The amplicon size is 108 bp.

Upon confirm the presence of desired amplicon, RFLP was performed. The digested fragments were loaded on 3% agarose gel and electrophoresed to separate the fragments corresponding to their respective size. The restriction enzyme employed for *COMT* Val158Met gene variants is endonuclease *Nla*III, which recognizes the 5'-CATG-3' sequence. The results obtained on the

agarose gel correspond approximately to the expected results as what Lajin and Alachkar (2011) proposed. All the bands were well-separated and the genotypes could be clearly distinguished (Figure 4.8). Only one band at 108 bp was observed for the homozygous wild type genotype (Val/Val). Meanwhile, the heterozygous genotype (Val/Met) showed three bands at 108 bp, 72 bp, and 36 bp. For the homozygous mutant genotype (Met/Met), two bands were seen at 72 bp and 36 bp.

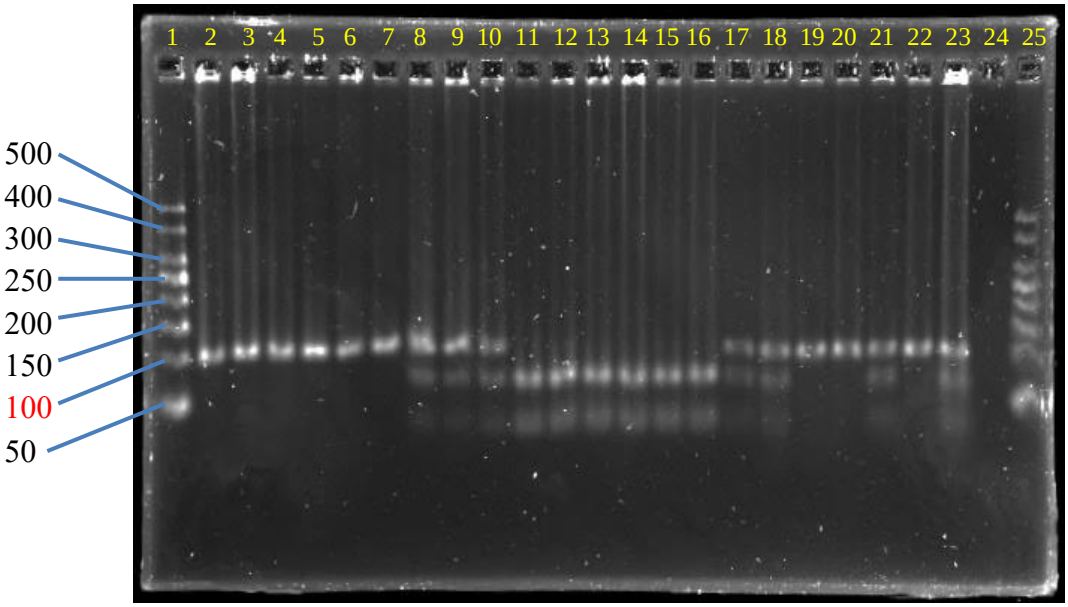


Figure 4.8: The three genotypes of *COMT* Val158Met gene variant on a 3% agarose gel. Lane 1 and lane 25 have been loaded with 50 bp DNA ladder. Lane 24 is designated to the negative control. The subsequent lanes 2-23 are loaded with samples. (Lanes 2-7, 19, 20, and 22: homozygous wild type; Lanes 8-10, 17, 18, 21, and 23: heterozygous; Lanes 11-16: homozygous mutant).

4.3.3 *COMT* Val158Met Genotypic and Allelic Frequencies of the Population

Upon completion of the RFLP process, the *COMT* Val158Met (rs4680) genotypes were identified from the agarose gel images. The results have been summarized in Table 4.7. The prevailing genotype is the Val/Val wild type genotype, which accounts for about 49% of the study population. No statistically significant difference was observed between gender and genotype distribution ($p = 0.977$).

The aim of the present study was to examine the possible involvement of *COMT* gene variants in the risk of developing hypertension. It was hypothesized that the mutant Met allele is a genetic risk factor for hypertension. Study has shown that individuals with homozygous mutant genotype have up to 4-fold variation in *COMT* enzyme activity, thereby resulting increased concentration of plasma catecholamines (Egan, et al., 2001). Thus, with the increased level of catecholamines in the body, it is speculated that they will bind to the α- or β-receptors and exert a series of cardiostimulatory effects, which include vasoconstriction, increased total peripheral resistance, and increased cardiac output (Goldberg, 1972).

In the present study, it was found that out of the 57 positive respondents, about 43.9% of them carry the heterozygous Val/Met genotype. On the other hand, there were up to 34 students (38.6%) from the control group who carried the

heterozygous genotype, which do not differ much from the positive responses. This data is presented in Table 4.8. The allelic frequency for *COMT* Val158Met was calculated to be 0.69 for the Val allele and 0.31 for the Met allele. These values are similar to that of other Chinese populations as well as Japanese population (Yeh, et al., 2010; Htun, et al., 2011). The results have been shown in Table 4.9. The detailed calculations are attached in Appendix B.

Table 4.7: Distribution of *COMT* Val158Met genotypes across gender.

Genotype	**Gender (n = 145)**		**p-value**
	Male	Female	
Wild type (Val/Val)	29 (50.0%)	42 (48.3%)	
Heterozygous	23(39.7%)	36 (41.4%)	0.977
(Val/Met)	6 (10.3%)	9 (10.3%)	
Mutant (Met/Met)			

Table 4.8: Genotype frequency of *COMT* Val158Met in positive response and control group.

Genotype	**Frequency (n = 145)**		**Total**
	Positive response	Control group	
Val/Val	25 (43.9%)	46 (52.3%)	71 (49.0%)
Val/Met	25 (43.9%)	34 (38.6%)	59 (40.7%)
Met/Met	7 (12.2%)	8 (9.1%)	15 (10.3%)

Table 4.9: Allele frequency of *COMT* Val158Met in positive response and control group.

