ASSOCIATION OF TRADITIONAL CHINESE MEDICINE (TCM) BODY CONSTITUTION, WAIST CIRCUMFERENCE AND FASTING BLOOD GLUCOSE WITH *HNF1A* AND *CAPN10* POLYMORPHISMS

SEE YING MEI

BACHELOR OF SCIENCE (HONS)

BIOMEDICAL SCIENCE

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

MAY 2023

ASSOCIATION OF TRADITIONAL CHINESE MEDICINE (TCM) BODY CONSTITUTION, WAIST CIRCUMFERENCE AND FASTING BLOOD GLUCOSE WITH *HNF1A* AND *CAPN10* POLYMORPHISMS

By

SEE YING MEI

A project report submitted to the Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

In partial fulfilment of the requirements for the degree of

Bachelor of Science (Hons) Biomedical Science

May 2023

ABSTRACT

ASSOCIATION OF TRADITIONAL CHINESE MEDICINE (TCM) BODY CONSTITUTION, WAIST CIRCUMFERENCE AND FASTING BLOOD GLUCOSE WITH *HNF1A* AND *CAPN10* POLYMORPHISMS

SEE YING MEI

Traditional Chinese Medicine Body Constitution (TCM BC) is a classification of individual's body condition influenced by acquired and inborn factors based on Chinese medicine theory. These factors may disturb and cause Yin and Yang imbalance, leading to increased susceptibility to certain diseases. Metabolic syndrome (MetS) is a group of illnesses caused by dysregulation biochemical pathways. MetS is strongly related to abdominal obesity and diabetes. Susceptibility to MetS may differ according to demographic distribution and BMI. TCM BC leads the foundation for diagnosis, treatment and disease prevention. The relationship between TCM BC with abdominal obesity and diabetes was included in this study. Gene variant can increase risk of MetS. Association of metabolic gene variants with abdominal obesity, diabetes and TCM BC was included in this study. Convenience sampling was used, and 102 subjects were recruited with informed consent in this study. Demographic distribution and COVID-19 history were self-declared by respondents. TCM BC was determined using questionnaire. Waist circumference, fasting blood glucose level and anthropometric measurement were measured. Blood was drawn for DNA extraction to genotype rs735396 and rs2975760. SPSS 26.0 was used for data analysis. The prevalence of abdominal obesity and diabetes

in this study were 34.31% and 1.96%, respectively. Both studied variants were found significant with minor allele frequency > 0.05. Gender, age, place of origin and anthropometric measurement had significant difference in waist circumference and fasting blood glucose level. There was no significant association between TCM BC and gene variants studied as well as between demographic distribution, anthropometric measurements and COVID-19 history with TCM BC. Association between TCM BC with waist circumference and fasting blood glucose level cannot be ruled out as majority study subjects have either gentleness or combinations of body constitutions. Larger sample size and involvement of population with broader variation are recommended in future study.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my greatest gratitude to my FYP supervisor, Dr. Teh Lai Kuan, for giving me opportunity to participate in her research. She has provided tremendous guidance, encourage, motivation and knowledge throughout my study. I appreciate her time spent even with hectic schedule, patience, help and sacrifice she has made during my study. I am blessed to have Dr. Teh with me during my study.

Besides, I would like to acknowledge the laboratory officer, Mr. Saravanan A/L Sivasangaran, Cik Fatin Masturah Binti Mohd Isa, Ms. Hemaroopini A/P Subramaniam and Puan Natrah Binti Khalid for their assistance during the bench work.

In addition, I wish to thank my bench mates for their cooperation and knowledge sharing. I would also thank my friends for their assistance and motivational support during my FYP.

My sincere appreciation also extends to my beloved family members for their unlimited moral support and understanding throughout my FYP. Last but not least, I wish to thank all the volunteers who participated in this study.

DECLARATION

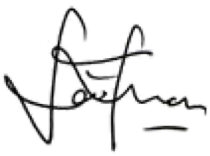
I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

See Ying Mei

APPROVAL SHEET

This project report entitled **"ASSOCIATION OF TRADITIONAL CHINESE MEDICINE BODY CONSTITUTION, WAIST CIRCUMFERENCE AND FASTING BLOOD GLUCOSE WITH *HNF1A* AND *CAPN10* POLYMORPHISMS"** was prepared by SEE YING MEI and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Biomedical Science at Universiti Tunku Abdul Rahman.

Approved by:



_____

Dr. Teh Lai Kuan Date: 27.04.2023

Supervisor

Department of Allied Health Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 27.04.2023

PERMISSION SHEET

It is hereby certified that **SEE YING MEI** (ID No: **20ADB06171**) has completed this final year project report entitled "ASSOCIATION OF TRADITIONAL CHINESE MEDICINE BODY CONSTITUTION, WAIST CIRCUMFERENCE AND FASTING BLOOD GLUCOSE WITH *HNF1A* AND *CAPN10* POLYMORPHISMS" under the supervision of Dr. Teh Lai Kuan from the Department of Allied Health Sciences, Faculty of Science.

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

Yours truly,

(SEE YING MEI)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

A	Adenine
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ARMS	Amplification refractory mutation system
ddH_2O	Autoclaved distilled water
bp	Base pair
BC	Body constitution
BCQ	Body constitution questionnaire
BMI	Body mass index
Ca^{2+}	Calcium ion
CAPN10	Calpain-10
CCMQ	Constitution in Chinese Medicine Questionnaire
cAMP	Cyclic adenosine monophosphate
C	Cytosine
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FPG	Fasting plasma glucose
GLUT2	Glucose transporter 2
GLUT4	Glucose transporter 4
G	Guanine
HbA1c	Haemoglobin A1c

HNF1A	Hepatocyte Nuclear Factor 1 Alpha
HDL	High density lipoprotein
IGR	Impaired glucose regulation
F_I	Inner forward primer
R_I	Inner reverse primer
IP3	Inositol triphosphate
IL-1	Interleukin-1
JIS	Joint Interim Statement
MODY	Mature-onset diabetes of the young
mRNA	Messenger ribonucleic acid
MetS	Metabolic syndrome
MAP1	Microtubule-associated proteins 1
MAF	Minor allele frequency
NCEP-ATP III	National Cholesterol Education Program-Adult Treatment Panel III
NHLBI	National Heart, Lung, and Blood Institute
NIH	National Institutes of Health
F_O	Outer forward primer
R_O	Outer reverse primer
PCR	Polymerase chain reaction
x g	Relative centrifugal force
SERC	Scientific and Ethical Review Committee
SNP	Single nucleotide polymorphism
T	Thymine
TCM	Traditional Chinese Medicine

TAE	Tris- Acetic acid- EDTA
TE	Tris-EDTA
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
UV	Ultraviolet
UPR	Unfolded protein response
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Traditional Chinese Medicine Body Constitution (TCM BC) is the classification of an individual's body condition based on the theory of traditional Chinese medicine from the Yellow Emperor's Inner Classic. Different factors including inherited or acquired factors may lead to different body constitutions that drive the susceptibility of an individual susceptible to certain diseases including inherited or acquired factors (Li, et al., 2017). The contributing acquired factors include dietary factors, environmental factors, emotional status, and lifestyle (Sun, et al., 2014). Meanwhile, inheritable factors are referring to inherited traits that make an individual susceptible to certain diseases. The inherited traits are also included the single nucleotide polymorphism (SNP) that present in certain regulatory genes in predisposing to certain diseases (Shastry, 2007). Inherited traits are unmodifiable and permanent. Therefore, there is a need to put more emphasis on lifestyle modification to avoid acquired factors that are harmful to health (Hsu, et al., 2022).

Based on TCM BC, there are a total of nine body constitution types which can be classified into Neutral, Qi Deficiency, Yang Deficiency, Yin Deficiency, Damp-heat, Qi Stagnation, Phlegm-dampness, Blood Stasis, and Special Diathesis. An individual is known to have a neutral body constitution when their internal organs are functioning optimally and developing disease resistance. Both the Yin and Yang deficiency body constitution types are implying of a state of imbalanced body condition. In TCM, Yin and Yang always need to be

maintained in an equilibrium state. A person with excessing of Yang would be showing symptoms of heatiness including fever, headaches, insomnia, and even abscesses may be seen in a damp-heat body constitution. Conversely, Yin is contradicting the Yang. Excessive Yin may lead to water retention or chills. Different TCM BC may influence the susceptibility to metabolic syndrome such as diabetes mellitus (You, et al., 2017). In addition, TCM possesses great potential in managing the acquired factors that may predispose to metabolic syndrome (Xu, et al., 2018).

According to National Heart, Lung, and Blood Institute (NHLBI) (2022), metabolic syndrome refers to a group of illnesses that increase the risk of getting coronary heart disease, diabetes, stroke, and other health complications. A large waist circumference, high blood pressure, high blood glucose levels, high blood triglyceride, and low high-density lipoprotein (HDL) level are the five criteria related to metabolic syndrome. If an individual has three or more of the above conditions, the individual is considered to have metabolic syndrome (National Heart, Lung, and Blood Institute, 2022). The attention of this study was to look into the waist circumference and fasting blood glucose level to investigate abdominal obesity and diabetes mellitus, respectively.

According to the National Health and Morbidity Survey (2019), the prevalence rate of abdominal obesity and diabetes in Malaysia was high, with one out of two adults and one out of five adults, respectively. One of the significant risk factors for abdominal obesity and diabetes is the genetic factor. There is a polygenic contribution that increases the risk for both obesity and diabetes

(Romao and Roth, 2008). The *HNF1A* and *CAPN10* genes are known to be the genes associated with diabetes, particularly in affecting insulin secretion (Murea, Ma, and Freedman, 2012). It had been commonly reported diabetic patients with family history with at least one parent had type 2 diabetes mellitus (Klein, et al., 1996). However, lifestyle and obesity are environmental factors that may alter genetic susceptibility (Murea, Ma, and Freedman, 2012). In addition, the National Health and Morbidity Survey (2019) also revealed diabetes is closely related to abdominal obesity. According to World Health Organization (2022), type 2 diabetes mellitus was reported to constitute up to 95% of diabetic patients primarily due to obesity and physical inactivity. In type 2 diabetes mellitus, patients were found with resistance to insulin.

Mature-onset diabetes of the young (MODY) is one of the most prevalent kinds of monogenic diabetes which is inherited as an autosomal dominant pattern (Hoffman, et al., 2022). Patients with MODY may misdiagnose as having type 1 diabetes mellitus as the clinical presentation typically presented before the age of 25 (McDonald and Ellard, 2013). MODY is due to the defects in the growth of pancreatic islet cells that reduce insulin output. The defects and phenotype could be varied depending on the expression of *HNF1A* and patients usually have heterozygous mutations in *the HNF1A* gene (Hoffman, et al., 2022). In this study, the polymorphisms of *the HNF1A* gene were studied in relation to abdominal obesity and diabetes mellitus. The association of the acquired factors with TCM BC in relation to metabolic syndrome was investigated in this study.

1.1 Significance of Study

This study drives attention to the relationship between TCM BC with abdominal obesity and diabetes among Malaysians. This will provide insights into better marker diagnosis in the planning of preventive measures and treatments. Lifestyle modification to change TCM BC would be significant in the prevention of abdominal obesity and diabetes. In addition, the SNP in the gene in relation to abdominal obesity and diabetes may act as the prognostic marker for the diagnosis of abdominal obesity and diabetes.

1.2 Objective of Study

In this study the objectives are:

1. To determine the prevalence of abdominal obesity and diabetes.
2. To identify the relationship between demographic distribution, anthropometric measurement, and COVID-19 history in the past one month with waist circumference and fasting blood glucose level.
3. To identify the relationship between TCM body constitution with waist circumference and fasting blood glucose level.
4. To compare the waist circumference and fasting blood glucose among different rs735396 and rs2975760 variants.
5. To determine the association between TCM body constitutions and the variants rs735396 and rs2975760.
6. To associate the demographic distribution, anthropometric measurement, and COVID-19 history in the past one month with TCM body constitution.

CHAPTER 2

LITERATURE REVIEW

2.1 Metabolic Syndrome

The combination of obesity, hypertension, dyslipidemia, and insulin resistance is known as metabolic syndrome (MetS). MetS is a group of symptoms and the elements of MetS can be resulted due to strong heredity (Cornier, et al., 2008.). Type 2 diabetes mellitus (T2DM) is also more likely to develop in patients with metabolic syndrome. In addition to physical inactivity, aging, and hormonal imbalance, MetS present abdominal obesity and insulin resistance as its main underlying risk factors. High metabolic risk markers are found among those with two diabetic parents or one parent and a first- or second-degree cousin is insulin resistant. Hence, MetS may consist of a collection of unconnected risk factors or a constellation of risk variables (Grundy, et al., 2005).

2.1.1 Prevalence of Metabolic Syndrome in Malaysia

Metabolic syndrome was reported to affect 25 to 40% of the adult population of Malaysia as increasing with age. Obese kids who also at another risk of getting MetS. Predominant of them are of Indian descent, followed by Malay and Chinese descent. It was also discovered that socioeconomic factors such as living in metropolitan regions, being unemployed, having a lower income, having less education, and working shifts were associated with a higher frequency of metabolic syndrome (Lim and Cheah, 2016). Due to the high prevalence rate of MetS among Malaysia population, this had presented that management of metabolic syndrome in Malaysia is lacking and ineffective. An

earlier study reported that BMI, diabetes, and hyperlipidemia were strongly correlated with metabolic syndrome in women. Meanwhile, diabetes and hypertension were significantly correlated with metabolic syndrome in men. Metabolic syndrome was strongly correlated to lifestyle and cardio-metabolic risk factors (Manaf, et al., 2021).

Asian countries were also reported with a high extent of MetS incidence. However, differences in lifestyle habits and ethnicities have contributed to the distribution of MetS. There is a rising trend of MetS prevalence reported in Singapore, China, and Malaysia when using the Asian-adapted definitions on the Adult Treatment Panel III (ATP-III) criteria of the National Cholesterol Education Program (NCEP). For instance, the body fat distribution among populations in Europe or North America regions in defining obesity cannot be accepted by Asian populations because the quantity and distribution of body fat in Asians are different. Hence, it was discovered that the Joint Interim Statement (JIS) "Harmonized" criterion definition was more useful for estimating the proportions of MetS in Asian populations (Manaf, et al., 2021).

2.1.2 Prevalence of Metabolic Syndrome in Younger Population

Metabolic syndrome is not just a problem for adults, but also among children. This had been seen in Korean children and teenagers with a high prevalence of MetS as well as the frequency of metabolic syndrome in the younger population is also rising. (Park, et al., 2021; Cornier, et al., 2008). In United States, the prevalence of metabolic syndrome has rapidly increased along with obesity. There is an increase of 1.5 times MetS among children and adolescents. Hence,

metabolic syndrome is becoming more common among younger people as well as not just the elderly (Cook, et al., 2003; Ogden, et al., 2002). MetS can result in insulin resistance or insulin deficiency which may give rise to diabetes mellitus (World Health Organization, 2022).

2.2 Diabetes Mellitus

Diabetes mellitus is a chronic condition resulting either from insufficient insulin production by the pancreas or inefficient insulin utilization by the body. Insulin is a hormone to control blood sugar levels. Uncontrolled blood sugar level frequently results in hyperglycemia, which may cause substantial harm to body systems. Diabetes mellitus may contribute to renal failure, heart attacks, strokes, blindness, and lower limb amputation. Type 2 diabetes can be prevented by maintaining an appropriate diet, engaging in regular physical activity, maintaining optimal body weight, and abstaining from tobacco use. According to World Health Organization (2022), diet, exercise, medication, and proper treatment can help to treat diabetes and prevent its complications. Type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and steroid-induced diabetes are a few subtypes of diabetes mellitus. The primary subtypes of diabetes mellitus are type 1 and type 2 diabetes mellitus. Each subtype has a unique etiology, presentation, and therapy (Sapra and Bhandari, 2022).

Type 1 diabetes mellitus (T1DM) is due to the absence of insulin production by pancreatic β-cells. This can be due to autoimmune destruction of the pancreatic β-cells leading to the absence of insulin production. Hereditary flaws in

pancreatic β-cells' ability to sense glucose as well as other genetic or acquired disorders are also the factors leading to T1DM (Sims, Mirmira and Evans-Molina, 2020).

Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes and is characterized by insulin resistance by target tissues. This condition may aggravate when there is a progressive loss of β cells in insulin secretion. Genetic predisposition can be one of the risks leading to T2DM. Other than genetic factors, obesity, and a sedentary lifestyle are also contributing to T2DM. Appropriate physical activity may help in delaying the onset of T2DM, including skeletal muscle contraction can increase blood flow into the muscle and subsequently improve plasma glucose absorption. In addition, exercise lowers the infamous intra-abdominal fat that causes insulin resistance. A moderate-intensity exercise may enhance 40% more glucose uptake giving to higher insulin sensitivity and glucose uptake and reducing or even eliminating inflammation and oxidative stress that may be the risk factors for T2DM (Galicia-Garcia, et al., 2020).

The fasting plasma glucose (FPG) test is one of the laboratory diagnosis methods for T2DM (Centers for Disease Control and Prevention, 2023). This diagnosis is a single point in time that is used to determine the blood glucose level. The test is carried out in the morning after the subject has been fasting for at least 8 hours with only consuming small amounts of water (Davidson, et al., 2021; NIDDK, 2022). The fasting glucose level should fall less than 5.55 mmol/L in non-diabetes individuals. This level would rise between 100 to 125

mg/dL (5.55-6.94 mmol/L) for prediabetics while more than 126 mg/dL (6.99 mmol/L) for diabetic individuals.

Inheritable diabetes in monogenic forms can lead to decreased insulin release from pancreatic cells due to a single gene mutation. These types of diabetes, are genetically diverse, including mitochondrial diabetes, permanent or temporary neonatal diabetes, and maturity-onset diabetes of the young (MODY). MODY was resulted in an autosomal dominant pattern leading to the main deficiency of insulin production (Yau, et al., 2021).

2.2.1 Prevalence of Diabetes Mellitus in Malaysia

Diabetes mellitus had been reported as one of the three most prevalent non-communicable diseases in the Asia Pacific region. Malaysia has been reported as one of the countries with the highest prevalence rate of diabetes in the Western Pacific (Mafauzy, et al., 2016). The prevalence of diabetes in Malaysia had raised from 11.2% in 2011 to 13.4% in 2015 and then to 18.3% in 2019. This makes Malaysia known as the "Sweetest Nation in Asia" which accounted for approximately 1 in 5 of the population's adults. According to National Health and Morbidity Survey in 2019, there is estimated 3.5 million adults in Malaysia aged 18 and above were having diabetes in 2015 but the number rose to 3.9 million in 2019. The highest prevalence rate of diabetes mellitus was discovered in Negeri Sembilan (33.2%), followed by Perlis (32.6%), and Pahang (25.7%). In Malaysia, T2DM prevalence has increased to 20.8% in individuals over the age of 30, which accounted for approximately 2.8 million people (Akhtar, et al., 2022; Ministry of Health Malaysia, 2019). By expectation,

diabetes would affect 7 million Malaysian adults aged 18 and above by 2025 at a prevalence rate of up to 31.3%. This may pose a major public health risk. Population growth, aging populations, urbanization, and obesity are the contributing factors to the high prevalence rate (Akhtar, et al., 2022).