Allele	**Frequency (n = 145)**	
	Positive response	Control group
Val	0.66	0.72
Met	0.34	0.28
Total	1.00	1.00

4.3.4 *COMT* Mutant Genotype (Met/Met) Does Not Confer Risk for Hypertension

The mathematical concept developed by Godfrey Hardy and Wilhelm Weinberg can be used to estimate the frequency of alleles in a population. It has become the foundation for studying the population genetics (Tooley, Fry and Gonzalez, 1985). For a population to be in a state of equilibrium, it is assumed that no mutation is occurring and random-mating is present. The population is large, in which there is no gene flow by migration in or out of the population. Besides that, it is also assumed that natural selection, which favors a particular allele, does not operate on the population (O'Neil, 2012).

When this mathematical relationship was applied to the present study, it was found that the Met allele is not a genetic risk factor for hypertension. The hypotheses were established as follow:

H_0: No mutation occurs in the population; the Met allele is not a genetic risk factor.
H_1: Mutation occurs in the population; the Met allele is a genetic risk factor.

Since the calculated χ^2 in both positive response and control group are less than tabulated χ^2 (3.84), the H_0 is accepted. The Met allele does not confer a genetic risk to hypertension. The calculations involved in drawing this conclusion are shown in Table 4.10 and Table 4.11. These results are similar to what was established in a study carried out by Ge, et al. (2015). In their

study, insignificant deviations were recorded in the *COMT* (rs4680) genotypic and allelic distributions from that predicted by the Hardy-Weinberg equilibrium ($p > 0.05$). Moreover, they also reported that the frequency of each genotype was almost identical between the two study populations.

Table 4.10: HWE calculation for the control group.

Genotype	**Observed (O)**	**Expected (E)**	**(O-E)**	**$(O-E)^2/E$**
Val/Val	46	43.1	2.9	0.1951
Val/Met	34	35.8	-1.8	0.0905
Met/Met	8	9.1	-1.1	0.1330
Total	88		(p = 0.05)	$\chi^2 = 0.4186$

Table 4.11: HWE calculation for the positive response group.

Genotype	**Observed (O)**	**Expected (E)**	**(O-E)**	**$(O-E)^2/E$**
Val/Val	25	27.9	-2.9	0.3014
Val/Met	25	23.2	1.8	0.1397
Met/Met	7	5.9	1.1	0.2051
Total	57		(p = 0.05)	$\chi^2 = 0.6462$

4.3.5 *COMT* Val158Met Variant (rs4680) Is Not Associated with Hypertension

The *COMT* Val158Met variant was assessed to explore the relationship between the functional variant (158-Met) and hypertension. In our study, no significant association was found between the *COMT* polymorphism (rs4680) and hypertension. The results have been presented in Table 4.12.

Table 4.12: Association between the *COMT* Val158Met variant and hypertension.

Blood pressure	**Homozygous wild type**	**Heterozygous**	**Homozygous mutant**	**Total**	**χ^2, p-value**
Normal	46 (52.3%)	34 (38.6%)	8 (9.1%)	88 (60.7%)	1.072 0.585
Hypertension	25 (43.9%)	25 (43.9%)	7 (12.2%)	57 (39.3%)	

To our knowledge, this is the first study addressing the genetic association between *COMT* Val158Met and hypertension in Malaysian population. Our results are in agreement with a recent cross sectional study conducted in China (Bian, et al., 2015). In their study, the subjects studied were mainly Chinese with age ranged from 30 to 95 years. The authors reported that *COMT* 158-Met was negatively correlated with blood pressure. However, many similar studies have shown a positive association between *COMT* polymorphism and hypertension.

COMT 158-Met has been found to have a reduced *COMT* enzyme activity. Under the circumstances, a higher risk of hypertension is speculated due to a spontaneous elevation of peripheral dopamine level. For example, Met/Met homogenous individuals in Sweden and Taiwan had been reported to have raised systolic and diastolic pressure as compared to the other two genotypes (Annerbrink, et al., 2008; Yeh, et al., 2010). They interpreted it as the consequence of increased concentrations of dopamine and estrogen due to lower *COMT* activity. In Japan, Htun, et al. (2011) also observed higher blood pressure and higher incidence rate of hypertension in Met/Met homozygotes. These observations were consistent in an *in vivo* study, in which lower *COMT* activity was noted in hypertensive rats (Masuda, Tsunoda and Imai, 2006). Nevertheless, an opposite conclusion was made by Hagen, et al. (2007) as elevated blood pressure was noted more often in Val/Val carriers rather than Met/Met carriers.

In the current study, no distinct association was found between *COMT* Val158Met polymorphism and hypertension. A possible explanation for these contradicting results could be due to the relatively small sample size as compared to other studies. Apart from that, involvement of only university students in the target population also limits the generalization of our findings. Since the study subjects were only a cohort of university students and sample size is small, so it may not be compared to the general population. Lastly, the literature suggests that heterogeneity in the genetic background, sample size, age range, and sex ratio also could attribute to these divergent findings (Annerbrink, et al., 2008).

4.4 *DRD2 Taq1A* Polymorphism in Hypertension

4.4.1 *DRD2 Taq1A* Genotyping

In present study, besides *COMT* gene, *DRD2 Taq1A* variant was also assessed to investigate if there is an association with hypertension. The target DNA sequence is the 3' translated region of exon 8 of the *ANKK1* gene (located downstream of the *DRD2* gene), with an amplicon size of 501 bp. An example of gel image has been presented in Figure 4.9.

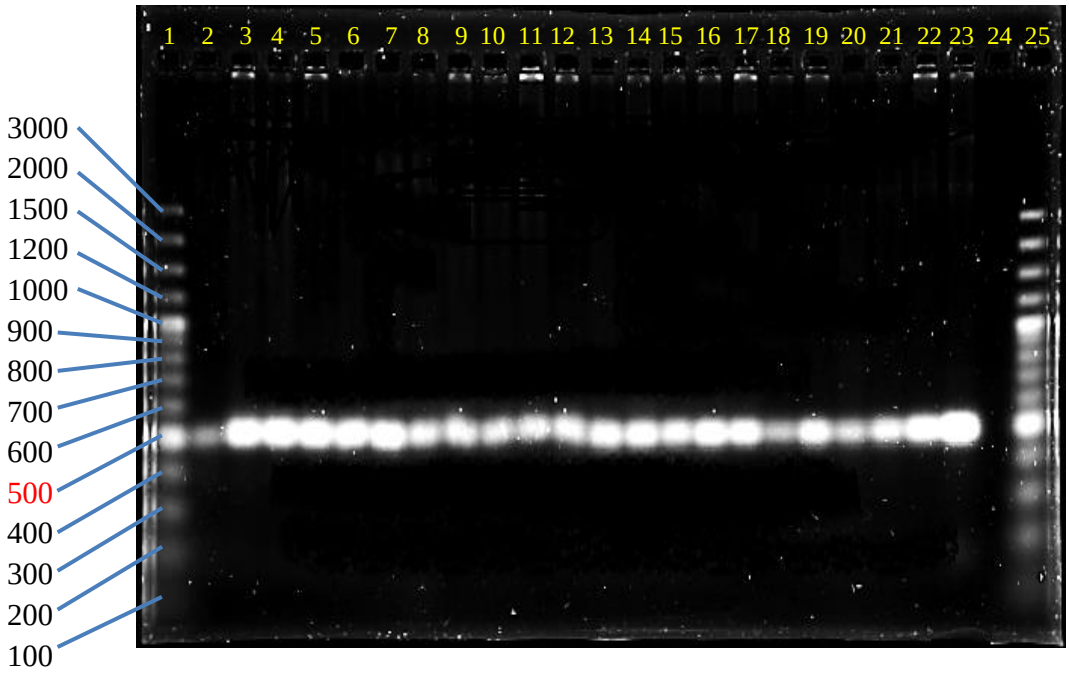


Figure 4.9: The PCR amplicons of targeted *DRD2* gene fragment run on a 2% agarose gel. The 100 bp DNA ladder is loaded into lane 1 and lane 25. The subsequent lanes 2-23 are loaded with PCR products. Lane 24 is loaded with negative control. The amplicon size is 501 bp.

The restriction enzyme used for *DRD2 Taq1A* gene variants is *Taq*I, which recognizes the 5'-TCGA-3' sequence. Only one band at 501 bp was observed for the homozygous mutant genotype (T/T) (Figure 4.10). Meanwhile, the heterozygous genotype (C/T) showed three bands at 501 bp, 310 bp, and 191 bp. For the homozygous wild type genotype (C/C), two bands were seen at 310 bp and 191 bp.

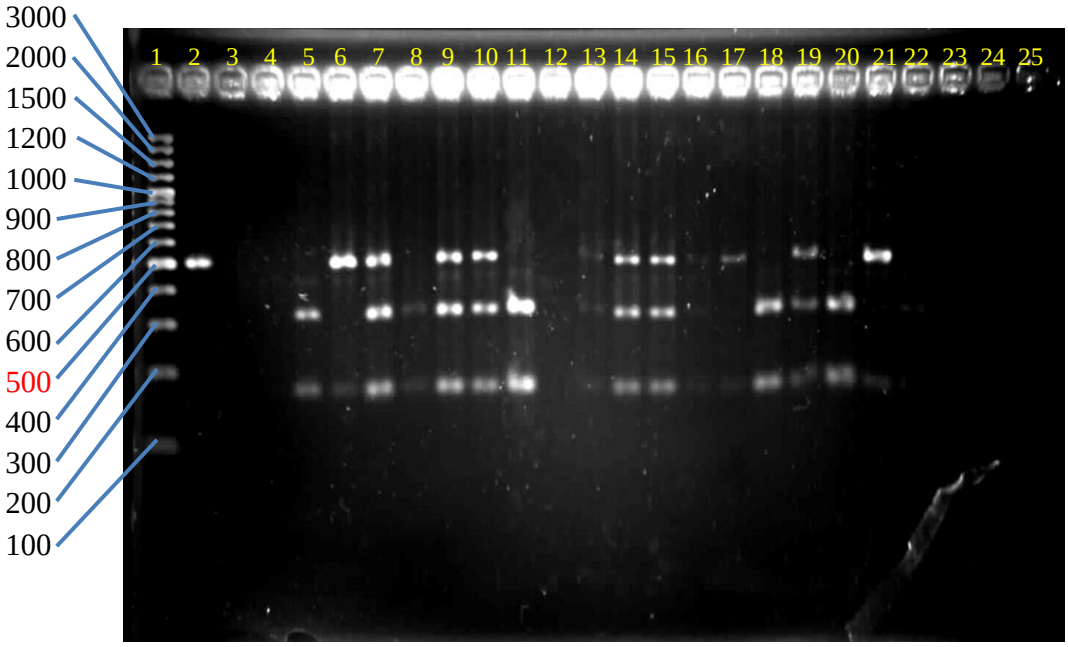


Figure 4.10: The three genotypes of *DRD2 Taq1A* gene variant on a 3% agarose gel. Lane 1 has been loaded with 100 bp DNA ladder. Lane 22 is designated to the negative control. Lane 3, 4, 12, 23, 24, and 25 are blank. The remaining lanes are loaded with samples. (Lanes 5, 8, 11, 18 and 20: homozygous wild type; Lanes 7, 9, 10, 13-16, and 19: heterozygous; Lanes 1, 6, 17, and 21: homozygous mutant).