2.2.2 Pathophysiology of Diabetes Mellitus

Diabetes mellitus can be resulted by insulin dysregulation. The β-cells produce pre-proinsulin and undergoes a structural change during maturation to produce proinsulin. Proinsulin enters into immature secretory vesicles and is cleaved into C-peptide and insulin. Elevated glucose levels trigger the release of insulin from granules. A glucose sensor for β-cells, the glucose transporter 2 (GLUT2) which is known as a solute carrier protein would help absorption of glucose by β-cells when circulating glucose levels rise. Once glucose is ingested, glucose catabolism is initiated. This raises the intracellular ATP/ADP ratio, which causes the ATP-dependent potassium channels in the plasma membrane to be closed. In order to allow Ca^{2+} to enter the cell, the membrane depolarizes and opens the voltage dependent Ca^{2+} channels. The priming and fusing of the secretory insulin-containing granules to the plasma membrane are due to an increase in the intracellular Ca^{2+} concentration, which leads to insulin exocytosis. The most significant messenger-enhancing insulin release is by cAMP (Fu, Gilbert and Liu, 2013). By reducing intracellular ion Ca^{2+} reservoirs and raising intracellular Ca^{2+} concentrations, cAMP triggers the mobilization of insulin-containing secretory vesicles. Release of insulin from β-cells is affected when the β-cells are malfunction (Galicia-Garcia, et al., 2020).

Death of β-cells is associated with β-cell dysfunction. Recent research reported a more intricate network of interactions between the environment and many biochemical pathways that may lead to dysfunction of β-cells in T2DM. Hyperglycemia and hyperlipidemia may lead to insulin resistance and chronic inflammation and these harmful stresses that act on β-cells may eventually result in the loss of islet integrity. Obesity-related lipotoxicity, glucose toxicity, and glucolipotoxicity cause oxidative stress and metabolic stress that harm beta cells. Stress resulting from high levels of saturated free fatty acids can activate the unfolded protein response (UPR) pathway that lead to changes in physiological Ca^{2+} mobilization, activation of proapoptotic signals, degradation of proinsulin mRNA, and release of interleukin-1 (IL-1), which attracts macrophages and intensifies inflammation within islets. Eventually, this will result in loss of islet integrity, obstruct cell-to-cell communication within pancreatic islets, cause poor regulation of insulin and glucagon release, and finally aggravating hyperglycemia. **Figure 2.1** summarises the pathogenesis of T2DM. Failure of pancreatic islet cell may lead to insulin resistance and associated with T2DM (Galicia-Garcia, et al., 2020).

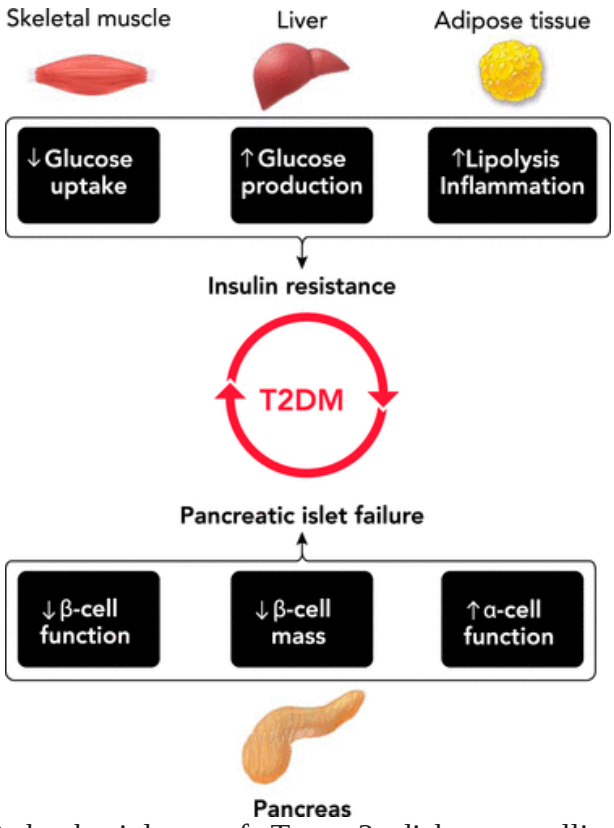


Figure 2.1: Pathophysiology of Type 2 diabetes mellitus (Javeed and Matveyenko, 2018).

2.2.3 TCM Body Constitution with Diabetes Mellitus

An individual's TCM body constitution was determined by the harmony of Yin and Yang. The reduction of the material and energy levels are referred to as Yin and Yang deficiencies, respectively. Unbalanced BCs are more likely to predisposition to specific diseases and experience different disease progression. Some clinical research using BCQ presented associations between various BCs with some disorders, including diabetes, breast cancer, schizophrenia, menopausal symptoms in women, and pregnancies (Lee, et al., 2022). A cross-sectional study discovered that phlegm stasis BCs, yin deficiency, and yang deficiency are strongly associated with diabetes (Tsai, et al., 2014). Another investigation revealed a cross-sectional link between diabetic retinopathy and yang deficiency (Lee, et al., 2015). In impaired glucose regulation (IGR)

participants, damp-heat and phlegm-dampness TCM body constitution is substantially correlated with aberrant serum cytokines and may be used to predict the development of diabetes (You, et al., 2017). The most common body constitution types seen in diabetics are yin deficiency and phlegm-dampness (Bai, et al., 2021).

2.3 Genetic Factors with T2DM: *HNF1A* Gene

HNF1A gene is known as hepatocyte nuclear factor 1 alpha gene. It encodes transcription factors in the liver. The *HNF1A* controls the expression of numerous genes including albumin, α1-antitrypsin, and β-fibrinogen, as well as pancreatic genes that are involved in glucose metabolism and transportation through the regulation of pyruvate kinase and the glucose transporter GLUT2. Therefore, *HNF1A* may be crucial for the control of GLUT2 transcription (Ban, et al., 2002). Besides, *HNF1A* also controls the expression of the insulin gene in the liver, in controlling metabolic processes and function of β-cells (Galán, et al., 2010).

2.3.1 Mutation in *HNF1A* Gene

Loss of HNF1A protein function will affect hepatocyte proliferation. Mutation in *HNF1A* gene may cause maturity-onset diabetes of the young (MODY). MODY is a collection of disorders defined by unusually high blood sugar levels (Miyachi, et al., 2022). The mutation in *HNF1A* gene leads to insulin resistance or failure of the autoimmune pancreatic β-cells and frequently developed before the age of 30. The most prevalent form of MODY is known as HNF1A-MODY or MODY3. This is characterized by autosomal dominant inheritance that will

lead to progressive failure of β-cell due to the presence of mutation in one of the copies of *HNF1A* gene. These mutations may cause an altered HNF1A protein to be produced that is unable to perform its function as transcription factor and may exhibit higher sensitivity to sulfonylureas leading to a lower renal threshold for glucose reabsorption (Galán, et al., 2010).

The mutation in *HNF1A* gene leads to the synthesis of altered HNF1A protein that is unable to perform its function, including being unable to form dimers, unable to enter the nucleus to interact with DNA, and unable to bind to DNA to regulate gene activity. Dimerization, DNA-binding, and transactivation domains are the three major functional domains of the HNF1A protein. The transactivation domain mutation causes minor alterations in the HNF1A protein structure (Zhao, et al., 2022). These will further inhibit the production of functional β-cells and subsequently unable to make insulin in response to blood sugar. The signs and symptoms of MODY are brought on by an increase in blood sugar (National Library of Medicine, 2019).

HNF1A is crucial for hepatocyte processes such as protein synthesis, lipid metabolism, detoxification, and glucose production and storage. A severely enlarged liver and gradual liver damage that results in hepatocyte degeneration are both brought on by an *HNF1A* deficiency. Plasma levels of liver enzymes are influenced by SNPs in the *HNF1A* gene, according to genome-wide association studies (Qian, et al., 2015). Rs735396 was included in this study to relate to the blood glucose level among Malaysia population.

2.3.2 rs735396 in *HNF1A* Gene

Human pancreatic islets with *HNF1A* mutations will encode more alpha and beta cells from stem cells but are prone to differentiate into alpha cells (Miyachi, et al., 2022). The rs735396 variant will also influence the waist circumference of an individual. The rs735396 polymorphism can change the levels of various indicators for the metabolic and inflammatory pathways, including C-reactive protein, and may indicate a hereditary propensity to obesity. The enhancer regulatory region gene rs735396 may influence the temporal interaction between several transacting factors and the *HNF1A* enhancer, modulating the expression of *HNF1A* (Dallali, et al., 2022). According to Morjane et al. (2017), rs735396 variant is associated with metabolic syndrome. On the other hand, a study found that the variant combinations in the *HNF1A* gene which includes the rs735396 polymorphism will reduce the risk of T2DM in Chinese population (Wang, et al., 2007).

2.4 *CAPN10* Gene

CAPN10 gene encodes for calpains which are intracellular, nonlysosomal proteases. Calpains can hydrolyze substrates that are crucial to calcium-regulated signaling pathways. There are approximately 14 isoforms of calpains present in many tissues, and some are significant in the regulation of diabetes. The calcium ion is essential for insulin release from β -cells in response to secretagogues. Calpain-10 (CAPN10) protein is involved in insulin processing, insulin secretion, and insulin action. In non-diabetic family members of individuals with a high risk for T2DM, the CAPN10 affects insulin sensitivity and glucose homeostasis (Prajapat and Bhattacharya, 2016). Studies found

genetic variation in *CAPN10* was responsible for 14% of the population-attributable risk for type 2 diabetes among Mexican Americans (Horikawa, et al., 2000) Calpain-10 is a crucial regulator of insulin secretion as it is associated with the etiology of T2DM. CAPN10 protein is implicated in insulin-stimulated glucose absorption in human skeletal muscle cells. CAPN10 protein processes microtubule-associated protein 1 family protein and regulates their binding activities to microtubules and actin filaments. Actin reorganization, coordination, and dynamics may be affected by MAP1 family processing defects associated with CAPN10 deficiency (Hatta, et al., 2018).

Inhibition of calpains will prevent insulin production as calpain-10 contribute to the actin rearrangement necessary for glucose-stimulated insulin release (Ling, et al., 2009). Hence, a deficiency of calpain-10 expression will lead to insulin resistance and impaired insulin secretion. According to a study (Sáez, et al., 2008), the genetic interaction between the *CYP19* and *CAPN10* genes increases susceptibility to T2DM. Calpain-10 may have a direct regulatory impact on the glucose absorption mechanism because it did not affect insulin-stimulated glycogen production or insulin signaling via protein kinase B when its expression was suppressed (Brown, et al., 2007).

CAPN10 protein promotes the translocation of glucose transporter 4 (GLUT4). Reduced CAPN10 protein expression reduces actin rearrangement, glucose uptake, and GLUT4 vesicle translocation in adipocytes in response to insulin. Additionally, skeletal muscle insulin-stimulated glucose absorption is impaired by targeted inhibition of CAPN10 protein expression. Furthermore, calpain

inhibition, rather than targeted suppression, reduces β-cell actin rearrangement and insulin release. A long-term treatment (48 hours) of mouse islets to calpain inhibitors showed a reduction of glucose-stimulated insulin production. Short-term exposure to calpain inhibitors increases insulin secretion by rapid exocytosis of insulin granules (Hatta, et al., 2018). Mutations in the *CAPN10* gene were found to affect the severity of gestational diabetes mellitus in women, and a significant number of women with higher grades of gestational diabetes mellitus have the mutation in *CAPN10* gene (Zhang, et al., 2019).

2.4.1 rs2975760 in *CAPN10* Gene

A recent study shows that the rs2975760 mutation in *CAPN10* gene will cause the development of diabetes as the SNP is located in the locus of *CAPN10* gene that is associated with diabetes and higher risk in getting T2DM (Nam, et al., 2018; Song, et al., 2004). Study mentioned that the C allele of the *CAPN10* rs2975760 polymorphism will increase the risk of T2DM (Yan, et al., 2014). The TT genotype is the wild type while the CC and TC genotypes are the mutant genotype (Zheng, et al., 2021). The rs2975760 polymorphism may either directly affect type 2 diabetes susceptibility (Evans, et al., 2001). Study also found that the rs2975760 will affect the grade of gestational diabetes. Pregnant women with mutant genotypes of *CAPN10* may be recognized as having an elevated risk of gestational diabetes mellitus (Zhang, et al., 2019). Grade B and D diabetes was found strongly associated with CC and TC genotypes of rs2975760 However, study also claims that the rs2975760 may not be individually associated with diabetes but suggested that the rs2975760

polymorphism is linked with other polymorphism on *CAPN10* gene in leading to the association with diabetes (Bodhini, et al., 2011). The likelihood ohhf mutant-type homozygotes would rise if external environmental factors or metabolic circumstances had an impact on heterozygotes (Zhang, et al., 2019). Therefore, the effect of this SNP may need further elucidation.

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals, Reagents, and Instruments

The chemicals, reagents, instruments, and consumables that are being used throughout the study were listed in **Table 3.1** and **3.2**, respectively.

Table 3.1: Chemicals and reagents used in this study.

Chemicals / reagents	**Manufacturer**
50 bp DNA ladder	GeneDireX, United States
Acetic acid	Synerlab, France
Agarose powder	Vivantis Technologies, Malaysia
DNA extraction kit	Favorgen Biotech Corp., Taiwan
DNA loading buffer	1st BASE, Singapore
EDTA	Orioner Hightech Sdn Bhd, Malaysia
Gel-Red Stain	Nippon Genetics Europe GmbH, Germany
Glucometer	Bayer, German
Master Mix	GeneDireX, United States
Primers	Integrated DNA technologies, United States
Tris	Thermo Fisher Scientific, United States
Tris-EDTA (TE) buffer	1st BASE, Singapore

Table 3.2: Instruments and consumables used in this study.

Instruments / consumables	Manufacturers
Analytical Balance	Shimadzu, Japan
Autoclave Machine	Hiramaya, Japan
Centrifuge Machine (Big)	Thermo Scientific, United States
Centrifuge Machine (Small)	Fisher Scientific, United States
EDTA Tube	Shandong Chengwu Medical Products Factory, China Chengdu Puth Medical Plastic Packaging, China
Freezer (-20 °C)	Pensonic, Malaysia
Fridge (4 °C)	Galaxy 202, Malaysia
Gel Cast	Major Science, United States
Gel Image Viewer	Biorad, United States
Gel Tank	Major Science, United States
Stadiometer	Seca, Germany
Hot Plate with Stirrer	Harmony, Japan
Kimwipes	Kimberly-Clark Corporation, United States
Lancet	ACCU-Chek, Malaysia
Measuring Cylinder	Favorit, Malaysia
Microwave	Panasonic, Japan
Nanodrop	Thermo Scientific, United States
Needles	Terumo, Japan
PCR Machine	Analytik Jena, Germany Hangzhou Bioer Technology Co., Ltd., China
Pipette P10	DLAB Scientific Co., Ltd., United Kingdom
Pipette P100	Biologix, United States
Pipette P1000	Biologix, United States
Power Supply	Major Science, United States
Schott Bottle	Kimble, United States Duran, Germany
Sewing Tape	Wintape Co., Ltd, China
Syringe	Terumo, Japan
Vortex	AITbiotech, Singapore
Water Bath Incubator	Memmert, Schwabach

3.2 Experimental Design

The study was conducted as the experimental design as shown in **Figure 3.1**

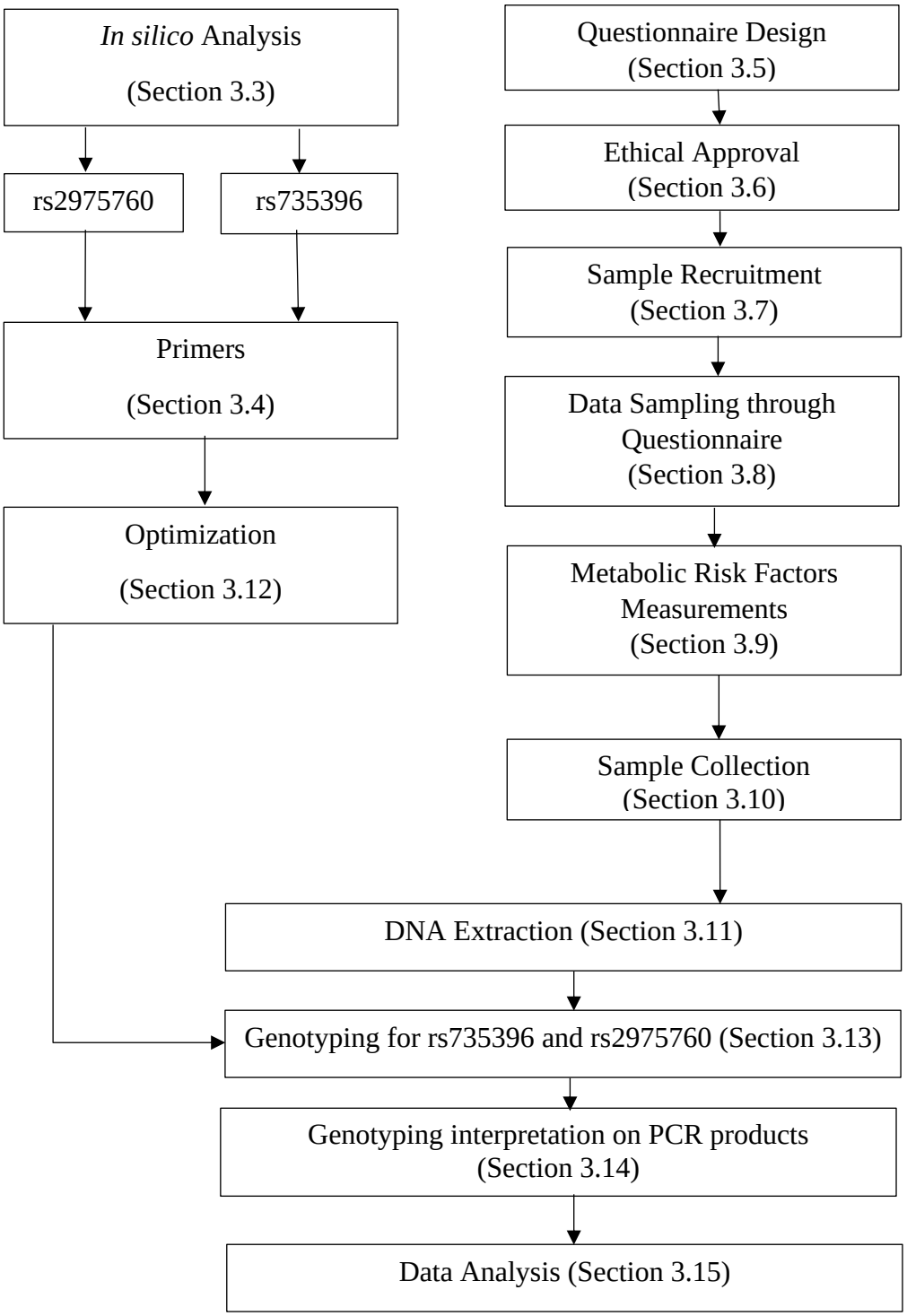


Figure 3.1: Overview of experimental design

3.3 *In silico* Analysis

The minor allele frequency for rs735396 and rs2975760 were checked through single nucleotide polymorphism (SNP) database. According to the National Library of Medicine in year 2022, the minor allele frequency reported for the C allele of rs735396 was ranging from 0.088 to 0.516 (National Library of Medicine, 2022b)., while the minor allele frequency reported for the C allele of rs2975760 was ranging from 0.046 to 0.230 (National Library of Medicine, 2022a). As both SNPs MAF> 0.05, indicating a significant SNP. Thus, this study included the SNP genotyping for rs735396 (T>C) and rs2975760 (T>C). Primers were designed for these two SNPs.