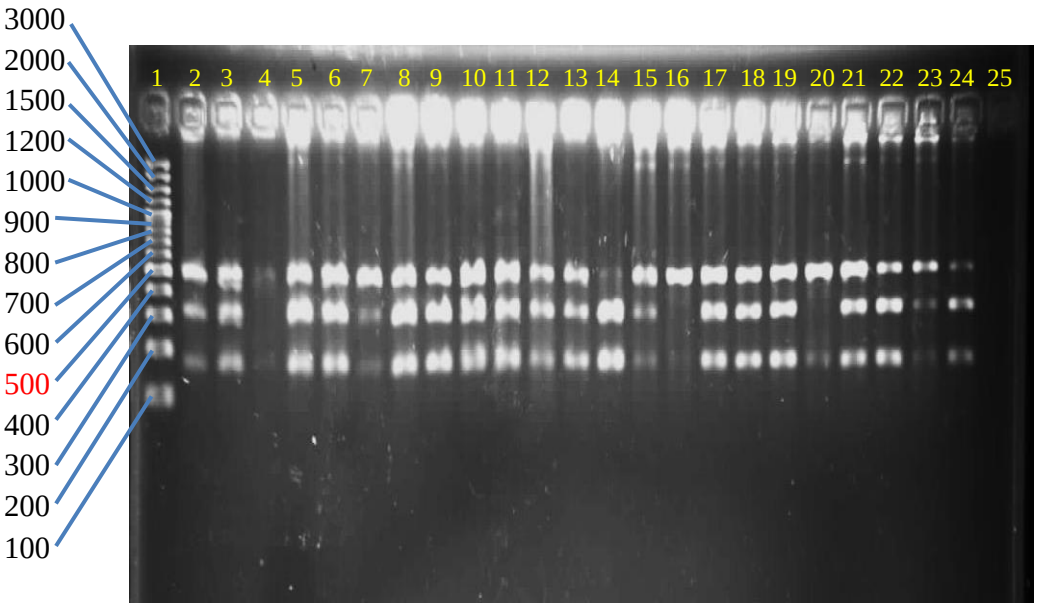


Figure 4.11: Gel image of *DRD2 Taq1A* gene variant. Lane 1 has been loaded with 100 bp DNA ladder. Lane 25 is designated to the negative control. The subsequent lanes 2-24 are loaded with samples. (Lanes 2, 3, 5-15, 17-19, and 21-24: heterozygous; Lanes 16 and 20: homozygous mutant).

Few faint bands were obtained for the heterozygous genotype (Figure 4.11). For instance, lanes 7, 14, 15, 23, and 24 have fainter bands as compared to other heterozygous genotypes with well-defined bands. This could be due to insufficient DNA template during PCR amplification. However, the template was amplified for 35 cycles in total. The amount of PCR product generated by right should be sufficient for the subsequent RFLP process. Nevertheless, if the concentration of the DNA template is extremely low in a PCR reaction, it is likely that the template may loss due to enzymatic degradation, clotting, or adsorption (Altshuler, 2006).

4.4.2 *DRD2 Taq1A* Genotypic and Allelic Frequencies of the Population

For the *DRD2 Taq1A* polymorphism, the predominant genotype in this study was heterozygous. It accounts for approximately 67.2% of the study population. No statistically significant difference was observed between gender and genotype distribution (p = 0.955). The results have been summarized in Table 4.13.

With the support of some publications, the mutant T allele (A1) was hypothesized to be a genetic risk factor for hypertension. In our population, there was no significant difference in genotypic frequency between positive response and control (Table 4.14). Moreover, the allelic frequency between positive response and control was almost similar too (Table 4.15). In general, the allelic frequency was calculated to be 0.55 for the C allele (A2) and 0.45 for the T allele (A1). This allelic frequency is similar to the Chinese population in Hong Kong studied by Thomas, et al. (2001). Furthermore, a study in Japan also found similar frequencies of the major and minor allele (Kawamura, et al., 2013).

Table 4.13: Distribution of *DRD2 Taq1A* genotypes across gender.

Genotype	**Gender (n = 134)**		**p-value**
	Male	Female	
Wild type (C/C)	11 (20.4%)	18 (22.5%)	
Heterozygous (C/T)	37 (68.5%)	53 (66.3%)	0.955
Mutant (T/T)	6 (11.1%)	9 (11.2%)	

Table 4.14: Genotype frequency of *DRD2 Taq1A* in positive response and control group.

Genotype	Frequency (n = 134)		Total
	Positive response	Control group	
C/C	9 (17.0%)	20 (24.7%)	29 (21.6%)
C/T	39 (73.6%)	51 (63.0%)	90 (67.2%)
T/T	5 (9.4%)	10 (12.3%)	15 (11.2%)

Table 4.15: Allele frequency of *DRD2 Taq1A* in positive response and control group.

Allele	Frequency (n = 134)	
	Positive response	Control group
C-allele	0.54	0.56
T-allele	0.46	0.44
Total	1.00	1.00

4.4.3 *DRD2* Mutant Genotype (T/T) Does Not Confer Risk for Hypertension

In this study, the *DRD2* genotypes conformed to the Hardy-Weinberg equilibrium in each of the groups investigated. It was found that the mutant T allele is not a genetic risk factor for hypertension. The hypotheses were established as follow:

H_0: No mutation occurs in the population; the T allele is not a genetic risk factor.

H_1: Mutation occurs in the population; the T allele is a genetic risk factor.

Since the calculated χ^2 in both positive response and control group are less than tabulated χ^2 (3.84), the H_0 is accepted. The T allele does not confer a genetic risk to hypertension. The calculations involved in drawing this conclusion are shown in Table 4.16 and Table 4.17. There were no significant differences in the *Taq1A* polymorphism genotype or allele distributions between positive response and control. These results are similar to what was established in a study conducted by Voisey, et al. (2009).

Table 4.16: HWE calculation for the control group.

Genotype	**Observed (O)**	**Expected (E)**	**(O-E)**	**$(O-E)^2/E$**
C/C	20	17.5	2.5	0.3571
C/T	51	54.4	-3.4	0.2125
T/T	10	9.1	0.9	0.0890
Total	81		(p = 0.05)	$\chi^2 = 0.6586$

Table 4.17: HWE calculation for the positive response group.

Genotype	**Observed (O)**	**Expected (E)**	**(O-E)**	**$(O-E)^2/E$**
C/C	9	11.5	-2.5	0.5435
C/T	39	35.6	3.4	0.3247
T/T	5	5.9	-0.9	0.1373
Total	53		(p = 0.05)	$\chi^2 = 1.0055$

4.4.4 *DRD2 Taq1A* Variant (rs1800497) Is Not Associated with Hypertension

Apart from the *COMT* Val158Met variant, *DRD2 Taq1A* variant was also assessed to study the relationship between these two polymorphisms and hypertension. Our results oppose the hypothesis that mutant T allele contributes to higher risk of getting hypertension. No significant association was found between the *DRD2 Taq1A* polymorphism (rs1800497) and hypertension. The results have been displayed in Table 4.18.

Table 4.18: Association between the *DRD2 Taq1A* variant and hypertension.

Blood pressure	**Homozygous wild type**	**Heterozygous**	**Homozygous mutant**	**Total**	**χ^2, p-value**
Normal	20 (69.0%)	51 (56.7%)	10 (66.7%)	81 (60.4%)	1.661 0.436
Hypertension	9 (31.0%)	39 (43.3%)	5 (33.3%)	53 (39.6%)	

Although studies concerning the relationship between *DRD2 Taq1A* polymorphism and hypertension are limited, previous study has been described the involvement of *DRD2* gene in the pathogenesis of hypertension in an *in vivo* study (Linthorst, et al., 1991). Furthermore, the T allele has been shown to be associated with reduced *DRD2* receptors capacity (Ariza, et al., 2012). Thus, with the reduced dopamine D2 receptors capacity, it is speculated that there will be impaired dopamine-mediated inhibition on the release of norepinephrine, leading to norepinephrine-mediated vasoconstriction (Thomas,

Tomlinson, and Critchley, 2000). In the meantime, Fang, et al. (2005) reported that there was statistical association between the *Taq1A* variant and risk of developing hypertension. A previous study by Rosmond, et al. (2001) also came with the same findings by showing that subjects with TT genotype (A1/A1) had a higher incidence rate of hypertension. On the contrary, an opposing conclusion was made by Thomas, et al. (2001), which reported that CC genotype (A2/A2) is associated with elevated blood pressure rather than the TT genotype.

In contrast to previous findings, our study showed that *Taq1A* polymorphism in human is not associated with hypertension ($p = 0.436$). The homozygous mutant TT genotype did not confer risk of hypertension. Therefore, the association between *DRD2 Taq1A* polymorphism (rs1800497) and hypertension is rather ambiguous. Further studies are required to acquire more insight into the association of *DRD2* polymorphism with hypertension. In addition, the divergence in findings would also suggest the likelihood of more than one dopaminergic mechanism is involved in the blood pressure regulation.

4.5 Genetic and Environmental Factors in the Etiology of Hypertension

Hypertension is known to be caused by interactions of multiple genetic and environmental factors. Nonetheless, in spite of the great extent of research, it is still difficult to identify the susceptibility genes for hypertension. There is growing evidence suggested that interactions between multiple genes and lifestyle behaviors play an important role in the increased incidence of hypertension (Kunes and Zicha, 2009). In modern society, sedentary lifestyle has become more common. The sedentary lifestyle, unhealthy dietary behavior, and concomitant obesity clearly heighten the risk of hypertension. However, the body response towards these environmental stimuli might be influenced by certain gene expression as well. For instance, a variant of TCF7L2 gene (rs12255372) has been reported to be protective for obesity (Klunder, et al., 2011). This is in agreement with the idea that hypertensive individuals not necessarily comes with obese.

In short, there is no doubt that blood pressure is greatly influenced by genetic and environmental factors. Due to this complex trait, it is not easy to identify the genes responsible for hypertension. Although some research data certain possibilities, it still requires some time to apply these findings in humans.

4.6 Future studies

Progressing from the present study, more cross-sectional studies can be conducted in other local universities to improve the accuracy and reliability of epidemiologic information on the prevalence of hypertension among Malaysian university students. Besides that, a follow-up study may be more useful in assessing how physical activity and dietary behavior correlate with hypertension. A standardized test to measure stress level may be useful as well.

Apart from that, a greater depth of gene expression study will be helpful in understanding the possible involvement of genetic variation in the pathophysiology of hypertension. A real-time PCR can be performed to quantify the gene expression or enzyme expression between the normotensive and hypertensive subjects. Lastly, besides the chosen SNPs in the present study, the known SNPs in other candidate genes can be assessed and evaluated to test for any associations with hypertension. For example, we have only studied the effects of genetic polymorphism in genes from the dopaminergic pathway. Further analysis on the contribution of other genetic polymorphisms in relevant pathways such as serotonergic, adrenergic, and noradrenergic pathway will be useful in identifying the corresponding genetic effects on the development of hypertension.