3.4 Primers

3.4.1 Primer Design

A multiplex PCR method, tetra-primer amplification refractory mutation system (ARMS) PCR was used in this study. A set of four primers were designed to detect the specific SNP, comprised of an outer forward, outer reverse, inner forward, and inner reverse primers. Nucleotide sequence for *HNF1A* gene and *CAPN10* genes were obtained from the NCBI database under the accession number NC_000012 and NC_000002 respectively. The location of the SNPs was identified, and the sequence was extracted for primers design. The primers were designed using primer design software, PRIMER1.

Two sets of primers were designed to detect rs735396 and rs2975760 respectively. The primer sequences are listed in **Table 3.3a** and **3.3b**. Each set of primer consists of 4 primers and the primer sequence was sent to Integrated DNA Technologies for primer synthesis.

Table 3.3a: rs735396 primers, targets and amplicon size

Primer sequence 5' → 3'	Amplification	Size
Forward inner primer (T allele): 475 GTGGGTGTGGGTGCCTGGTGGGTGTCT 501	Mutant IF-OR	193
Reverse inner primer (A allele): 529 GGACACTGCAGAGGCAAACAAGGCTGATG 501	Wild type OF-IR	263
Forward outer primer (5' - 3'): 267 CTACCTCGGCATCTCACCGGGGCTTCTC 294	Internal control OF-OR	400
Reverse outer primer (5' - 3'): 666 CCCAGGTGCCGTGGTTACTGGGAGGAAG 639		

Table 3.3b: rs2975760 primers, targets and amplicon size

Primer sequence 5' → 3'	Amplification	Size
Forward inner primer (T allele): 476 GACTGCAGGGCGCTCACGCTTGCGGT 501	Mutant IF-OR	200
Reverse inner primer (A allele): 529 TTAGCCTCACCTTCAAACGCCTTACTGCG 501	Wild type OF-IR	274
Forward outer primer (5' - 3'): 256 AAGGCAACTGGACTGACAGGCAGGCAGG 283	Internal control OF-OR	419
Reverse outer primer (5' - 3'): 674 TCACCATGGGAGTGAGCCTCTGGCATTG 647		

The SNP (T > C) was targeted for rs735396 at exon 9 of *HNF1A* gene at chromosome 12. The SNP is located at position 27296 of the gene as shown in **Figure 3.2a**. Meanwhile, the SNP (T > C) was targeted for rs2975760 at exon 12 of *CAPN10* gene at chromosome 2. The SNP is located at position 10021 of the gene as in figure 3.2b. The SNPs on the exons were chosen in this study as the exons are the coding region which will code for protein after the translation. The non-coding region which is the intron will be removed forming the mature mRNA and eventually the non-coding region is not translated. **Figure 3.2a** and **3.2b** showed the primers targeting the exon 9 and exon 12 in detecting rs735396 and rs2975760 respectively.

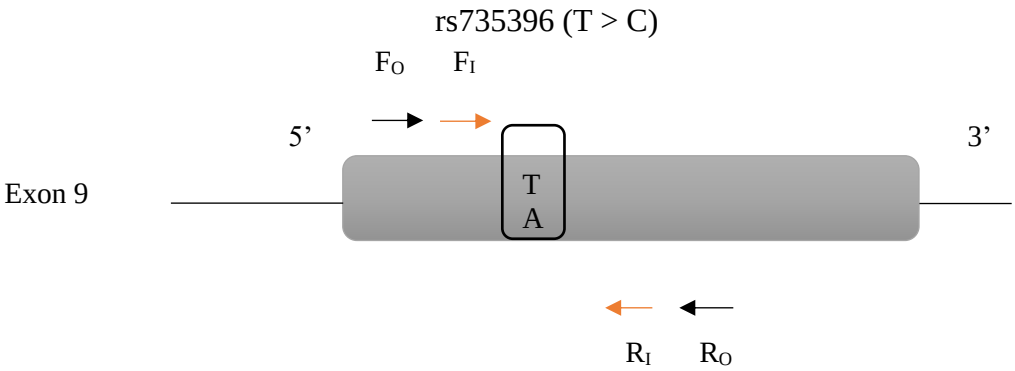


Figure 3.2a: Primers targeting the exon 9 to detect rs735396

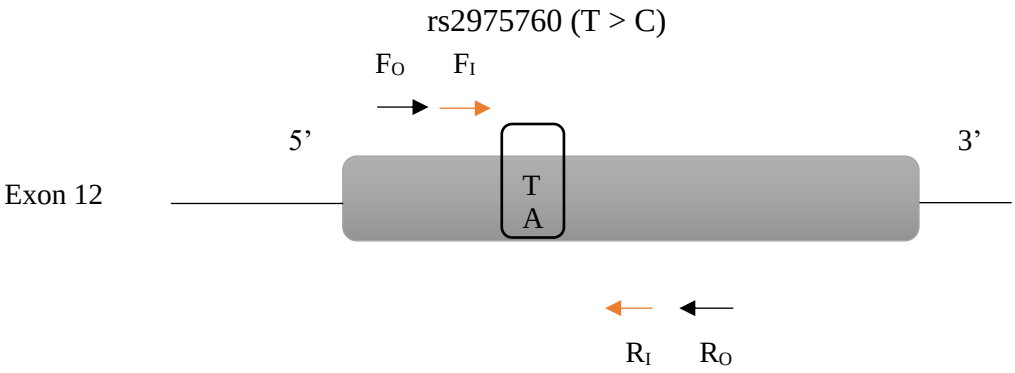


Figure 3.2b: Primers targeting the exon 12 to detect rs2975760

3.4.2 Preparation of Stock Primer

The primers were received in lyophilized form. The lyophilized primers were centrifuged to spin down and then reconstituted with TE buffer. A specific volume of TE buffer was added into the tube according to the specification sheet and the mixture was mixed by pulse vortex. The specification sheet of the primers was attached at Appendix A.

3.5 Development of Questionnaire

The questionnaire was adopted from the China Association of Chinese Medicine, published in 2009. The body constitution questionnaire consists of 63 questions asking about the frequency of experience of study subjects in near a month using a 5-point Likert-type scale. Google Form platform was used to develop the questionnaire to minimize the cost of printing and ease the data entry process. The questionnaire was divided into 10 sections: demographic data, questions of body constitution in different categories, Yang Xu, Yin Xu, Qi Xu, Tan Shi, Shi Re, Xue Yu, Te Bing, Qi Yu, and Ping He. The questionnaire was attached as Appendix H.

3.6 Ethical Approval

Prior to the commencement of the study, ethical approval was obtained from UTAR Scientific and Ethical Review Committee (SERC) (Re: U/SERC/221/2022). The ethical approval was attached as Appendix I.

3.7 Sample Recruitment

Poster with registration link was shared through social media and pasted at Block D, UTAR and in a Traditional Chinese Medicine clinic in Ipoh. Appointments were made with the participants who registered through the link. The walk-in patients in the clinic were also approached. The volunteers were briefed about the study. Informed consents were obtained prior sample collection. Samples were recruited based on the inclusion and exclusion criteria.

Inclusion criteria:

- 18 years old and above
- Fasting state
- Willing to involve in venepuncture and finger pricking

Exclusion criteria:

- Pregnancy
- Below 18 years old
- Not willing to involve in venepuncture and finger pricking

Sample size was calculated to meet 90% significance level with 20% drop off rate. According to Joint Interim Statement (JIS), the expected prevalence of metabolic syndrome is 43.4% (Ramli, et al., 2013). Therefore, using the formula to calculate the significant sample size by Naing, 2003. The significant sample size calculated was 67. In addition to the 67 significant sample size with 20% of drop off rate, the significant sample size was 81 samples.

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

$$n = \frac{(1.645^2).0.434.(1-0.434)}{0.1^2}$$

$$n = 67$$

Addition 20% Drop Off Rate:

n = 67 x 120%

n = 81

n = sample size

Z = statistic level for 90% level of confidence = 1.645

p = expected prevalence of metabolic syndrome (JIS) = 0.434

d = 10% allowable error = 0.1

3.8 Data Collection

All participants were required to fill up the TCM body constitution questionnaire either prior to their appointments or on the spot via QR code provided. The anthropometry measurements of the participants were measured using stadiometer for height, weighing scale for weight and measuring tape for waist circumference.

3.9 Metabolic Risk Factors Measurements

3.9.1 Waist Circumference Measurement

Measuring tape was used to measure the waist circumference of the subjects. The waist circumference was measured at the position between the last rib bone and the navel as shown in **Figure 3.3**.

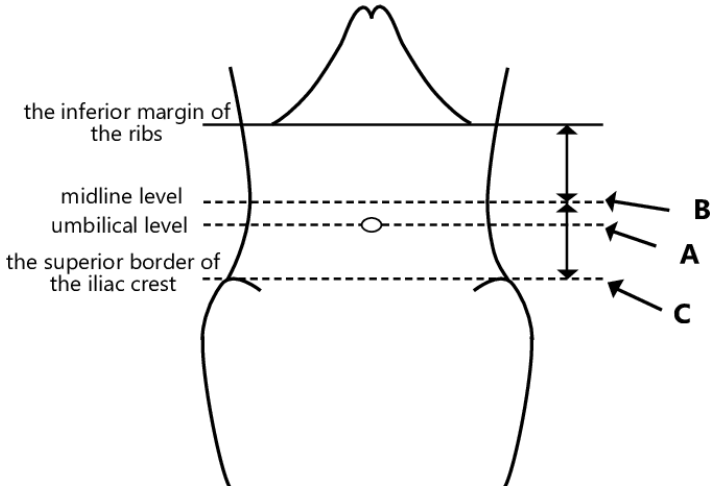


Figure 3.3: Measurement of waist circumference (Lemoncito, et al., 2010).

3.9.2 Fasting Blood Glucose Measurement

Capillary blood was obtained through finger pricking. The first blood was wiped off and the subsequent blood were used for the measurement. About 0.6 µL of capillary blood was loaded to glucose test strips to measure for fasting blood glucose was measured using Bayer Contour Plus Blood Glucose. Measurement was done within one minute.

3.10 Sample Collection

Whole blood sample was collected through venepuncture. A total of 6 mL of venous blood were collected through venepuncture performed by my supervisor, Dr. Teh Lai Kuan. The samples collected were stored in ethylenediaminetetraacetic acid (EDTA) vacutainer to prevent the blood from clotting.

3.11 DNA Extraction

3.11.1 DNA Extraction Protocol

DNA extraction was done by using Favorgen DNA Extraction kit. Venous blood sample was allowed to stand for the separation of buffy coat layer that contain the lymphocytes from the whole blood. A volume of 200 µL of the buffy coat was isolated and transferred to a new 1.5 mL microcentrifuge tube. The samples were added with 20 µL of Proteinase K and 200 µL of FABG buffer and mixed with vortex. The samples were then incubated at 60 °C for 15 minutes using water bath incubator. The samples were vortexed at every 5 minutes interval to speed up the lysis of cell membrane for the release of cell contents. The tubes were then spun down, followed by the addition of 200 µL of absolute ethanol (96%) and pulse-vortexed for 10 seconds to homogenise the solution. The solution was spun down and transferred to FABG mini column in collection tube and was centrifuged at 6,000 x g for 1 minute, followed by the addition of 400 µL W1 buffer prior the centrifugation at 17,000 x g for 30 seconds. The filtrate was discarded. A volume of 750 µL wash buffer was added to the column and was centrifuged at 17,000 x g for 30 seconds. The filtrate was discarded and the FABG mini column was centrifuged at 17,000 x g for 3 minutes to remove the remaining fluid. The FABG mini column was transferred to a new microcentrifuge tube and added with 100 µL elution buffer. The FABG mini column was incubated at room temperature for 10 minutes and centrifuged at 17,000 x g for 1 minute. A volume of 100 µL elution buffer was added to the mini column and incubated for another 10 minutes prior to the centrifugation at 17,000 x g for 1 minute to elute all extracted DNA. The extracted DNA was stored at 4 °C and the FABG mini column was discarded (Favorgen, n.d.).

3.11.2 Concentration and Purity Analysis of Extracted DNA

The concentration and the purity of the extracted DNA was measured using nanodrop and gel electrophoresis. The nanodrop machine was first blank with 1 μL of elution buffer. Then, 1 μL of extracted DNA was placed on the nanodrop machine for the measurement. The accepted purity of the extracted DNA is determined using A_{260}/A_{280} ratio within the range of 1.8 to 2.1 (Lucena-Aguilar, et al., 2016). In addition, the extracted DNA that have concentration greater than 25 ng/μL is accepted in this study.

The genomic gel electrophoresis was used to check the purity of the extracted DNA. The extracted DNA was diluted to a concentration of 25 ng/μL using autoclaved distilled water. The diluted working DNA was mixed with the loading dye prior loading into the agarose gel. The agarose gel was mixed with the 10X diluted GelRed before solidifying. The gel was ran using 90 V power supply for 30 minutes. The gel was viewed using gel image viewer. Samples with thick band of genomic DNA without smearing indicating no degradation or fragmentation would be used for preparation of working DNA (Abdel-Latif and Osman, 2017).

3.11.3 Preparation of working DNA

The extracted genomic DNA would be kept as stock DNA and also used to prepare working DNA at 25 ng/μL by diluting the samples with autoclaved distilled water (ddH_2O) (Nakayama, et al., 2016). All the working DNA were prepared at a final volume of 30 μL using the formula below.

$$M_1V_1 = M_2V_2$$

M_1 = Concentration of extracted DNA

V_1 = Volume of extracted DNA

M_2 = Final concentration of working DNA (25 ng/μL)

V_2 = Volume of working DNA (10 μL)

3.12 Optimization for genotyping of rs735396 and rs2975760

PCR optimization was done to check for the ideal annealing temperature. To determine the best annealing temperature for the efficacy of PCR, gradient PCR reaction was carried out that cover a range of melting temperature (Obradovic, et al., 2013). For rs735396, the annealing temperature used were 59.0 °C, 61.4 °C, 63.2 °C, 65.5 °C, 68.1 °C, 70.5 °C, 72.3 °C and 74.6 °C. For rs2975760, the annealing temperature used were 59.0 °C, 61.9 °C, 64.0 °C, 66.0 °C, 68.0 °C, 70.0 °C, 72.1 °C and 74.6 °C. From the product of the PCR, the samples that showed the optimum amplification was chosen as the annealing temperature for the cycling condition. The optimization was performed using the Genepro thermal cycler which is with gradient function from Bioer Technology.

3.13 Genotyping for rs735396 and rs2975760

Both rs735396 and rs2975760 SNPs were detected using Tetra-Primer Amplification Refractory Mutation System (Tetra-Primer ARMS) PCR system, which is a simple and economical method in detecting SNPs (Medrano and Oliveira, 2014). Before PCR preparation, primer mix for rs735396 and rs2975760 were prepared as mentioned in **Table 3.4a** and **Table 3.4b**.

Table 3.4a: Primer mix preparation for rs735396

Primers	Initial Concentration (µM)	Final Concentration (µM)	Volume (µL)
F_o	100	4	2
R_o	100	4	2
F_I	100	4	2
R_I	100	4	2
ddH_2O	-	-	42
Total			50

Table 3.4b: Primer mix preparation for rs2975760

Primers	Initial Concentration (µM)	Final Concentration (µM)	Volume (µL)
F_o	100	4	2
R_o	100	4	2
F_I	100	4	2
R_I	100	4	2
ddH_2O	-	-	42
Total			50

Genotyping of rs735396 and rs2975760 were prepared in final volume of 10 µL consisting of PCR buffer, dNTPs, respective primers, and genomic DNA. PCR reaction for rs735396 and rs2975760 detection was prepared as in **Table 3.5a** and **Table 3.5b** respectively.

PCR was conducted using Biometra thermal cycler with the cycling condition consisting of pre-denaturation, denaturation, annealing, extension, and final extension as in **Table 3.6a** and **Table 3.6b**.

Table 3.5a: rs735396 PCR mix preparation

Reagents	Initial Concentration	Final Concentration	Volume (µL)
Master Mix	2X	1X	5
Primer Mix	-	-	1
Extracted DNA	25 ng/µL	50 ng/µL	2
PCR Water	-	-	2
Total			10

Table 3.5b: rs2975760 PCR mix preparation

Reagents	Initial Concentration	Final Concentration	Volume (µL)
Master Mix	2X	1X	5
Primer Mix	-	-	1
Extracted DNA	25 ng/µL	50 ng/µL	2
PCR Water	-	-	2
Total			10

Table 3.6a: PCR cycling condition for rs735396

Events	Temperature (°C)	Time	Cycle
Pre-denaturation	95.0	3 minutes	1
Denaturation	95.0	30 seconds	30
Annealing	62.0	30 seconds	30
Extension	72.0	30 seconds	30
Final extension	72.0	3 minutes	1

Table 3.6b: PCR cycling condition for rs2975760

Events	Temperature (°C)	Time	Cycle
Pre-denaturation	95.0	3 minutes	1
Denaturation	95.0	30 seconds	30
Annealing	64.0	30 seconds	30
Extension	72.0	30 seconds	30
Final extension	72.0	3 minutes	1

3.14 Genotyping Interpretation on PCR Products

Gel electrophoresis was run for PCR products for genotyping using 2.0% agarose gel. A small 2.0% agarose gel was prepared at the volume of 20 mL of 1X TAE buffer, while a large 2.0% agarose gel was prepared at 30 mL of 1X TAE buffer. The amount of agarose powder added was calculated using the formula as below.

$$2.0\% = \frac{\text{Amount of agarose powder (g)}}{\text{Volume of TAE buffer}} \text{ x } 100\%$$

The PCR product and 1 µL 50 bp ladder was loaded into the agarose gel at different well. The 50 bp ladder was loaded in the first well followed by the PCR products. A volume of 5 µL PCR product was mixed with 1 µL loading

dye prior loading into the agarose gel. The agarose gel was mixed with the 10X diluted GelRed before solidifying. The GelRed enable the PCR product to be visible under ultraviolet (UV) light. GelRed is a relatively safe material to stain nucleic acid as compared to ethidium bromide (Vivantis, n.d.). The gel was ran using 90 V power supply for 40 minutes. After the gel electrophoresis, the gel was viewed using the Biorad gel image viewer. Genotype was interpreted based on the amplicon size as shown in **Table 3.7**. All the samples would show an internal control band to ensure the proper PCR amplicon. Internal control for rs735396 was at 400 bp while the internal control for rs2975760 was at 419 bp. Bands should not be detected in the lane loaded with non-template control to indicate that there is no contamination present, which might give rise to false positive results.