CHAPTER 5

CONCLUSION

The present study can conclude that hypertension is prevalent among the university students of Universiti Tunku Abdul Rahman (UTAR). A total of 37.2% university students were found to be prehypertensive and 2.1% were hypertensive. Furthermore, out of the 57 positive responses, more males (63.2%) were found to be hypertensive than females (36.8%), which showed a statistically significant association ($p < 0.05$). In this representative group of university students or young adults, the rates of hypertension awareness are low.

Demographic analysis revealed that majority of students (56.6%) had a healthy range of body weight, and there was no statistical significance between BMI and hypertension ($p = 0.137$). Besides that, no significant association was found between family history of hypertension, physical activity, consumption of fried foods, stress and risk of hypertension ($p > 0.05$). However, in this population, women were reported significantly experienced higher level of stress than men.

In terms of genetic factors, the *COMT* Val158Met and *DRD2 Taq1A* polymorphisms were found to be not associated with hypertension ($p > 0.05$). The *COMT* wild type genotype had the highest frequency of 49% in this

cohort of students. For the *DRD2* gene, the prevailing genotype was heterozygous (67.2%). Both Met and T mutant allele were hypothesized to be genetic risk factors for hypertension. Nonetheless, in this study, both *COMT* and *DRD2* genotypes have shown conformity to Hardy-Weinberg equilibrium. In conclusion, our results reject the hypothesis that *COMT* Val158Met (rs4680) and *DRD2 Taq1A* (rs1800497) polymorphisms act as a genetic susceptibility factor for hypertension. The mutations of both genetic variants are not independently associated with the prevalence of hypertension.

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APPENDICES

Appendix A

Examples of Fried Food in Malaysia

1. Fried chicken
2. French fries
3. Fried tofu
4. Fried keropok
5. Youtiao (fried dough or bread)
6. Fish and chips
7. Deep fried onion rings
8. Deep fried pizza
9. Fried mushrooms
10. Fried nuggets
11. Fried popiah
12. Deep fried curry puff
13. Fried banana
14. Deep fried sweet potato
15. Deep fried fish
16. Fried cempedak
17. Fried ice cream
18. Fried shrimp

19. Fried pumpkin chips

20. Deep fried hotdog

Appendix B

Calculation of allelic frequencies:

Val allele = $\frac{(2 \times number\ of\ wild\ type) + number\ of\ heterozygous}{n \times 2}$

= $\frac{(2 \times 71) + 59}{145 \times 2}$

= 0. 69

Met allele = $\frac{(2 \times number\ of\ mutant) + number\ of\ heterozygous}{n \times 2}$

= $\frac{(2 \times 15) + 59}{145 \times 2}$

= 0. 31

Appendix C

1

UNIVERSITI TUNKU ABDUL RAHMAN
DEPARTMENT OF BIOMEDICAL SCIENCE

FINAL YEAR PROJECT October 2015
YEAR 3 TRIMESTER 2

CONSENT FORM

Name of Researcher : Gan Kelvin

ID no. : 1205788

Project Title:

Prevalence of hypertension in UTAR students and association of catechol-O-methyltransferase (COMT) (Val158Met) and Dopamine D2 receptor rs1800497 (Taq1A) polymorphisms and hypertension.

Aim / Purpose of the Research:

You are participating in a research to study the association of COMT (Val158Met) and Dopamine D2 receptor rs1800497 (Taq1A) polymorphisms and hypertension in a cohort of students in UTAR. The objectives are:

a) To study the prevalence of hypertension among UTAR students.
b) To assess the association between consumption of fried foods, physical activities, stress and risk of hypertension.
c) To evaluate the prevalence of COMT (Val158Met) and Dopamine D2 receptor rs1800497 (Taq1A) polymorphisms in the studied samples.
d) To investigate the association COMT (Val158Met) and Dopamine D2 receptor rs1800497 (Taq1A) polymorphisms and hypertension.

Procedure, Risk and Discomfort:

Thank you for your interest in the present study. The aim of the study is to understand the association between consumption of fried foods, physical activities, stress and risk of hypertension. Furthermore, the association of genes and hypertension is being studied due to the limitation of molecular genetics study on hypertension. The volunteer will be requested to complete the questionnaire. The questionnaire consists questions about disease you may have had in the past, family history, your health behaviors, foods you eat, exercise, medications you take, stress and how you feel about life. The study is designed to measure health and should not cause pain. Your mouth wash sample will be collected. The information about you is confidential. We may re-contact you if necessary to clarify the provided information. We hope that the results of the study will provide better understanding on the risk of hypertension in Universiti Tunku Abdul Rahman (UTAR) students. If you have questions, you may reach Dr Phoon Lee Quen (phoonlq@utar.edu.my) from Department of Biomedical Science, Faculty of Science, UTAR.

[*Donors are advised not to consume any food or drink 30 min prior to sample collection.*]
Firstly, you are required to rinse your mouth with mineral water to cleanse the mouth from food particles. After cleaning, you are required to rinse again with saline solution provided. This mouth rinse will be collected in a disposable paper cup as sample. This sample which contains your buccal cells or cheek cells will be used for genomic extraction for subsequent genotyping work. Buccal cell collection poses minimal risk and discomfort to donor as it is a non-invasive method to collect sample from donor. Blood pressure reading will be taken down by using sphygmomanometer. The blood pressure was measured on the left arm after the donor has rested for 5 minutes in a seated position.

Confidentiality:
Serial number will be assigned as to protect your personal information and the result obtained. The info is used for research purpose only and would not be revealed to any other parties.

Consent and Signature:
Please tick the respective box:
☐ I have read and understood the condition stated above and I agree to voluntarily participate in this research study.
☐ I give my permission for you to re-contact me if you need to clarify information.

Signature _____ Date _____

This questionnaire paper consists of 4 sessions on 10 printed pages.

Demographics:

Please fill in the following information and answer the questions provided.

Name : _____

Contact number : _____

E-mail : _____

Faculty : _____

Course : _____

Age: _____ *[Please state]*

Current Height (cm):

Current Weight (kg):

BMI (kg/m^2) = _____

Race:
a) Malay
b) Chinese
c) Indian
d) Others (state : _____)

Gender:
a) Male
b) Female

Educational level:
a) Foundation
b) Degree study
c) Postgraduate program

BMI (kg/m^2)		Weight Categories	Health Risk
WHO	Asian		
30 & above	27.5 & above	Obese	High risk of developing heart disease, high blood pressure, stroke and diabetes
25 to 29.9	23 to 27.4	Overweight	Moderate risk of developing the above diseases
18.5 to 24.9	18.5 to 22.9	Healthy Range	Low risk of developing the above diseases
Below 18.5	Below 18.5	Underweight	At risk of developing nutritional deficiency diseases and osteoporosis

Measurement readings (Filled up by the Researcher)

	1st reading		2nd reading		3rd reading		Mean
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	
Blood pressure (mmHg)							
Heart Rate (bpm)							

This questionnaire paper consists of 4 sessions on 10 printed pages.

This chart reflects blood pressure categories defined by the **American Heart Association**.

Blood Pressure Category	Systolic mm Hg (upper #)	Diastolic mm Hg (lower #)
Normal	less than 120	less than 80
Prehypertension	120 – 139	80 – 89
High Blood Pressure (Hypertension) Stage 1	140 – 159	90 – 99
High Blood Pressure (Hypertension) Stage 2	160 or higher	100 or higher
Hypertensive Crisis (Emergency care needed)	Higher than 180	Higher than 110

Answer the following questions and "circle" your choice:

Part A: Health History

1. Do you suffer from high blood pressure (hypertension)?

a) Yes	b) No	c) Unsure

If yes, please state when were you diagnosed to have hypertension?

_____

[If yes, please answer No 2 – 4]

2. Compared to 12 months ago, is your blood pressure:

a) Better	b) Same	c) Worse
d) I do not know	e) I didn't get my blood pressure measurement 12 months ago	

3. Have you been prescribed any medication to lower your blood pressure?

a) Yes	b) No	c) Unsure

4. Do you take all your prescribed medications?

a) Yes	b) No	c) Unsure

If yes, please state how many different medicines a day are you taking (approximate number)?

_____

5. Do you have/had any form of vascular disorders? (e.g., Heart attack, stroke, thrombosis, etc.)
   * Thrombosis- formation of a blood clot inside blood vessel, obstructing blood flow through circulatory system.

a) Yes	b) No	c) Unsure

If yes, please state the disorder and when does it occur?

_____

6. Do you have/had any form of neurologic disease (seizures, epilepsy, etc.)

a) Yes	b) No	c) Unsure

If yes, please state the disorder and when does it occur?

_____

7. Do you have/had any form of homeostatic disorders? (eg. Hypoxia, dialysis, etc.)
*Homeostatic- Property of a system in which variables are regulated so that internal conditions remain stable relatively constant

a) Yes	b) No	c) Unsure

If yes, please state the disorder and when does it occur?

_____

8. Do you have/had any form of Glaucoma- eye disease (loss of vision) or Subconjunctival Haemorrhage (blood spot on the retina)?

a) Yes	b) No	c) Unsure

If yes, please state the disorder and when does it occur?

_____

9. Have you ever suffered from any of the following conditions? (E.g. Diabetes Mellitus, High Cholesterol and Kidney diseases)

a) Yes	b) No	c) Unsure

If yes, please state the condition and when does it occur?

_____

10. Do you have/had any form of psychiatric disorders? (E.g. Depression, anxiety, OCD, etc.)

a) Yes	b) No	c) Unsure

If yes, please state the disorder and when does it occur?

_____

11. Do you have any other health problem?

If yes, please state:

_____

_____

_____

This questionnaire paper consists of 4 sessions on 10 printed pages.

12. Do you have any family member or close relative who is/was suffering from hypertension?

a) Yes	b) No	c) Unsure

If yes, please state who(s), what is his or her age: _____

_____

13. Do you have any family member or close relative who is/was suffering from Diabetes Mellitus?

a) Yes	b) No	c) Unsure

If yes, was it type 1 or type 2 Diabetes Mellitus?

a) Type 1	b) Type 2	c) Unsure

14. Does anybody in your family have a history of Heart disease (heart attack, stroke, heart failure, etc.)?

a) Yes	b) No	c) Unsure

If yes, please state who(s), type of disease and his or her age:

_____

_____

15. Does anybody in your family have a history of Neurologic disease (seizures, epilepsy, etc.)

a) Yes	b) No	c) Unsure

If yes, please state who(s), type of disease and his or her age:

_____

_____

16. Do you have/had any form of substance use or withdrawal in the last 3 months?

I am currently taking:

I. Alcohol (If yes, how much do you drink?) : _____ _____ per day / _____ per week / _____ per month	Yes	No
II. Smoking (If yes, how much do you smoke?) : _____	Yes	No

I have withdrawn from:

I. Alcohol (If yes, when did you quit?) _____	Yes	No
II. Smoking (If yes, when did you quit?) _____	Yes	No

_____

This questionnaire paper consists of 4 sessions on 10 printed pages.