For rs735396, amplicon size at 263 bp was detected for T allele, which is the wild type allele of the rs735396. Meanwhile, amplicon size at 193 bp was used to detect C allele, the minor allele of rs735396. For rs2975760, amplicon size at 274 bp was detected for T allele, which is the wild type allele for rs2975760. Meanwhile, amplicon size at 200 bp was detected for C allele, the minor allele for rs2975760.

Table 3.7: Amplicon size for rs735396 and rs2975760

	rs735396		**rs2975760**	
	Genotype	**Amplicon size (bp)**	**Genotype**	**Amplicon size (bp)**
Wild type	TT	263	TT	274
Heterozygous	TC	193 and 263	TC	200 and 274
Mutant	CC	193	CC	200

3.15 Statistical Analysis

Data collected from the TCM BC questionnaire, demographic data, waist circumference, fasting blood glucose level, BMI and genotypes were analysed using IBM SPSS Statistics 26 software. Both Shapiro-Wilk and Kolmogorov-Smirnov test were performed on waist circumference and fasting blood glucose level to determine the normality. As the waist circumference and fasting blood glucose level were not normally distributed, therefore the non-parametric test, Kruskal-Wallis test was used to determine the relationship between categorical data (Traditional Chinese Medicine Body Constitution, demographic distribution, BMI, COVID-19 History, genotypes) and continuous data (waist circumference and fasting blood glucose level). Besides, Fisher's exact test was used to associate two categorical data as the sample size is small.

CHAPTER 4

RESULTS

4.1 Study Subject and Prevalence of Abdominal Obesity and Diabetes

A total of 102 study subjects were successfully recruited in this study based on the inclusion and exclusion criteria. This fulfilled the calculated sample size of 81 including 20% drop-off rate and fit into 90% confidence interval. Fasting blood glucose levels were measured using Bayer Contour Plus Glucose Meter and a level more than 6.9 mmol/L was diagnosed as diabetes. In this study, 2 out of the 102 subjects were diagnosed as diabetic with a prevalence rate of 1.96%. The demographic distribution of each study subject was included in Appendix B. The fasting blood glucose level of each study subject was included in Appendix C.

According to the International Diabetes Federation (2006), a waist circumference of more than 90 cm and more than 80 cm were considered abdominal obesity for men and women, respectively. Abdominal obesity increases the risk of developing diabetes. In this study, there was a total of 16 out of 51 females (31.4%) and 19 out of 51 males (37.3%) were considered to have abdominal obesity in this study. The waist circumference of each study subject was included in Appendix C.

4.2 Genomic DNA Analysis

All the 102 whole blood sample were centrifuged to isolate buffy coat layer for DNA extraction using Favorgen DNA Extraction Kit. Concentration and purity

of the extracted DNA were checked using Nanodrop and genomic gel electrophoresis as in **Figure 4.1**. The concentration and purity (A_{260}/A_{280} ratio) of all the samples were tabulated in Appendix D. The mean of the extracted genomic DNA concentration was 53.46 ng and A_{260}/A_{280} purity was 1.80.

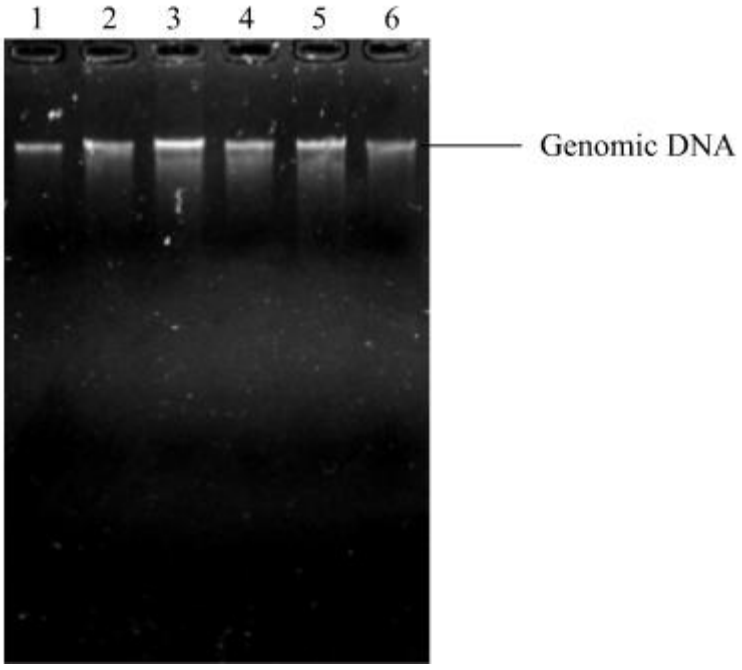


Figure 4.1: Representative genomic gel electrophoresis for genomic DNA at 90 volts for 30 minutes using 2.0% of agarose gel

Lane 1: sample 077; Lane 2: sample 081; Lane 3: sample 082; Lane 4: sample 083; Lane 5: sample 084; Lane 6: sample 085

4.3 Genotype of *HNF1A* and *CAPN10*: rs735396 and rs2975760

Genotyping of rs735396 and rs2975760 was conducted using Tetra-primer ARMS-PCR and was analyzed using agarose gel electrophoresis at the voltage of 90 volts for 35 minutes with 2.0% agarose gel. Electrophoresis was run in parallel with 50 bp ladder from GeneDireX to identify the approximate molecular size. **Figure 4.2a** shows representative gel image for genotyping of rs735396. Lane 4 showed three amplicons with amplicon size at 400 bp for detection of internal control that amplify target *HNF1A* gene, 193 bp, and 263

bp for amplification of wild type T allele and mutant C allele, respectively. Presence of internal control band (400 bp) and wildtype, T allele band (193 bp) as in lanes 2 and 3, presenting as wild type. The presence of the internal control band (400 bp) with the mutant allele band (263 bp) indicates a homozygous mutant. Whereas the presence of the internal control band (400 bp) with both wildtype allele band (193 bp) and mutant allele band (263 bp) indicates heterozygous.

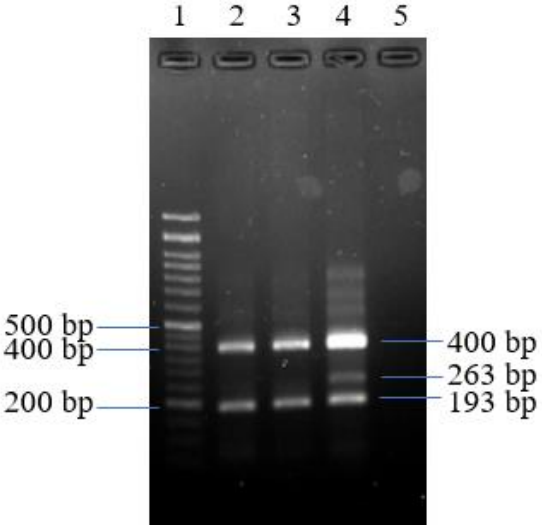


Figure 4.2a: Representative gel electrophoresis for genotyping of rs735396

Lane 1: 50 bp ladder; Lane 2: sample 050; Lane 3: sample 090; Lane 4: sample 095; Lane 5: Non-template control (NTC)

Figure 4.2b shows a representative gel image for genotyping of rs2975760. The amplicon size of 419 bp was amplified internal control for the target *CAPN10* gene. Detection of wild type, T allele and mutant, C allele was amplified at 200 bp and 274 bp, respectively. Lanes 2, 4,5, and 7 presented an internal control band (419 bp) and wild type allele band (200 bp), indicating wild type. The presence of internal control band (419 bp) with mutant allele band (274 bp), indicates a homozygous mutant. Whereas the presence of

internal control band (419 bp) with wildtype allele band (200 bp) and mutant allele band (274 bp) as in lanes 3 and 6 indicates heterozygous for rs2975760. Negative control was run in parallel in genotyping by replacing DNA samples with water. Lane 8 showed the absence of a band in non-template control depicting that the PCR was carried out without any contamination. The genotype for all the samples was included in Appendix E and agarose gel electrophoresis in Appendix F.

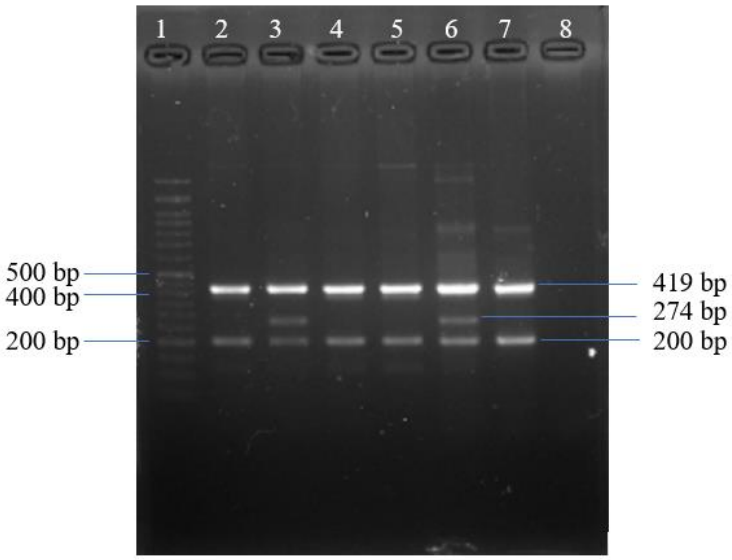


Figure 4.2b: Representative gel electrophoresis for genotyping of rs2975760

Lane 1: 50 bp ladder; Lane 2: sample 007; Lane 3: sample 008; Lane 4: sample 009; Lane 5: sample 010; Lane 6: sample 011; Lane 7: sample 012; Lane 8: Non-template control (NTC)

4.4 Genotypic and Allelic Frequency for rs735396 and rs2975760

Genotypic and allelic frequencies for rs735396 and rs2975760 were presented in **Table 4.1**. For rs735396, 22.55% (n = 23) study subjects were genotyped as homozygous wildtype (TT) and 21.57% (n = 22) study subjects were homozygous mutant (CC). The heterozygous genotype (TC) is composed of the highest percentage rate of 55.88% (n = 57). The allelic frequency for the major

allele (T allele) was 0.5 and the minor allele (C allele) was 0.5. For rs2975760, homozygous wildtype (TT) composed the highest percentage rate of 87.25% (n = 89). On the other hand, 2.94% (n = 3) study subjects were genotyped as homozygous mutant (CC) and 9.80% (n = 10) study subjects were heterozygous genotype (TC). The allelic frequency of the major allele (T allele) was 0.92 and the minor allele (C allele) was 0.08. Both polymorphisms were found as significant variants as the minor allele frequency (MAF) was found more than 0.05.

Table 4.1: Genotypic and allelic frequency for rs735396 and rs2975760.

	Genotypic frequency, n (%)			**Allelic frequency**	
				Major	**Minor**
rs735396	**TT**	**TC**	**CC**	**T**	**C**
(T>C)	23	57	22	0.50	0.50
n = 102	(22.55%)	(55.88%)	(21.57%)		
rs2975760	**TT**	**TC**	**CC**	**T**	**C**
(T>C)	89	10	3	0.92	0.08
n = 102	(87.25%)	(9.80%)	(2.94%)		

4.5 Relationship between Waist Circumference and Fasting Blood Glucose Level

Pearson correlation was conducted to determine the relationship between waist circumference and fasting glucose level ($p < 0.10$, 90% CI). The correlation coefficient (R^2) value for waist circumference and fasting blood glucose level was 0.325, denoting a weak positive correlation between waist circumference and fasting blood glucose level as shown in **Figure 4.3**.

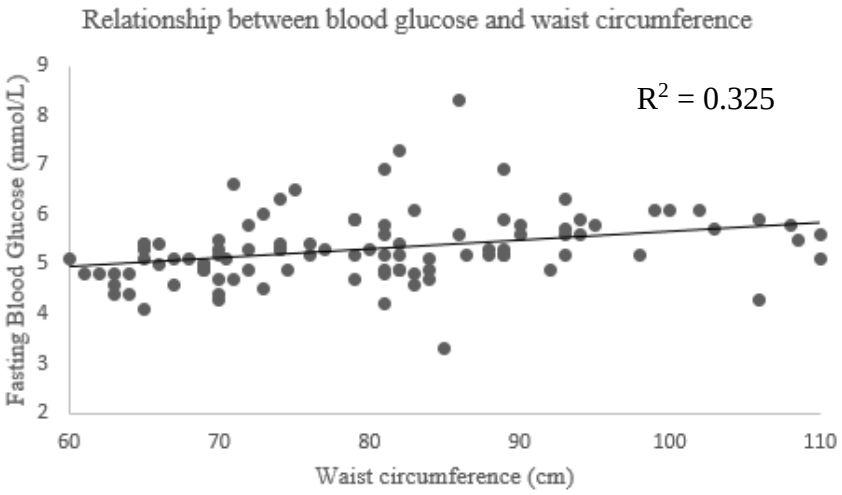


Figure 4.3: Relationship between waist circumference and fasting blood glucose level

4.6 Normality Test for Waist Circumference and Fasting Blood Glucose Level

Normality tests of waist circumference and fasting blood glucose level were conducted using Kolmogorov-Smirnov and Shapiro-Wilk tests. Both waist circumference and fasting blood glucose level were not normally distributed with p-value less than 0.10 (**Table 4.2**). Thus, the analyses were proceeded with non-parametric test, Kruskal-Wallis test.

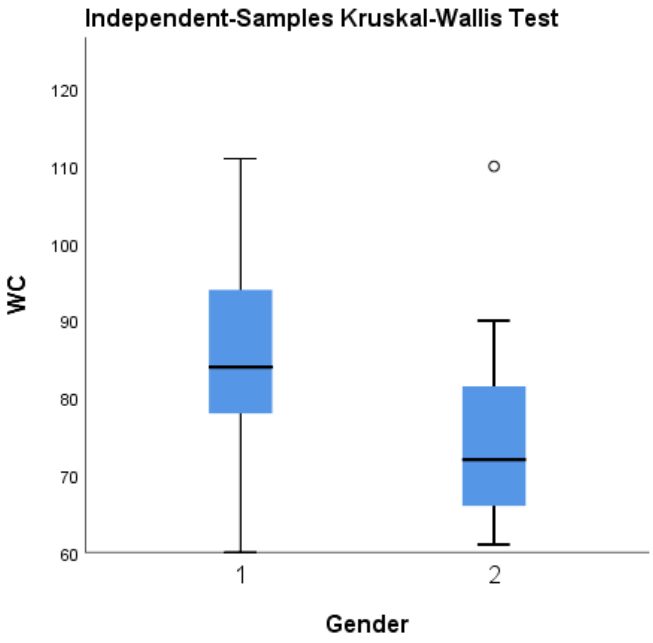
Table 4.2: Kolmogorov-Smirnov and Shapiro-Wilk normality tests on waist circumference and fasting blood glucose level.

	Kolmogorov-Smirnov		**Shapiro-Wilk**	
	df	**p-value**	**df**	**p-value**
Waist circumference (cm)	102	0.031	102	0.001
Fasting blood glucose level (mmol/L)	102	0.001	102	0.000

df = degree of freedom

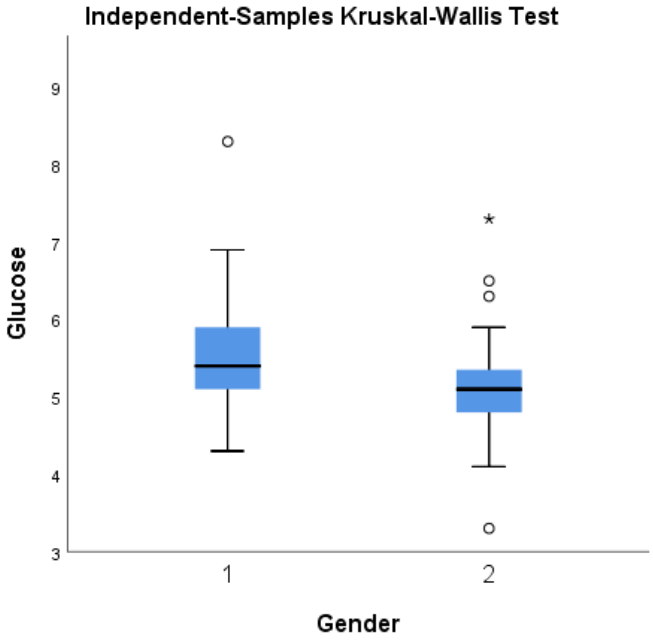
4.7 Demographic Distribution in relation to Waist Circumference and Fasting Blood Glucose Level

Table 4.3 shows the demographic data including gender, age, ethnicity, and place among 102 study subjects. There were equal numbers in both genders, 51 males (50.00%) and 51 females (50.00%) participated in this study. The Kruskal-Wallis H test showed that there was a significant difference in waist circumference between different gender at $p = 0.000$, with a mean rank waist circumference of 65.68 for males and 37.32 for females. **Figure 4.4** shows the distribution of waist circumference among study subjects with different gender. Females have lower waist circumferences at a mean of 74.29 cm compared to males with a mean waist circumference of 86.49 cm. There was also a significant difference in fasting blood glucose between different gender at $p = 0.003$, with a mean rank fasting blood glucose of 60.27 for males and 42.73 for females. Females were found to have lower fasting blood glucose levels compared to males. **Figure 4.5** shows the distribution of fasting blood glucose level among study subjects with different gender.



1: Male; 2: Female

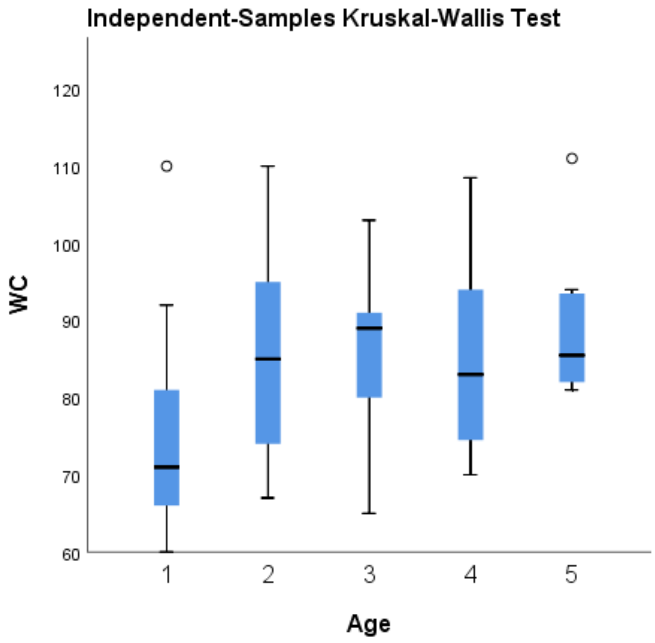
Figure 4.4: Waist circumference against gender among 102 study subjects



1: Male; 2: Female

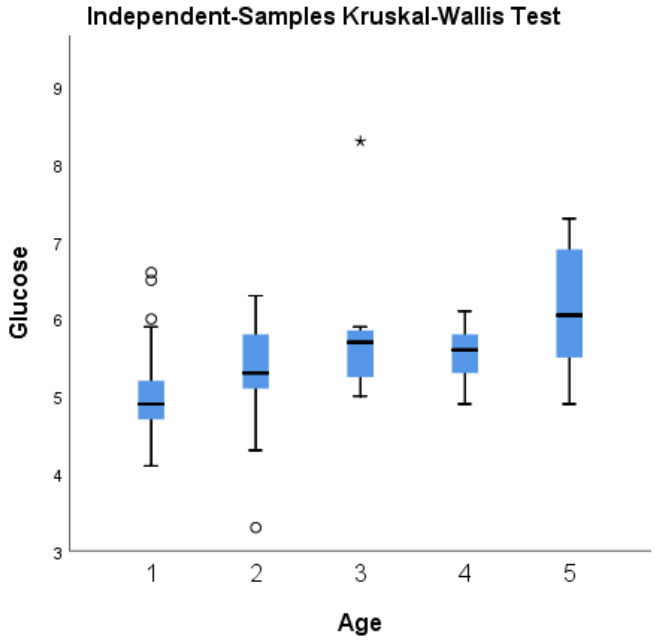
Figure 4.5: Fasting blood glucose level against gender among 102 study subjects

The age of the study subjects ranged between 19 to 86 and was categorized into 5 groups, 29 and below, 30 – 39, 40 – 49, 50 – 59, and 60 and above. There were 49 subjects under the age group below 29 (48.04%), followed by 18 subjects from the age group of 30-39 (17.65%), 10 subjects from the age group of 40-49 (9.80%), 17 subjects from the age group 50-59 (16.67%) and 8 subjects from the age group above 60 (7.84%). The Kruskal-Wallis H test showed a significant difference in waist circumference between different age groups at p = 0.000, with a mean rank waist circumference of 36.51 for age group 29 and below, 64.39 for age group 30 – 39, 62.80 for age group 40 – 49, 63.82 for age group 50 – 59 and 74.00 for age group 60 and above. Subjects from the age group 29 and below has the lowest waist circumference at a mean rank of 36.51 while the subjects from the age group 60 and above have the highest waist circumference at a mean rank of 74.00. **Figure 4.6** shows the distribution of waist circumference among study subjects with different age groups. The Kruskal-Wallis H test showed that there was a significant difference in fasting blood glucose between different age group at p = 0.000, with a mean rank fasting blood glucose of 35.40 for age group 29 and below, 56.28 for age group 30 – 39, 67.10 for age group 40 – 49, 70.09 for age group 50 – 59 and 80.38 for age group 60 and above. Subjects from the age group 29 and below were found to have the lowest fasting blood glucose while the subjects from the age group 60 and above were found to have the highest fasting blood glucose. **Figure 4.7** shows the distribution of fasting blood glucose level among study subjects with different age groups.



1: 29 and below; 2: 30 – 39; 3: 40 – 49; 4: 50 – 59; 5: 60 and above

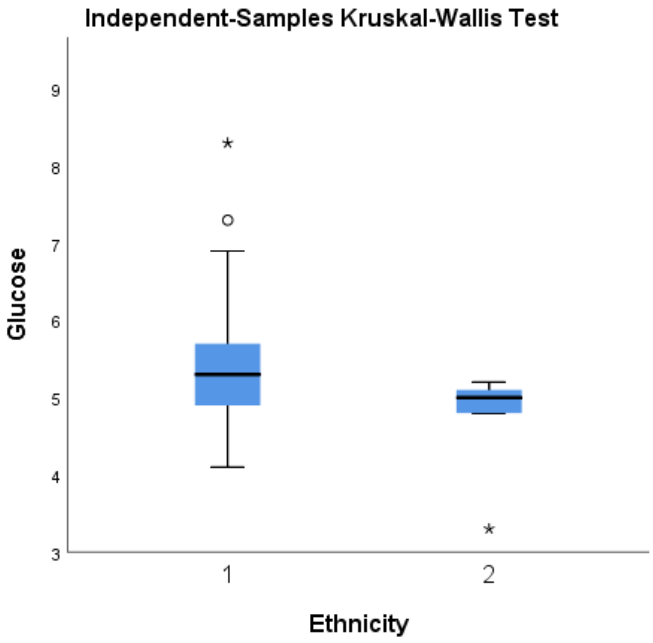
Figure 4.6: Waist circumference against age among 102 study subjects



1: 29 and below; 2: 30 – 39; 3: 40 – 49; 4: 50 – 59; 5: 60 and above

Figure 4.7: Fasting blood glucose level against age among 102 study subjects

Out of 102 subjects, 95.10% (n = 97) of the study subjects were Chinese, and 4.90% (n = 5) were from other ethnicities. Other ethnicities include Malay, Indian, and Chinese-Filipino. Kruskal-Wallis test presented no significant difference in waist circumference but with significance in fasting blood glucose level as the p-value at 0.852 and 0.070, respectively among different ethnicities. The mean rank waist circumference was 52.70 for Chinese and 28.20 for other ethnicities. Subjects who were Chinese were found to have higher fasting blood glucose than other ethnicities. **Figure 4.8** shows the distribution of fasting blood glucose level among study subjects with different ethnicities.

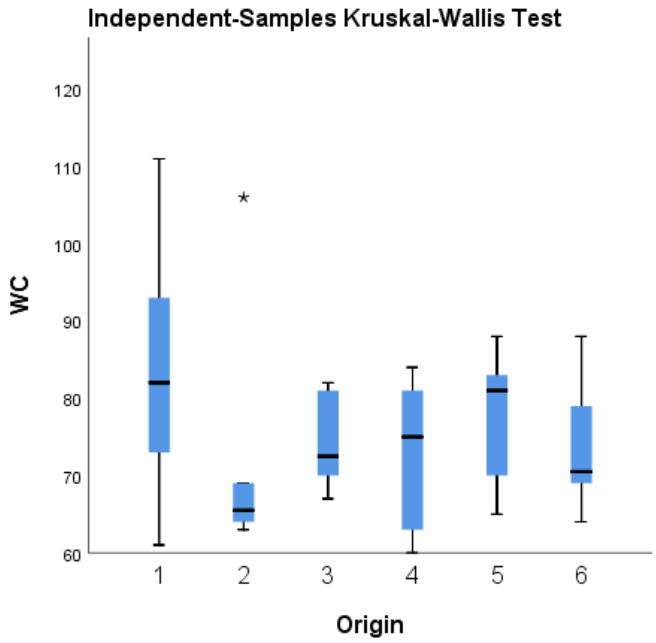


1: Chinese; 2: Other ethnicities

Figure 4.8: Fasting blood glucose level against ethnicity among 102 study subjects

The respondents are from 11 different states in Malaysia. There were 66 subjects from Perak (64.71%), 5.88% (n = 6) from Penang, 5.88% (n = 6) from Johor, 4.90% (n = 5) from Kuala Lumpur, 5.88% (n = 6) from Selangor and 12.75% (n = 13) from other places of origin. Other places of origin include Melaka,

Terengganu, Pahang, Sabah, Negeri Sembilan, and Labuan. The Kruskal-Wallis H test showed that there was a significant difference in waist circumference between places of origin at p = 0.010, with a mean rank waist circumference of 59.42 for subjects from Perak, 25.92 for subjects from Penang, 38.33 for subjects from Johor, 34.40 for subjects from Kuala Lumpur, 47.83 for subjects from Selangor and 37.46 for subjects from other places of origin. Subjects from Perak have the highest waist circumference at a mean rank of 59.42 while subjects from Penang were found to have the lowest waist circumference at a mean rank of 25.92. **Figure 4.9** shows the distribution of waist circumference among study subjects with different place of origin.

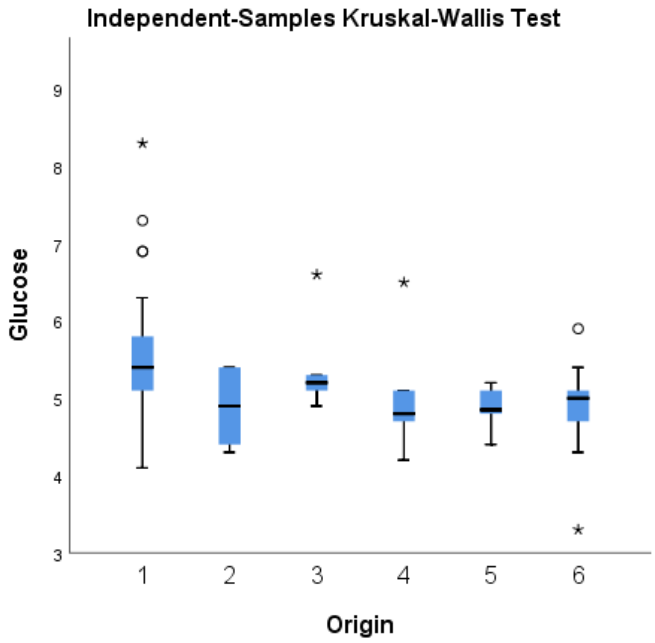


1: Perak; 2: Penang; 3: Johor; 4: Kuala Lumpur; 5: Selangor; 6: Others

Figure 4.9: Waist circumference against place of origin among 102 study subjects

The Kruskal-Wallis H test showed that there was a significant difference in fasting blood glucose level between places of origin at p = 0.001, with a mean rank fasting blood glucose level of 60.34 for subjects from Perak, 32.58 for

subjects from Penang, 53.00 for subjects from Johor, 34.60 for subjects from Kuala Lumpur, 26.67 for subjects from Selangor and 32.62 for subjects from other places of origin. Subjects from Perak were found to have the highest fasting blood glucose level at a mean rank of 60.34 while subjects from Selangor were found to have the lowest fasting blood glucose level at a mean rank of 26.67. **Figure 4.10** shows the distribution of fasting blood glucose level among study subjects with different place of origin.



1: Perak; 2: Penang; 3: Johor; 4: Kuala Lumpur; 5: Selangor; 6: Others

Figure 4.10: Fasting blood glucose level against place of origin among 102 study subjects

Table 4.3: Demographic distribution with waist circumference and fasting blood glucose level among 102 study subjects

	N	Waist circumference (cm) (mean ± SD)	Mean rank	p-value[a]	Fasting blood glucose level (mmol/L) (mean ± SD)	Mean rank	p-value[a]
Gender							
Male	51	86.49 ± 12.54	65.68	0.000*	5.51 ± 0.72	60.27	0.003*
Female	51	74.29 ± 9.75	37.32		5.12 ± 0.61	42.73	
Age							
≤ 29	49	74.06 ± 9.72	36.51		5.01 ± 0.49	35.40	
30 – 39	18	86.22 ± 13.40	64.39	0.000*	5.30 ± 0.70	56.28	0.000*
40 – 49	10	84.90 ± 11.84	62.80		5.74 ± 0.90	67.10	
50 – 59	17	85.65 ± 12.80	63.82		5.58 ± 0.34	70.09	
≥ 60	8	89.25 ± 9.59	74.00		6.14 ± 0.79	80.38	
Ethnicity							
Chinese	97	80.51 ± 12.96	51.62	0.852	5.35 ± 0.68	52.70	0.070*
Others**	5	78.10 ± 8.35	49.10		4.68 ± 0.70	28.20	
Place of Origin							
Perak	66	83.73 ± 13.08	59.42		5.50 ± 0.67	60.34	
Penang	6	72.17 ± 15.25	25.92		4.88 ± 0.43	32.58	
Johor	6	74.17 ± 5.58	38.33	0.010*	5.38 ± 0.56	53.00	0.001*
Kuala Lumpur	5	72.60 ± 9.56	34.40		5.06 ± 0.78	34.60	
Selangor	6	78.00 ± 7.92	47.83		4.87 ± 0.26	26.67	
Others ***	13	74.19 ± 7.57	37.46		4.85 ± 0.60	32.62	

*Significant data with p-value lesser than 0.1.

** Other ethnicities include Malay, Indian and Chinese-Filipino

*** Other places of origin includes Sabah, Melaka, Terengganu, Negeri Sembilan, Pahang and Labuan

[a] Kruskal-Wallis analyses were performed to compare mean rank

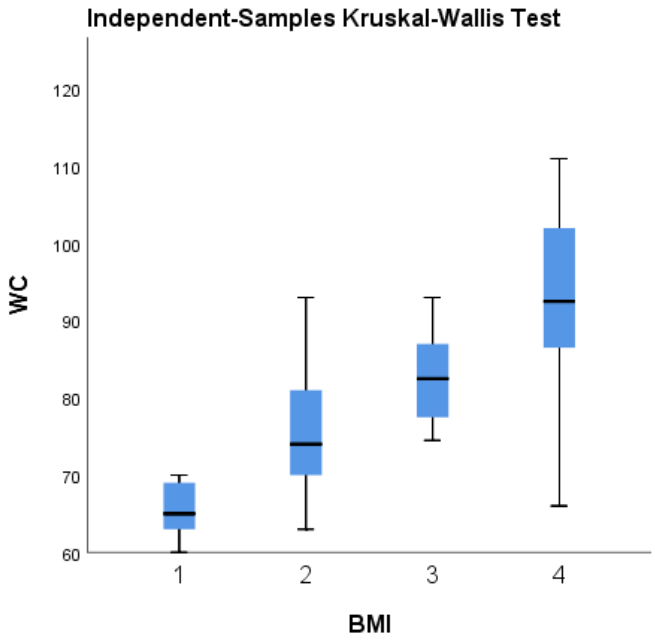
4.8 Anthropometric Measurement and COVID-19 History in relation to Waist Circumference and Fasting Blood Glucose Level.

Anthropometric measurements including height and weight and COVID-19 history were analyzed using the Kruskal-Wallis test. The mean and standard deviation of waist circumference and fasting blood glucose level are tabulated in **Table 4.4**. Anthropometric measurement, height and weight were used to calculate the body mass index (BMI) of the subjects with the formula below:

$$\text{BMI} = \frac{\text{Weight (kg)}}{[\text{Height}]^2\ (\text{m}^2)}$$

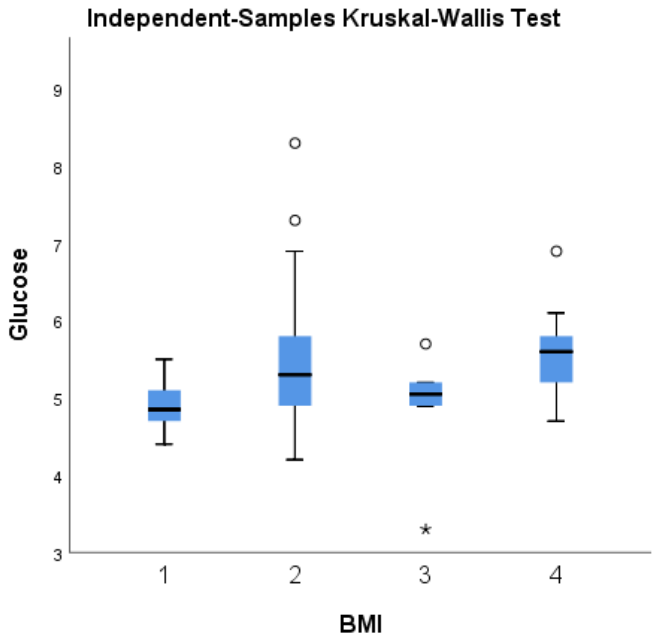
Based on the BMI value, the subjects were categorised into four different categories which are underweight, normal weight, overweight and obese. BMI value lesser than 18.5 was considered as underweight, 18.5-22.9 as normal, 23-24.9 as overweight and any BMI above 25 as obese. Based on **Table 4.4**, there were a total of 14 subjects (13.73%) were categorised as underweight, 46 subjects (45.10%) as normal, 8 subjects (7.84%) as overweight and 34 subjects (33.33%) as obese. There was significant difference in waist circumference between different BMI categories at $p = 0.000$ with a mean rank waist circumference of 12.29 for underweight, 39.80 for normal, 60.94 for overweight, 79.43 for obese. Subjects who were underweight were found to have lowest waist circumference at mean rank 12.29 while subjects who were obese were found to have highest waist circumference at mean rank 79.43. **Figure 4.11** shows the distribution of waist circumference among study subjects with different BMI categories. The Kruskal-Wallis H test showed that there was significant difference in fasting blood glucose between different BMI categories at $p = 0.002$ with a mean rank fasting blood glucose of 27.79 for underweight,

52.86 for normal, 37.25 for overweight, 61.34 for obese. Subjects who were underweight were found to have lowest fasting blood glucose level at mean rank 27.79 while subjects who were obese were found to have highest fasting blood glucose level at mean rank 61.34. **Figure 4.12** shows the distribution of fasting blood glucose level among study subjects with different BMI categories.



1: Underweight; 2: Normal; 3: Overweight; 4: Obese

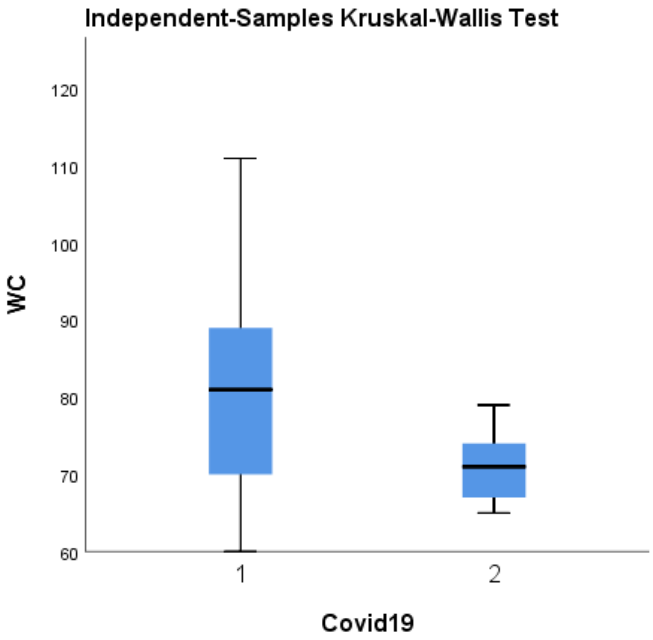
Figure 4.11: Waist circumference against BMI categories among 102 study subjects



1: Underweight; 2: Normal; 3: Overweight; 4: Obese

Figure 4.12: Fasting blood glucose level against BMI categories among 102 study subjects

Out of 102 subjects, 5 subjects (4.90%) had COVID-19 in the past month while 97 subjects (95.10%) did not have COVID in the past month. The Kruskal-Wallis H test showed that there was a significant difference in waist circumference between different categories of COVID-19 history at p = 0.087 with a mean rank waist circumference of 29.40 for subjects with COVID -19 history in the past month and 52.64 for subjects without COVID -19 history in the past one month. Subjects with a COVID-19 history in the past month were found to have lower waist circumferences than subjects without a COVID-19 history in the past month. The Kruskal-Wallis H test showed that there was no significant difference in fasting blood glucose between different categories of COVID-19 history at p = 0.529. **Figure 4.13** shows the distribution of waist circumference among study subjects with different COVID-19 history in the past month.