17. Have tests on your urine always been normal?

_____

18. Within the last 2 years have you had any abnormal specialist investigations? E.g. electrocardiogram (EKG) or x-rays.

_____

Part B: Physical Activity

1. Do you engage in regular physical activity?

a) Yes (Please state the activities: _____	b) No

2. How many minutes per day?

a) 10 - 20	b) 20 - 30	c) 30 - 40	d) 40 - 60	e) >60

3. How many times per week?

a) 1	b) 2	c) 3	d) 4
e) 5	f) 6	g) 7	h) More (please state : _____)

4. What level of physical activities do you have while commuting to and from work/university?

a) Using motorized transportation, or no work (0 minute of walking or cycling)	**b)** Walking or cycling 1-29min	**c)** Walking or cycling >30min

5. Do you suffer from any form of disability that makes it difficult for you to exercise?

a) Yes	**b)** No	**c)** Unsure

Part C: Dietary behavior and nutrition intake

1. Do you consider yourself to be healthy?

a) Yes	**b)** No	**c)** Unsure

2. Have you recently gained or lost weight?

a) Yes	**b)** No	**c)** Unsure

If yes, please explain whether it was weight gain or weight loss and what changes you made that led to the change in weight.

_____

_____

This questionnaire paper consists of 4 sessions on 10 printed pages.

3. Have you ever had concerns about your weight?

a) Yes	b) No

4. Have you ever tried to lose weight in the past?

a) Yes	b) No

If yes, please explain: _____

5. Food usage questionnaire items (Please circle your answer):

a.	Usually eat salty	Very salty	A little salty	Modestly	Little salty	Not salty
		(a)	(b)	(c)	(d)	(e)
b.	Add salt/ soy sauce on cooked dishes	Always	Frequently	Seldom	Never	
		(a)	(b)	(c)	(d)	
c.	Add salt/ soy sauce before tasting	Always	Frequently	Seldom	Never	
		(a)	(b)	(c)	(d)	
d.	Frequency salt usage on thick broth/ soup	More than a tea-spoon	As much as a tea-spoon	Half a tea-spoon	Little salt	No salt
		(a)	(b)	(c)	(d)	(e)
e.	Frequency soy sauce on pan-fried/ deep-fried food	Always	Sometimes	Never		
		(a)	(b)	(c)		
f.	Frequency soy sauce on broiled meat or fish	Always	Sometimes	Never		
		(a)	(b)	(c)		
g.	Frequency of salted vegetables	More than 3 a day	1-2 a day	3-6 a week	2-8 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
h.	Frequency of food seasoned with salty seasoning	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
i.	Frequency of ham, sausage/ bacon	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
j.	Frequency of semi-cooked	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
k.	Frequency of instant food	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
l.	Frequency of canned food	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)

m.	Frequency of fried food	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
n.	Frequency of salted snack	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
o.	Frequency of taking meat	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)
p.	Frequency of taking vegetables	More than 1 a day	3-6 a week	1-2 a week	2-3 a month	Less than 1 a month
		(a)	(b)	(c)	(d)	(e)

6. How often did you drink the beverage? (Please tick)

	Never or less than once per month	1 – 3 per month	1 per week	2 - 4 per week	5-6 per week	1 per day	2 - 3 per day	4 – 5 per day	6+ per day
Instant or brewed coffee, not decaffeinated									
Green tea (Not decaffeinated- instant, bottled, and brewed)									
Espresso and espresso drinks, not decaffeinated (Latte, Mocha, Americano)									
Black tea such as Lipton, or Earl Grey (Not decaffeinated- instant, bottled, and brewed)									
Mountain Dew, Red Bull and other highly caffeinated sodas									
Regular colas and root beer (With caffeine, not diet)									
Diet colas and diet root beer (With caffeine)									

This questionnaire paper consists of 4 sessions on 10 printed pages.

Part D: Stress Management

1. How would you rate your stress level?

a) None	b) Mild	c) Moderate	d) High	e) Very high

2. Do you manage stress well?

a) Yes	b) No

3. How do you manage stress? (Check all that apply)

a) Exercise	b) Social relationships	c) Hobbies	d) Prayer/ Spiritual activities	e) Family relationships

If others, please state: _____

4. In the past month have you:

Response scale:
1 Rarely or none of the time (less than one day)
2 Some or a little of the time (1 to 2 days)
3 A moderate amount of time (3 to 4 days)
4 Most of the time
5 Refused / unable to answer

Answer the following questions and "circle" your choice:

Had periods of time when you feel down or depressed?	1	2	3	4	5
Felt less interested in doing things you normally like to do?	1	2	3	4	5
Mood swings, increased irritability and/or loss of concentration?	1	2	3	4	5
Been worrying excessively about a number of things?	1	2	3	4	5
Felt very nervous or anxious or suddenly experienced a lot of physical symptoms (e.g., heart racing, sweating?	1	2	3	4	5
Had a fear of losing control of yourself?	1	2	3	4	5
Avoided social situations for fear of what others may think or say about you?	1	2	3	4	5
Been afraid of being alone?	1	2	3	4	5
Had repeated thoughts or images in your head that are difficult to dismiss?	1	2	3	4	5
Felt compelled to complete certain behaviors repeatedly (e.g., checking to make sure you locked the doors, washing your hands again and etc.)	1	2	3	4	5
Thought a lot about an upsetting event from the past?	1	2	3	4	5
Found yourself preoccupied with food, weight or body image?	1	2	3	4	5
Been concerned about your academic performance?	1	2	3	4	5

5. Have you been in therapy before or received any prior professional assistance for emotional, psychological relationship issues?

a) Yes	b) No

If yes, please describe, starting with most recent/current:

_____

_____

6. Have you ever been diagnosed with a psychological condition (e.g.: clinical depression)?

a) Yes	b) No

If yes, please describe:

_____

_____

Thank you for taking the time to complete this form. Your responses will be treated as private and confidential.

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This questionnaire paper consists of 4 sessions on 10 printed pages.

PREVALENCE OF HYPERTENSION IN UNIVERSITY STUDENTS AND ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE (VAL158MET) AND DOPAMINE D2 RECEPTOR (TAQ1A) POLYMORPHISM WITH HYPERTENSION

By

GAN KELVIN

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