1: Had COVID-19 history in the past one month; 2: No COVID-19 history in the past one month

Figure 4.13: Waist circumference against COVID-19 history in the past one month among 102 study subjects

Table 4.4: Body mass index and COVID-19 history on waist circumference and fasting blood glucose level.

	N	Waist circumference (cm) (mean ± SD)	Mean rank	p-value[a]	Fasting blood glucose level (mmol/L) (mean ± SD)	Mean rank	p-value[a]
Body mass index (BMI)							
Underweight (<18.5)	14	65.36 ± 3.22	12.29		4.85 ± 0.37	27.79	
Normal (18.5-22.9)	46	75.03 ± 7.02	39.80	0.000*	5.40 ± 0.80	52.86	0.002*
Overweight (23-24.9)	8	82.69 ± 5.93	60.94		4.91 ± 0.66	37.25	
Obese (>25)	34	93.29 ± 10.54	79.43		5.49 ± 0.50	61.34	
COVID-19 history in the past one month							
Yes	5	71.20 ± 5.00	29.40	0.087*	5.14 ± 0.48	43.40	0.529
No	97	80.87 ± 12.88	52.64		5.32 ± 0.70	51.92	

*Significant data with p-value lesser than 0.1

[a] Kruskal-Wallis analyses were performed to compare mean rank

4.9 TCM Body Constitution in relation to Waist Circumference and Fasting Blood Glucose Level.

The response rate for the body constitution questionnaire is 97.06%. Out of 102 study subjects, 99 study subjects were included in the study as three study subjects failed to fill up the questionnaire. Hence, the study on TCM body constitution in relation to waist circumference and fasting blood glucose level had involved 99 study subjects.

The waist circumference and fasting blood glucose level were compared on different TCM BC using Kruskal-Wallis. The TCM BC of each subject was included in Appendix G. **Table 4.5** shows the waist circumference and fasting blood glucose level between gentleness, other single body constitutions (yang-deficiency, yin-deficiency, qi-deficiency, qi-stagnation, phlegm-dampness, damp heat, blood stasis, and special diathesis), the combination of 2 body constitutions, a combination of 3 body constitutions and combination of more than 3 body constitutions. There were 27.27% (n = 27) of the subjects are gentleness, 20.20% (n = 20) were having other single body constitutions, 20.20% (n = 20) had 2 body constitutions, 11.11% (n = 11) had 3 body constitutions and 21.21% (n = 21) have more than 3 body constitutions. There were three subjects excluded from the TCM body constitution as the body constitution questionnaire was not submitted. There was no significant difference in waist circumference ($p = 0.460$) and fasting blood glucose level ($p = 0.563$) between different TCM BC.

Table 4.5: TCM body constitutions in relation to waist circumference and fasting blood glucose level

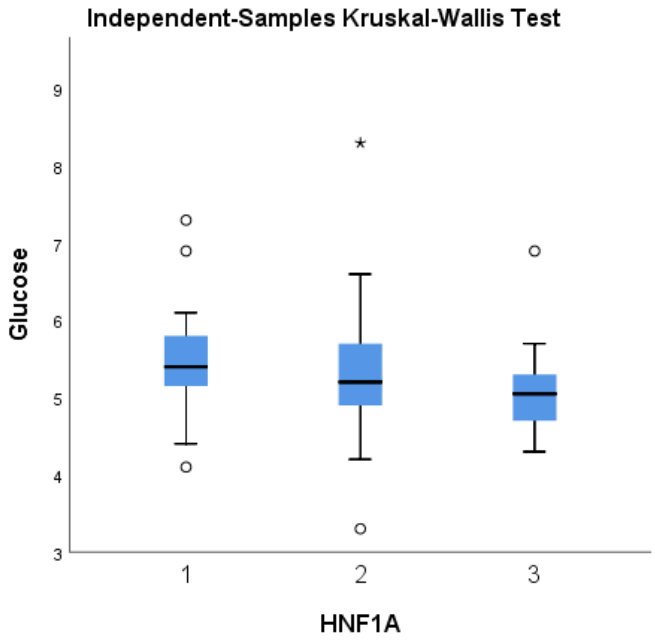
	N	Waist circumference (cm) (mean ± SD)	p-value[a]	Fasting blood glucose level (mmol/L) (mean ± SD)	p-value[a]
Body constitutions					
Gentleness	27	83.61 ± 13.28		5.30 ± 0.70	
Other single body constitution*	20	81.00 ± 14.39		5.45 ± 0.67	
2 Body Constitutions	20	79.71 ± 13.24	0.460	5.35 ± 0.45	0.563
3 Body Constitutions	11	78.88 ± 10.60		5.44 ± 1.16	
>3 Body Constitutions	21	76.10 ± 9.45		5.10 ± 0.46	

*Other single body constitution includes Yang-deficiency, Yin-deficiency, Qi-deficiency, Qi-stagnation, Blood stasis, Phlegm dampness, Damp heat and Special diathesis

[a] Kruskal-Wallis analyses were performed to compare mean rank

4.10 rs735396 and rs2975760 in relation to Waist Circumference and Fasting Blood Glucose Level.

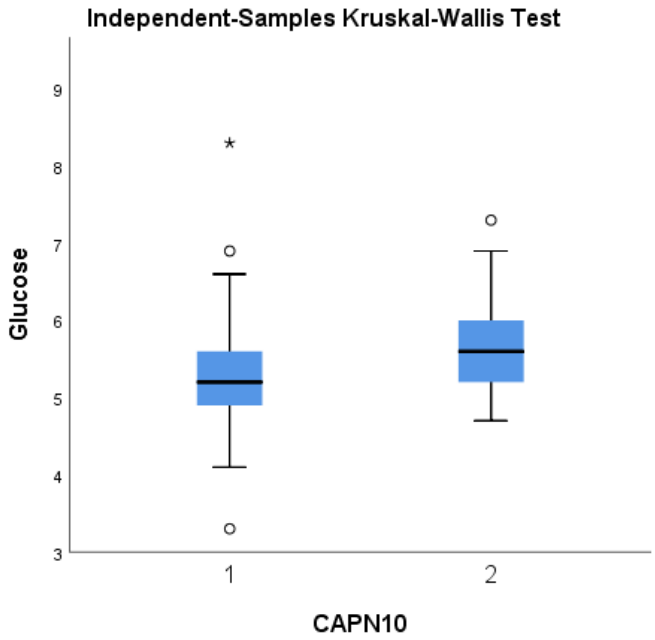
Based on the genotyping results for both rs736396 and rs2975760, all 102 subjects were categorized into 3 groups which were wildtype, heterozygous and homozygous mutant as in **Table 4.6**. The Kruskal-Wallis H test showed that there was no significant difference in waist circumference between different genotypes in rs735396 at $p > 0.1$. However, the Kruskal-Wallis H test showed that there was a significant difference in fasting blood glucose between different genotypes in rs735396 at $p = 0.049$ with a mean rank fasting blood glucose of 59.91 for wildtype (TT), 52.98 for heterozygous (TC) and 38.86 for homozygous mutant (CC). Subjects who were with the wild type genotype were found to have the lowest fasting blood glucose level at a mean rank of 59.91 while subjects who were with homozygous mutant were found to have the highest fasting blood glucose level at a mean rank of 38.86. **Figure 4.14** shows the distribution of fasting blood glucose level among study subjects with different genotypes of rs735396.



1: Wildtype (TT); 2: Heterozygous (TC); 3: Homozygous mutant (CC)

Figure 4.14: Fasting blood glucose level against genotypes for *HNF1A* (rs735396) among 102 study subjects

There was no significant difference in fasting blood glucose level between different genotypes in rs2975760 at p = 0.422. However, the Kruskal-Wallis H test showed that there was a significant difference in fasting blood glucose between different genotypes in rs2975760 at p = 0.053 with a mean rank fasting blood glucose of 52.40 for wildtype (TT) and 45.35 for heterozygous (TC) and homozygous mutant (CC). Subjects who were with wildtype genotype were found to have higher fasting blood glucose levels while subjects who were with heterozygous and homozygous mutant were found to have lower fasting blood glucose levels. **Figure 4.15** shows the distribution of fasting blood glucose level among study subjects with different genotypes of rs2975760.



1: Wildtype (TT); 2: Heterozygous (TC) and Homozygous mutant (CC)

Figure 4.15: Fasting blood glucose level against genotypes for *CAPN10* (rs2975760) among 102 study subjects

Table 4.6: rs735396 and rs2975760 in relation to waist circumference and fasting blood glucose level

	N	Waist circumference (cm) (mean ± SD)	Mean rank	p-value[a]	Fasting blood glucose level (mmol/L) (mean ± SD)	Mean rank	p-value[a]
rs735396							
Wildtype (TT)	23	82.43 ± 15.24	53.91		5.49 ± 0.72	59.91	
Heterozygous (TC)	57	79.75 ± 12.11	50.70	0.905	5.34 ± 0.70	52.98	0.049*
Homozygous mutant (CC)	22	79.93 ± 11.34	51.05		5.07 ± 0.55	38.86	
rs2975760							
Wildtype (TT)	89	80.91 ± 13.34	52.40		5.26 ± 0.66	49.34	
Heterozygous (TC) and Homozygous mutant (CC)	13	76.85 ± 6.94	45.35	0.422	5.72 ± 0.77	66.31	0.053*

* Significant data with p-value lesser than 0.1.

[a] Kruskal-Wallis analyses were performed to compare mean rank

4.11 Association of TCM Body Constitution with rs735396 and rs2975760

Fisher's exact test was performed to evaluate the association between TCM body constitution and the polymorphisms of metabolic genes, *HNF1A* and *CAPN10*. Based on **Table 4.7**, there was no association between TCM BC with rs735396 and rs2975760 with p-values of 0.692 and 0.430, respectively.

Table 4.7: Association between TCM BC and the polymorphisms of metabolic genes

Variables		TCM body constitutions, n (%)					df	p-value
		Gentleness	**Other single body constitution**	**2 body constitutions**	**3 body constitutions**	**>3 body constitutions**		
Variant rs735396	TT	6 (27.3)	3 (13.6)	7 (31.8)	3 (13.6)	3 (13.6)	8	0.692
	TC	13 (23.6)	14 (25.5)	9 (16.4)	6 (10.9)	13 (23.6)		
	CC	8 (36.4)	3 (13.6)	3 (13.6)	3 (13.6)	5 (22.7)		
Variant rs2975760	TT	24 (27.9)	17 (19.8)	17 (19.8)	12 (14.0)	16 (18.6)	4	0.430
	TC, CC	3 (23.1)	3 (23.1)	2 (15.4)	0 (0.0)	5 (38.5)		

4.12 Association between Demographic Distribution, Anthropometric Measurement, and COVID-19 History with TCM Body Constitution

Fisher's exact test was performed to evaluate the association between demographic distribution, anthropometric measurement, and COVID-19 history with TCM body constitution. Based on **Table 4.8**, there was no association between TCM BC with age ($p = 0.105$), gender ($p = 0.406$), ethnicity ($p = 0.406$), place of origin ($p = 0.920$), BMI ($p = 0.237$), and COVID-19 history ($p = 0.708$).

Table 4.8: Association between demographic distribution and COVID-19 history with TCM body constitution

Variables		TCM body constitutions n (%)					df	p-value
		Gentleness	**Other single body constitution**	**2 body constitutions**	**3 body constitutions**	**>3 body constitutions**		
Age	29 and below	11 (22.4)	9 (18.4)	11 (22.4)	5 (10.2)	13 (26.5)	16	0.105
	30 – 39	6 (37.5)	2 (12.5)	2 (12.5)	2 (12.5)	4 (25.0)		
	40 – 49	2 (18.2)	4 (36.4)	2 (18.2)	2 (18.2)	1 (9.10)		
	50 – 59	7 (46.7)	1 (6.7)	4 (26.7)	0 (0.0)	3 (20.0)		
	60 and above	1 (12.5)	4 (50.0)	0 (0.0)	3 (37.5)	0 (0.0)		
Gender	Male	16 (32.7)	9 (18.4)	8 (16.3)	8 (16.3)	8 (16.3)	4	0.406
	Female	11 (22.0)	11 (22.0)	11 (22.0)	4 (8.0)	13 (26.0)		
Ethnicity	Chinese	25 (26.6)	19 (20.2)	17 (18.1)	12 (12.8)	21 (22.3)	4	0.601
	Others*	2 (40.0)	1 (20.0)	2 (40.0)	0 (0.0)	0 (0.0)		
Place of origin	Perak	16 (25.4)	16 (25.4)	11 (17.5)	8 (12.7)	12 (19.0)	20	0.920
	Penang	2 (33.3)	0 (0.0)	3 (50.0)	0 (0.0)	1 (16.7)		
	Johor	2 (33.3)	1 (16.7)	1 (16.7)	0 (0.0)	2 (33.3)		
	Kuala Lumpur	1 (20.0)	0 (0.0)	2 (40.0)	0 (0.0)	2 (40.0)		
	Selangor	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)		
	Others**	4 (30.8)	2 (15.4)	1 (7.7)	3 (23.1)	3 (23.1)		
BMI	Underweight	3 (21.4)	1 (7.1)	2 (14.3)	2 (14.3)	6 (42.9)	12	0.237
	Normal	7 (15.2)	12 (26.1)	10 (21.7)	7 (15.2)	10 (21.7)		
	Overweight	3 (42.9)	2 (28.6)	1 (14.3)	0 (0.0)	1 (14.3)		
	Obese	14 (43.8)	5 (15.6)	6 (18.8)	3 (9.4)	4 (12.5)		
COVID-19 history in the past one month	Yes	26 (27.7)	19 (20.2)	19 (20.2)	11 (11.7)	19 (20.2)	4	0.708
	No	1 (20.0)	1 (20.0)	0 (0.0)	1 (20.0)	2 (40.0)		

* Other ethnicity includes Indian, Malay and Chinese-Filipino

** Other places of origin include Melaka, Terengganu, Pahang, Sabah, Negeri Sembilan and Labuan

CHAPTER 5

DISCUSSION

5.1 Prevalence of Diabetes and Abdominal Obesity among 102 Study Subjects

In this study, the mean for the fasting blood glucose level was 5.32 ± 0.69 mmol/L. By using 6.9 mmol/L as the cut-off value to diagnose diabetes, 2 out of the 102 subjects were diagnosed as diabetic with a prevalence rate of 1.96%. This was found lower than the prevalence rate reported in the National Diabetes Registry Report (2019) at 18.3%. This low prevalence rate of diabetes in this study may be due to younger subjects with age 29 and below being involved in this study compared to older subjects.

The mean for the waist circumference was 80.39 ± 12.78 cm. The mean waist circumference was 86.49 ± 12.54 cm and 74.29 ± 9.75 cm for males and females, respectively. According to the previous study in Malaysia, the prevalence of abdominal obesity was reported at 37.4% for males and 66.4% for females based on 90.0 cm for males and 80.0 cm for females cut-off value (Ahmad, et al., 2016). The prevalence rate of abdominal obesity obtained in this study was 34.31%, and the prevalence was 37.3% and 31.4% for males and females, respectively. The prevalence for males was found consistent with the earlier study but the prevalence rate of abdominal obesity for females was lesser than the earlier study. The difference in the prevalence between males and females is probably due to the higher consciousness of females in maintaining their body shape and body weight as compared to males (Voges, et al., 2019). Besides, the

previous study was conducted in Tanjung Karang in which the majority of the population is Malays (Pejabat Daerah/Tanah Kuala Selangor, 2019), while most of the study subjects involved in this study were Chinese. Malays tend to have a higher waist circumference due to their high sugar content diet (Balasubramanian, et al., 2020). In addition, Malays consume fewer fruits and vegetables is one of the factors contributing to the higher prevalence of abdominal obesity (Tan, Dunn and Yen, 2011).

5.2 Genotyping of rs735396 and rs2975760

Tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was used for the genotyping of rs735396 and rs2975760. In this PCR reaction, two allele-specific amplicons were generated using the outer and inner primers, in which one amplicon was for wild type allele and the other pair was for the mutant allele (Medrano and De Oliveira, 2014). For rs735396, primers were designed to target T and C nucleotides. The inner reverse primer (R_I) which carries a mutated 3' end (C nucleotide) would not be able to bind to and amplify the wildtype allele which carried either A or T nucleotide. It anneals to the mutant allele, C or G nucleotide, and produces an amplicon with the size of 193 bp. The inner forward primer (F_I) identifies T nucleotide, wild type allele, and amplifies an amplicon with size 263 bp. In genotyping of rs2975760, the inner reverse primer (R_I) which carry a mutant 3' end (C nucleotide) would not be able to bind to and amplify the wildtype allele which carried A and T nucleotide. However, it binds to the mutated allele which carries C and G nucleotides, producing an amplicon with a size of 200 bp. The inner forward primer (F_I) which carries a T nucleotide binds to the wildtype

allele and amplifies an amplicon with size 274 bp, and vice versa. Genotyping for polymorphisms was performed in a single tube.

According to the National Institutes of Health (NIH), the minor allele frequency (MAF) for the C allele was 0.5113 in the Asian population for rs735396. In this study, the MAF obtained for the C allele was 0.50 for rs735396 which was consistent with MAF by NIH for the Asian population. The MAF for the C allele reported in this study was also consistent with the study reported in the Korean population at 0.56 (Vinayagamoorthy, et al., 2014). Thus, our findings showed MAF for rs735396 is consistent with earlier reported MAF in the Asian population and hence is significant polymorphism with MAF > 0.5.

For rs2975760, the MAF for C allele was at 0.1510 in the Asian population as reported by NIH. In this study, the MAF obtained for the C allele was 0.08 for rs2975760 which was lower than the MAF reported by NIH in the Asian population and the MAF reported in a previous study among Asian Indians at 0.20 among healthy individuals, 0.21 among subjects with type 2 diabetes mellitus and 0.23 among subjects with gestational diabetes mellitus （Khan, et al., 2014）. The difference between MAF in this study and the statistics reported by NIH is probably due to the narrow variation in study subjects. The study subjects in this study were mainly young Chinese and the study subjects cannot implicate the Asian population comprehensively. Moreover, the ethnicity difference in this study and previous studies may lead to a huge difference in MAF. The previous study was performed among the Indian population while this study focused on the Chinese population. The MAF found in this study was found close to the MAF value reported in the Asian population.

However, a larger sample size can be included to validate this finding. In overall, MAF for both rs735396 and rs2975760 may be varied among different populations from different continents.

5.3 Relationship between Waist Circumference and Fasting Blood Glucose Level

In this study, Pearson correlation presented a weak positive linear relationship with an R^2 value of 0.325 between waist circumference and fasting blood glucose level, indicating waist circumference changes in proportionate to fasting blood glucose. Waist circumference is one of the indicators of obesity and central obesity is thought to be closely related to diabetes mellitus (Santoso, et al., 2022). Central obesity is due to high visceral fat and visceral fat secretes adipokines that will induce impaired glucose tolerance (ElKafrawi, Shoaib, and Elghanam, 2017). A higher content of visceral fat in an individual will lead to higher waist circumference which is associated with impaired glucose tolerance and eventually lead to diabetes. Our study presented waist circumference had a relationship with fasting blood glucose as revealed by a study that waist circumference is a stronger indicator of diabetes mellitus compared with BMI (Wang, et al., 2005). A study in Iran showed that waist circumference is a strong indicator in determining central obesity where it is one of the risk factors for type 2 diabetes mellitus (Veghari, et al., 2014). However, a study conducted in Mexico shows that waist circumference is not associated with impaired fasting blood glucose in children and teenagers (Jáuregui-Ulloa, et al., 2021).

5.4 Demographic Distribution in relation to Waist Circumference and Fasting Blood Glucose Level

5.4.1 Gender

In this study, gender was found significantly different in both fasting blood glucose and waist circumference. Male has relatively higher waist circumference and fasting blood glucose level as consistent with the earlier reported findings. A study conducted in Hong Kong showed that female has a lower waist circumference as compared to male with a mean waist circumference of 74.9 cm and 80.8 cm respectively (Ko, 1997). Higher waist circumference in male can be attributed to male having more central distribution of fat while female has a more peripheral distribution of fat as influenced by different sex hormones (Chang, Varghese, and Singer, 2018).

Female tends to present with normal fasting blood glucose level. This could explain why female always has normal glucose metabolism as female are more insulin-sensitive as compared to male (Kautzky-Willer, et al., 2015). In addition, the endogenous estrogen in females also plays another important role in glucose homeostasis and thus protects female from high fasting blood glucose. Oestrogen preserves the pancreatic cells' ability to function by preventing apoptosis, modifying their role in insulin resistance, and keeping their insulin content high. By preventing lipogenesis, estrogen receptor activation reduces the damage to the cells due to excess lipids (Alemany, 2021). Studies showed that early menopause and premature ovarian insufficiency will lead to a higher risk of type 2 diabetes (Tramunt, et al., 2020). This is also in agreement with the study conducted among the Indian population, which revealed that the

fasting blood glucose of males is higher than female which were at the mean of 4.16 mmol/L and 3.93 mmol/L respectively (Anish, et al., 2013).

5.4.2 Age

In this study, our findings reported significant difference between age with waist circumference and fasting blood glucose Our study reported that waist circumference will increase by age in both male and female. This was found consistent with earlier finding. According to previous study, the waist circumference for the subjects below 30 years old is between 82.89 cm and 91.53 cm while the waist circumference for the subjects in the age group of 50 and above is between 95.95 cm and 102.71 cm in the Venezuelan population (Bermudez, et al., 2021). The increment in the waist circumference could be due to the loss of lean tissue which can start as early as age 45 (Janssen, et al., 2000). The total and regional fat distribution will differ at different age. In older individuals, decreased physical activity and basal metabolic rate while the energy intake remained the same, all these will contribute to the increase in adiposity (Enzi, et al., 1986). The abdominal fat, particularly the visceral fat tends to increase while the lower body subcutaneous fat will tend to decrease (Kuk, et al., 2009).

A study conducted by Khan et al. in 1970 among Pakistan population revealed that age was significantly associated with fasting blood glucose level which is found in agreement with our study. Previous study had showed that the glycated hemoglobin level rose with age, but no association was found between fasting plasma glucose levels and age (Kilpatrick, Dominiczak and Small, 1996).

According to the national diabetes statistics reported by Centers for Disease Control and Prevention (2020), there was about 25% of diabetic individual in 2015 were over 65 years. The increment of fasting blood glucose with age may be multi-factorial. Due to aging, the body's ability in regulating glucose level will progressively diminish. The secondary influences of body fat and physical fitness will lead to poor glucose tolerance (Shimokata, et al., 1991). The changes in glucose tolerance are more prominent in individuals who were 60 and above (Shimokata, et al., 1991). The deterioration of glucose tolerance may be due to the rise in hepatic insulin clearance as well as the decline in total insulin clearance in elderly and subsequently lead to decline in insulin secretion and action (Basu, et al., 2003). Moreover, aging may cause changes in the glycemic index values of food, insulin levels and insulin sensitivity (Ko, Wai and Tang, 2006). The deterioration in pancreatic β-cell activity may result from the elderly's disproportionately high proinsulin to C-peptide levels (Gama, et al., 2000). Hence, these can attribute to high fasting blood glucose level as seen in this study, especially when the age is more than 40.

5.4.3 Ethnicity

This study grouped the ethnic group of study subjects into two groups, which were Chinese and other ethnicities including Indian, Malay, and Chinese-Filipino. There was no significant difference between ethnicity and waist circumference, which is found to contradict a study conducted among Malaysian workers in Peninsular Malaysia. An earlier study mentioned that Indian workers are prone to have a greater waist circumference (Shafiee, et al., 2022). Another study conducted among Asian pre-pubertal children also show

significant differences in waist circumference among four different ethnic groups (Liu, et al., 2011). However, due to only one Indian subject in this study, this small sample size and low variation in ethnicity may contribute to the contradiction with earlier studies.

Our study showed a significant difference between the ethnic group and fasting blood glucose levels. Similar findings were also seen in a study conducted in Singapore in which a significant association was presented between ethnicity and fasting blood glucose level (Venkataraman, et al., 2012). Different ethnic groups presented variations in HbA1c and fasting plasma glucose in which Malay and Indians had higher HbA1c levels compared to Chinese. Another study conducted in Singapore revealed Chinese are highly insulin sensitive while Indians are weakly insulin-sensitive (Tan, et al., 2015). The finding by Tan et al. was contradicting our study as our study shows that the Chinese have relatively higher fasting blood glucose levels than other ethnicities. This contradiction could be probably due to the small sample size of other ethnic groups and the predominant study population is Chinese in our study.

5.4.4 Place of Origin

In this study, there were significant differences in waist circumference and fasting blood glucose among groups of different places of origin. Perak showed the highest waist circumference and fasting blood glucose level. This was consistent with earlier findings. Our study showed the subjects from urban areas such as Kuala Lumpur and Penang have relatively lower waist circumferences. Studies conducted in China showed a significant difference between waist

circumference and place of origin, where subjects from rural areas are prone to abdominal obesity (Shen, et al., 2019). The population in an urban area has lower waist circumference and lower risk of abdominal obesity due to their higher educational level as the educational level has an inverse association with the waist circumference (Boing and Subramanian, 2015). In addition, Penang was found to have relatively higher educational attainment levels than Perlis, Kedah, and Perak (Organisation for Economic Co-operation and Development, n.d.). The findings from a study in India showed contradictory results where subjects from rural areas have a lower waist circumference than urban areas (Pradeepa, et al., 2015). The main factor contributing to the higher waist circumference in urban environments due to increased availability of unhealthy meals or fast food and the sedentary lifestyle (Samadoulougou, et al., 2022).

Based on the results obtained in this study, the subjects from urban areas including Kuala Lumpur, Penang, and Selangor were found with relatively lower fasting blood glucose levels. This inconsistent finding is where the prevalence of pre-diabetes and diabetes are higher in the urban area due to unhealthy and sedentary lifestyles (McAlexander, et al., 2022; Ghassab-Abdollahi, et al., 2023). The inconsistent findings could be attributed to the predominant study subjects in this study being young adults who are university students that tend to have unhealthy lifestyles, such as preferring to have supper or late evening dinner.

5.5 Anthropometric Measurement and COVID-19 History in relation to Waist Circumference and Fasting Blood Glucose Level.

5.5.1 Anthropometric Measurement

In this study, the BMI of the subjects was calculated from the height and weight measurements. The subjects were grouped into four groups based on their BMI, which were underweight, normal, overweight, and obese. Our study showed that obese subjects had the largest waist circumference while the underweight subjects had the smallest waist circumference. A previous study on the Belgian population found that there was a strong positive linear correlation between BMI and waist circumference with Pearson's correlation coefficient and Spearman's rank correlation coefficient of 0.87 (Wilmet, et al., 2017). This could be explained as obesity is caused by the accumulation of fat due to the consumption of excess calories. The waist circumference will increase as a direct result of fat buildup, especially in the abdominal part. BMI and waist circumference is directly proportional to each other, nevertheless, waist circumference remains the best reference in determining obesity as BMI does not depict the distribution of fats or muscle in an individual (Gierach, et al., 2014; Darsini, et al., 2020).

In this study, there were significant differences in fasting blood glucose levels between different BMI categories. Our study showed that subjects who were obese had the highest fasting blood glucose while the subjects who were underweight had the lowest fasting blood glucose level. This was found in agreement with earlier findings. A previous study in Jharkhand, India revealed that fasting blood glucose level has a significant positive correlation with BMI

(Agrawal, et al., 2017). Another study in India also showed a positive correlation between fasting blood glucose with BMI category with Pearson's correlation coefficient of 0.26 (Vittal, Praveen, and Deepak, 2010). This could be justified as obesity impairs β-cell sensitivity to glucose and results in peripheral resistance to insulin-mediated glucose absorption (Vittal, Praveen, and Deepak, 2010).

5.5.2 COVID-19 History

Waist circumference was found significantly different with the history of COVID-19 in the past month. Subjects who do not have COVID-19 history were having larger waist circumference. Contradicting findings were seen as the study conducted among Danish patients hospitalized with community-acquired pneumonia revealed that as much as 82% of female and 65% of male patients with COVID-19 will have increased waist circumference (Ryrsø, et al., 2022). Our findings were found contradictory as the dominance of subjects without COVID-19 history in the past month. The reduction of waist circumference in subjects with a COVID-19 history may be because of COVID-19 on chemosensory functions which involves ageusia, anosmia, and parosmia. The altered chemosensory functions may eventually lead to loss of appetite in COVID-19 patients. In this study, COVID-19 history in the past month was found not significantly associated with fasting blood glucose levels. However, earlier studies showed that COVID-19 patients tend to have a direct or permanent injury in their β-cells which manufacture insulin. This injury may lead to insulin resistance where the ability of β-cells to absorb blood glucose is impaired. Acute insulin resistance or even transient insulin deficiency can be

overcome by insulin or other medications for a short time (Massachusetts General Hospital, 2022).

5.6 TCM Body Constitution in relation to Waist Circumference and Fasting Blood Glucose Level

Previous findings revealed that obesity is often accompanied by blood stasis (Kang, et al., 2019). The coagulation system is altered by excess body weight, including reduced fibrinolytic activity and increased plasma concentrations of clotting components. Both arterial and venous thrombosis are hypothesized to be affected by these changes in endothelial function and coagulation. Due to these mechanisms, central obesity is believed to increase the risk of venous stasis (Willenberg, et al., 2010). Previous findings revealed that fasting plasma insulin is a marker for insulin resistance and was considerably higher in impaired glucose regulation participants with Phlegm-damp, Damp-heat, or Qi-deficiency constitutions. Subjects with phlegm-dampness, damp-heat, or qi-deficiency were expected to have higher levels of blood glucose and were at higher risk for progression of diabetes (You, et al., 2017).

Based on the results from our study, TCM body constitution was not significantly associated with both waist circumference and fasting blood glucose level. The insignificance may be due to the small sample size recruited in the study, in addition, most of the subjects were found to be gentleness. In addition, most of the subjects were having a combination of body constitutions probably because they were ambiguous while answering the body constitution

questionnaire. Hence, the finding was not conclusive and further investigation was needed.

5.7 rs735396 and rs2975760 in relation to Waist Circumference and Fasting Blood Glucose Level

From the results obtained in this study, there was no significant association between variant rs735396 and waist circumference which is inconsistent with Dallali's findings but consistent with Morjane's findings. According to Dallali et al. in 2022, there was a significant association between variant rs735396 and a higher waist circumference in the Tunisian population. However, Morjane et al. (2017) revealed that there was no significant association between variant rs735396 and waist circumference among the Moroccan population. From the results obtained in this study, there was a significant association between rs735396 and fasting blood glucose level which is contradictory to the previous findings. The study conducted by Morjane et al. (2017) and Dallali et al. (2022) revealed that there was no significant association between rs735396 and fasting blood glucose level. Subjects with wild type genotype (TT) were found to have the highest fasting blood glucose level. The findings may depict other than polymorphism, rs735396, there could be other factors that may also influence the fasting blood glucose.

There was no significant difference between rs2975760 genotypes with waist circumference which was found consistent with the study by Saez et al. (2008). Another study also revealed that variant rs2975760 was not significantly associated with intra-abdominal fat area (Pihlajamäki, et al., 2006). This can be

explained that the waist circumference was not associated with rs2975760. The change in waist circumference is often led by the change in intra-abdominal fat. Our study showed a significant difference between rs2975760 genotypes with fasting blood glucose levels. Based on a previous study, inherited the rare allele C in rs2975760 from heterozygous parents to their offspring was detected with type 2 diabetes mellitus (Song, et al., 2004). This was consistent with the results obtained in our study where subjects with wildtype genotype had a lower fasting blood glucose level. Our study showed mutant allele (C nucleotide) is associated with type 2 diabetes where the fasting blood glucose will rise. The variant rs2975760 was associated with transcriptional regulation of calpain-10 expression (Evans, et al., 2001). Calpain-10 acts as the determinant for insulin exocytosis and thus, dysregulation in the expression of calpain-10 gene will directly alter the glucose metabolism and eventually influence the fasting blood glucose level (Turner, Cassell, and Hitman, 2005). A study conducted by Pihlajamäki, et al. (2006) also claimed that variant rs2975760 was associated with insulin exocytosis by the calpain-10.

5.8 Association of TCM body constitution with rs735396 and rs2975760

Based on the results obtained from this study, TCM body constitution was not significantly associated with both SNPs, rs735396 and rs2975760. This indicates that both variables are independent of each other. This is probably because the TCM body constitution can be changed over time by acquired factors such as dietary uptake, lifestyle, and environmental changes (Yap, et al., 2021). Therefore, an individual can have different body constitutions throughout their life. Polymorphism presented in genes is unchangeable as this

was inherited from parents. As SNPs tend to occur naturally, the distribution of SNPs is substantially non-random due to mutation hotspots, natural selection, and ascertainment biases (Amos, 2010). However, limited study was found on the association between TCM body constitution and the SNPs of metabolic genes, more studies are needed to prove the insignificance.

5.9 Association between Demographic Distribution and COVID-19 History with TCM Body Constitution

A study conducted by Bai, et al. (2021) showed that the different body constitution distribution was related to gender, especially among patients with unbalanced TCM body constitution. Both Yang deficiency and Blood Stasis were found higher in females whereas phlegm dampness and qi-deficiency were found higher in males. Yin predominates Yang in females and physiological processes like menstruation and childbirth tend to cause blood to stagnate. Conversely, males are more prone to smoke and drink alcohol, and they also prefer salty and oily foods, which can lead to an increase in phlegm-dampness in the body (Bai, et al., 2021).

A previous study revealed that there is a significant association between age and TCM body constitution where Yin deficiency, Yang deficiency, Qi deficiency, and Qi depression is more common in older adults (Ma, et al., 2022). Blood-stasis constitution was reported at a higher percentage among older age groups (Bai, et al., 2021). Elderly people often develop yang deficiency and blood stasis constitutions because as they age, their blood and qi circulations become irregular (Bai, et al., 2021).

There was no significant difference between TCM BC with age and gender which may be due to limited subjects who possess just a single type of body constitution. There were many study subjects with more than three types of body constitution which were not reliable. Other variables in demographic distribution which include ethnicity and place of origin also showed no significant association with TCM body constitution. Furthermore, there was no significant association between COVID-19 history and TCM body constitution. However, the findings have remained ambiguous as this study was not able to have study subjects with only a single constitution. The possible reason could be the study subjects might be ambiguous when answering the questionnaire. This may attribute to low accuracy when the score was calculated to analyze the type of body constitutions.

5.10 Limitations

The limitation of this study was the small sample size recruited where it was only able to meet 90% confidence interval. There was a 10% error that might occur in this study. In addition, the study subjects recruited were majority Chinese and in the age group of 29 and below which causes difficulties in determining the relation of demographic distribution with waist circumference and fasting blood glucose level.

5.11 Future Recommendations

More samples should be recruited to meet the minimum of 95% confidence interval. Furthermore, a waist-hip ratio should be considered as waist circumference alone may not contribute to a comprehensive analysis of

abdominal obesity. Besides, the answering session for CCMQ should be guided to ensure the accuracy of answering in avoiding a combination of body constitutions.

CHAPTER 6

CONCLUSIONS

In conclusion, the incidence of abdominal obesity and diabetes differs in individuals with different demographic distributions. Many factors including BMI, genetic variants, and TCM body constitutions may contribute to the risk of having abdominal obesity and diabetes The prevalence rate of diabetes obtained from this study was lower than the prevalence rate reported in the National Diabetes Registry Report in 2019 due to the imbalance age distribution of the subjects in this study. The prevalence rate of abdominal obesity was lower than the prevalence rate reported by the previous study because of the bias in the ethnicity of the subjects that were being recruited. Both identified SNPs were significant in this study population with MAF > 0.05. The minor allele frequency for rs735396 obtained in this study was close to the minor allele frequency reported by NIH whereas the minor allele frequency for rs2975760 obtained in this study differs from the reported minor allele frequency. Geographical distribution could be depicted as one of the factors in giving genetic variation among different populations. Gender, age, place of origin, BMI, and COVID-19 history in the past month were found to have significant differences in waist circumference. Meanwhile, gender, age, ethnicity, place of origin, BMI, rs735396, and rs2975760 were found to have significant differences in fasting blood glucose level at a 90% confidence interval. Age was found to be significantly associated with the TCM body constitution of the subjects studied. Hence abdominal obesity and diabetes were found to be

multifactorial. It is important to elucidate the risk factors in knowing the predisposition to MetS for better prevention as well as healthcare awareness.

REFERENCES

Abdel-Latif, A. and Osman, G., 2017. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. *Plant Methods*, 13, pp.1-9.

Agrawal, N., Agrawal, M.K., Kumari, T. and Kumar, S., 2017. Correlation between body mass index and blood glucose levels in Jharkhand population. *International Journal of Contemporary Medical Research*, 4(8), pp. 1633-6.

Ahmad, N., Adam, S.I.M., Nawi, A.M., Hassan, M.R. and Ghazi, H.F., 2016. Abdominal obesity indicators: waist circumference or waist-to-hip ratio in Malaysian adults population. *International Journal of Preventive Medicine*, 7(82).

Akhtar, S., Nasir, J.A., Ali, A., Asghar, M., Majeed, R. and Sarwar, A., 2022.Prevalence of type-2 diabetes and prediabetes in Malaysia: A systematic review and meta-analysis. *PLOS ONE*, 17(1), p. e0263139.

Alemany, M., 2021. Estrogens and the regulation of glucose metabolism. *World Journal of Diabetes*, 12(10), p. 1622.

Amos, W., 2010. Even small SNP clusters are non-randomly distributed: is this evidence of mutational non-independence?. *Proceedings of the Royal Society B: Biological Sciences*, 277(1686), pp. 1443-1449.

Anish, T.S., Shahulhameed, S., Vijayakumar, K., Joy, T.M., Sreelakshmi, P.R. and Kuriakose, A., 2013. Gender difference in blood pressure, blood sugar, and cholesterol in young adults with comparable routine physical exertion. *Journal of Family Medicine and Primary Care*, 2(2), p. 200.

Bai, F., Luo, H., Wang, L., Zhu, L., Guan, Y., Zheng, Y., Li, L. and Wang, Q., 2021. A Meta-Analysis of the Association between Diabetes Mellitus and Traditional Chinese Medicine Constitution. *Evidence-Based Complementary and Alternative Medicine*, 2021, pp. 1–14.

Bai, Q., Chuang, Y., Zhao, Y., Wang, Y., Ge, P., Xu, Y. and Bian, Y., 2021. The correlation between demographical and lifestyle factors and traditional Chinese medicine constitution among Macau elderly individuals. *Evidence-Based Complementary and Alternative Medicine*, 2021, pp. 1-9.

Balasubramanian, G.V., Chuah, K.A., Khor, B.H., Sualeheen, A., Yeak, Z.W., Chinna, K., Sundram, K. and Karupaiah, T., 2020. Associations of eating mode defined by dietary patterns with cardiometabolic risk factors in the Malaysia lipid study population. *Nutrients*, 12(7), p.2080.

Ban, N., Yamada, Y., Someya, Y., Miyawaki, K., Ihara, Y., Hosokawa, M., Toyokuni, S., Tsuda, K. and Seino, Y., 2002. Hepatocyte Nuclear Factor-1α Recruits the Transcriptional Co-Activator p300 on the GLUT2 Gene Promoter. *Diabetes*, 51(5), pp. 1409–1418.

Basu, R., Breda, E., Oberg, A.L., Powell, C.C., Dalla Man, C., Basu, A., Vittone, J.L., Klee, G.G., Arora, P., Jensen, M.D. and Toffolo, G., 2003. Mechanisms of the age-associated deterioration in glucose tolerance: contribution of alterations in insulin secretion, action, and clearance. *Diabetes*, 52(7), pp. 1738-1748.

Bermudez, V., Salazar, J., Martínez, M.S., Olivar, L.C., Nava, M., Rojas, M., Ortega, Á., Añez, R., Toledo, A., Rojas, J. and Chacín, M., 2021. Age-specific waist circumference cutoff-points for abdominal obesity diagnosis: a personalized strategy for a large Venezuelan population. *Journal of Diabetes & Metabolic Disorders*, 20(1), pp. 217-227.

Bodhini, D., Radha, V., Ghosh, S., Sanapala, K.R., Majumder, P.P., Rao, M.R.S. and Mohan, V., 2011. Association of calpain 10 gene polymorphisms with type 2 diabetes mellitus in Southern Indians. *Metabolism*, 60(5), pp. 681-688.

Boing, A.F. and Subramanian, S.V., 2015. The influence of area-level education on body mass index, waist circumference and obesity according to gender. *International Journal of Public Health*, 60(6), pp. 727-736.

Brown, A.E., Yeaman, S.J. and Walker, M., 2007. Targeted suppression of calpain-10 expression impairs insulin-stimulated glucose uptake in cultured primary human skeletal muscle cells. *Molecular Genetics and Metabolism*, 91(4), pp. 318–324.

Centers for Disease Control and Prevention (CDC), 2020. *National diabetes statistics report 2020.* [online] Available at: <https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf> [Accessed 26 April 2023].

Centers for Disease Control and Prevention (CDC), 2023. *Diabetes Tests*. [online] Available at: <https://www.cdc.gov/diabetes/basics/getting-tested.html#:~:text=Fasting%20Blood%20Sugar%20Test,higher%20indicates%20you%20have%20diabetes> [Accessed 26 April 2023].

Chang, E., Varghese, M. and Singer, K., 2018. Gender and sex differences in adipose tissue. *Current diabetes reports*, 18(9), pp. 1-10.

China Association of Chinese Medicine, 2009. *Constitution classification and determination. National Standards of Public Health Service.* [online] Available at: <http://www.360doc.com/content/18/0914/01/605353_786500160.shtml> [Accessed 26 February 2023].

Cornier, M.A., Dabelea, D., Hernandez, T.L., Lindstrom, R.C., Steig, A.J., Stob, N.R., Van Pelt, R.E., Wang, H. and Eckel, R.H., 2008. The metabolic syndrome. *Endocrine Reviews*, 29(7), pp. 777-822.

Cook, S., Weitzman, M., Auinger, P., Nguyen, M. and Dietz, W.H., 2003. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Archives of Pediatrics & Adolescent Medicine*, 157(8), pp.821-827.

Dallali, H., Hechmi, M., Morjane, I., Elouej, S., Jmel, H., Ben Halima, Y., Abid, A., Bahlous, A., Barakat, A., Jamoussi, H. and Abdelhak, S., 2022. Association of HNF1A gene variants and haplotypes with metabolic syndrome: a case–control study in the Tunisian population and a meta-analysis. *Diabetology & Metabolic Syndrome*, 14(1), p. 25.

Darsini, D., Hamidah, H., Notobroto, H.B. and Cahyono, E.A., 2020. Health risks associated with high waist circumference: A systematic review. *Journal of Public Health Research*, *9*(2).

Davidson, K.W., Barry, M.J., Mangione, C.M., Cabana, M., Caughey, A.B., Davis, E.M., Donahue, K.E., Doubeni, C.A., Krist, A.H., Kubik, M., Li, L., Ogedegbe, G., Owens, D.K., Pbert, L., Silverstein, M., Stevermer, J., Tseng, C.-W. and Wong, J.B., 2021. Screening for Prediabetes and Type 2 Diabetes. *JAMA*, 326(8), p. 736.

ElKafrawi, N.A., Shoaib, A.A. and Elghanam, M.H.A.E., 2017. Measurement of waist circumference as a screening tool for type 2 diabetes mellitus in female patients. *Menoufia Medical Journal*, 30(1), p. 168.

Enzi, G., Gasparo, M., Biondetti, P.R., Fiore, D., Semisa, M. and Zurlo, F., 1986. Subcutaneous and visceral fat distribution according to sex, age, and overweight, evaluated by computed tomography. *The American Journal of Clinical Nutrition*, 44(6), pp. 739-746.

Evans, J.C., Frayling, T.M., Cassell, P.G., Saker, P.J., Hitman, G.A., Walker, M., Levy, J.C., O'Rahilly, S., Rao, P.V.S., Bennett, A.J., Jones, E.C., Menzel, S., Prestwich, P., Simecek, N., Wishart, M., Dhillon, R., Fletcher, C., Millward, A., Demaine, A. and Wilkin, T., 2001. Studies of Association between the Gene for Calpain-10 and Type 2 Diabetes Mellitus in the United Kingdom. *The American Journal of Human Genetics*, 69(3), pp. 544–552.

Favorgen, n.d.. *FavorPrepTM Blood Genomic DNA Extraction Mini Kit.* [online] Available at: < http://www.favorgen.com/favorgen/serv_1/mem_t1/h_1/pdf/genomic/FABGK%20000%20001%20001_1%20001_2.pdf> [Accessed 25 December 2022].

Fu, Z., R Gilbert, E. and Liu, D., 2013. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Current Diabetes Reviews*, 9(1), pp. 25-53.

Galán, M., García-Herrero, C.-M., Azriel, S., Gargallo, M., Durán, M., Gorgojo, J.-J., Andía, V.-M. and Navas, M.-A., 2010. Differential Effects of HNF-1α Mutations Associated with Familial Young-Onset Diabetes on Target Gene Regulation. *Molecular Medicine*, 17(3-4), pp. 256–265.

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B., Ostolaza, H. and Martín, C., 2020. Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences*, 21(17), p. 6275.

Gama, R., Medina-Layachi, N., Ranganath, L., Hampton, S., Morgan, L. and Marks, V., 2000. Hyperproinsulinaemia in elderly subjects: evidence for age-related pancreatic β-cell dysfunction. *Annals of Clinical Biochemistry*, 37(3), pp. 367-371.

Ghassab-Abdollahi, N., Nadrian, H., Pishbin, K., Shirzadi, S., Sarbakhsh, P., Saadati, F., Moradi, M.S., Azar, P.S. and Zhianfar, L., 2023. Gender and urban–rural residency based differences in the prevalence of type-2 diabetes mellitus and its determinants among adults in Naghadeh: Results of IraPEN survey. *Plos one*, 18(3), p. e0279872.

Gierach, M., Gierach, J., Ewertowska, M., Arndt, A. and Junik, R., 2014. Correlation between body mass index and waist circumference in patients with metabolic syndrome. *International Scholarly Research Notices*, *2014*.

Grundy, S.M., Cleeman, J.I., Daniels, S.R., Donato, K.A., Eckel, R.H., Franklin, B.A., Gordon, D.J., Krauss, R.M., Savage, P.J., Smith Jr, S.C. and Spertus, J.A., 2005. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation*, 112(17), pp. 2735-2752.

Hatta, T., Iemura, S., Ohishi, T., Nakayama, H., Seimiya, H., Yasuda, T., Iizuka, K., Fukuda, M., Takeda, J., Natsume, T. and Horikawa, Y., 2018. Calpain-10 regulates actin dynamics by proteolysis of microtubule-associated protein 1B. *Scientific Reports*, 8(1).

Hoffman, L.S., Fox, T.J., Anastasopoulou, C. and Jialal, I., 2022. *Maturity Onset Diabetes in the Young.* [online] Available at: < https://www.ncbi.nlm.nih.gov/books/NBK532900/> [Accessed 26 April 2023].

Horikawa, Y., Oda, N., Cox, N.J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T.H., Mashima, H., Schwarz, P.E.H., del Bosque-Plata, L., Horikawa, Y., Oda, Y., Yoshiuchi, I., Colilla, S., Polonsky, K.S., Wei, S., Concannon, P., Iwasaki, N. and Schulze, J., 2000. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nature Genetics*, 26(2), pp. 163–175.

Hsu, M.F., Tang, P.L., Pan, T.C. and Hsueh, K.C., 2022. Different traditional Chinese medicine constitution is associated with dietary and lifestyle behaviors among adults in Taiwan. *Medicine*, 101(39), p. e30692.

International Diabetes Federation, 2006. *The IDF consensus worldwide definition of the metabolic syndrome.* [online] Available at: <https://www.idf.org/component/attachments/attachments.html?id=705&task=download> [Accessed 9 April 2023].

Janssen, I., Heymsfield, S.B., Wang, Z. and Ross, R., 2000. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology*, 89(1), pp. 81-88.

Jáuregui-Ulloa, E., Gaytán-González, A., Elizalde-Villarreal, M., González-Navarro, E., Ocampo-Chavarría, A. and López-Taylor, J., 2021. Waist Circumference Is Not Associated with Impaired Fasting Blood Glucose in a Sample of Mexican Children and Teenagers: Results from a State Screening Program. *Children*, 8(3), p. 172.

Javeed, N. and Matveyenko, A.V., 2018. Circadian etiology of type 2 diabetes mellitus. *Physiology*, 33(2), pp. 138-150.

Kang, B.K., Jang, S., Ko, M.M. and Jung, J., 2019. A study on the development of a Korean Metabolic Syndrome Questionnaire using blood stasis clinical data. *Evidence-Based Complementary and Alternative Medicine*, *2019*.

Kautzky-Willer, A., Kosi, L., Lin, J. and Mihaljevic, R., 2015. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes, Obesity and Metabolism*, 17(6), pp. 533-540.

Khan, I.A., Movva, S., Shaik, N.A., Chava, S., Jahan, P., Mukkavali, K.K., Kamineni, V., Hasan, Q. and Rao, P., 2014. Investigation of Calpain 10 (rs2975760) gene polymorphism in Asian Indians with gestational diabetes mellitus. *Meta Gene*, 2(2014), pp. 299-306.

Khan, S.H., Masood, U., Hanif, M.S., Bokhari, S.O.R.S. and Khan, M.J., 1970. Effect of age and gender on blood lipids and glucose. *Rawal Medical Journal*, 37(4), pp. 344.

Kilpatrick, E.S., Dominiczak, M.H. and Small, M., 1996. The effects of ageing on glycation and the interpretation of glycaemic control in Type 2 diabetes. *QJM: An International Journal of Medicine*, 89(4), pp. 307-308.

Klein, B.E., Klein, R., Moss, S.E. and Cruickshanks, K.J., 1996. Parental history of diabetes in a population-based study. *Diabetes Care*, 19(8), pp. 827-830.

Ko, G.T., Wai, H.P. and Tang, J.S., 2006. Effects of age on plasma glucose levels in non-diabetic Hong Kong Chinese. *Croatian Medical Journal*, 47(5), pp. 709-713.

Ko, G.T.C., Chan, J.C.N., Woo, J., Lau, E., Yeung, V.T.F., Chow, C.C., Wai, H.P.S., Li, J.K.Y., So, W.Y. and Cockram, C.S., 1997. Simple anthropometric indexes and cardiovascular risk factors in Chinese. *International Journal of Obesity*, 21(11), pp. 995-1001.

Kuk, J.L., Saunders, T.J., Davidson, L.E. and Ross, R., 2009. Age-related changes in total and regional fat distribution. *Ageing Research Reviews*, 8(4), pp. 339-348.

Lee, C.-H., Li, T.-C., Tsai, C.-I., Lin, S.-Y., Lee, I-Te., Lee, H.-J., Wu, Y.-C. and Su, Y.-C., 2015. Association between Albuminuria and Different Body Constitution in Type 2 Diabetes Patients: Taichung Diabetic Body Constitution Study. *Evidence-Based Complementary and Alternative Medicine*, 2015, pp. 1–8.

Lee, C.-H., Tsai, C.-I., Su, Y.-C., Lin, S.-Y., Lee, I-Te. and Li, T.-C., 2022. Traditional Chinese medicine body constitution predicts new-onset diabetic albuminuria in patients with type 2 diabetes: Taichung diabetic body constitution prospective cohort study. *Medicine*, 101(50), p. e32342.

Lemoncito, M.V., Paz-Pacheco, E., Lim-Abrahan, M.A., Jasul Jr, G., Isip-Tan, I.T. and Sison, C.M., 2010. Impact of waist circumference measurement variation on the diagnosis of metabolic syndrome. *Philippine Journal of Internal Medicine*, 8, pp.9-12.

Li, M., Mo, S., Lv, Y., Tang, Z. and Dong, J., 2017. A study of traditional Chinese medicine body constitution associated with overweight, obesity, and underweight. *Evidence-Based Complementary and Alternative Medicine*, 2017.

Lim, K.G. and Cheah, W.K., 2016. A review of metabolic syndrome research in Malaysia. *The Medical Journal of Malaysia*, 71(2017), pp. 20-8.

Ling, C., Groop, L., Guerra, S.D. and Lupi, R., 2009. Calpain-10 Expression Is Elevated in Pancreatic Islets from Patients with Type 2 Diabetes. *PLoS ONE*, 4(8), p. e6558.

Liu, A., Byrne, N.M., Kagawa, M., Ma, G., Kijboonchoo, K., Nasreddine, L., Koon Poh, B., Ismail, M.N. and Hills, A.P., 2011. Ethnic differences in body fat distribution among Asian pre-pubertal children: a cross-sectional multicenter study. *BMC Public Health*, 11, pp.1-7.

Lucena-Aguilar, G., Sánchez-López, A.M., Barberán-Aceituno, C., Carrillo-Avila, J.A., López-Guerrero, J.A. and Aguilar-Quesada, R., 2016. DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreservation and Biobanking*, 14(4), pp.264-270.

Ma, X., Tang, H., Zeng, J., Pan, X., Luo, X., Liao, J., Liang, D., Zhang, L., Zhou, S., Yin, M. and Ni, J., 2022. Traditional Chinese Medicine Constitution Is Associated with the Frailty Status of Older Adults: A Cross-Sectional Study in the Community. *Evidence-Based Complementary and Alternative Medicine*, *2022*.

Mafauzy, M., Zanariah, H., Nazeri, A. and Chan, S.P., 2016. DiabCare 2013: A cross-sectional study of hospital based diabetes care delivery and prevention of diabetes related complications in Malaysia. *The Medical Journal of Malaysia*, 71(4), pp. 177-185.

Manaf, M.R.A., Nawi, A.M., Tauhid, N.M., Othman, H., Rahman, M.R.A., Yusoff, H.M., Safian, N., Ng, P.Y., Manaf, Z.A., Kadir, N.B.A., Yanasegaran, K., Basir, S.M.A., Ramakrishnappa, S. and Ganasegeran, K., 2021. Prevalence of metabolic syndrome and its associated risk factors among staffs in a Malaysian public university. *Scientific Reports*, 11(1).

Massachusetts General Hospital. 2022. *Newly diagnosed diabetes in patients with COVID-19 may simply be a transitory form of the blood sugar disorder*. [online] Available at: <https://www.massgeneral.org/news/press-release/diabetes-in-patients-with-covid-may-simply-be-transitory> [Accessed 9 April 2023].

McAlexander, T.P., Malla, G., Uddin, J., Lee, D.C., Schwartz, B.S., Rolka, D.B., Siegel, K.R., Kanchi, R., Pollak, J., Andes, L. and Carson, A.P., 2022. Urban and rural differences in new onset type 2 diabetes: Comparisons across national and regional samples in the diabetes LEAD network. *SSM-Population Health*, 19(2022), p. 101161.

McDonald, T.J. and Ellard, S., 2013. Maturity onset diabetes of the young: identification and diagnosis. *Annals of clinical biochemistry*, 50(5), pp. 403-415.

Medrano, R.F.V. and De Oliveira, C.A., 2014. Guidelines for the tetra-primer ARMS–PCR technique development. *Molecular Biotechnology*, 56(7), pp. 599-608.

Miyachi, Y., Miyazawa, T. and Ogawa, Y., 2022. HNF1A Mutations and Beta Cell Dysfunction in Diabetes. *International Journal of Molecular Sciences*, 23(6), p. 3222.

Morjane, I., Kefi, R., Charoute, H., El Yaagoubi, F.L., Hechmi, M., Saile, R., Abdelhak, S. and Barakat, A., 2017. Association study of HNF1A polymorphisms with metabolic syndrome in the Moroccan population. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 11, pp. S853-S857.

Murea, M., Ma, L. and Freedman, B.I., 2012. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. *The Review of Diabetic Studies: RDS*, 9(1), p. 6.

Nam, J.S., Han, J.W., Lee, S.B., You, J.H., Kim, M.J., Kang, S., Park, J.S. and Ahn, C.W., 2018. Calpain-10 and Adiponectin Gene Polymorphisms in Korean Type 2 Diabetes Patients. *Endocrinology and Metabolism*, 33(3), p. 364.

Ministry of Health Malaysia (MOH), 2019. *National Health and Morbidity Survey 2019.* [online] Available at: < https://iptk.moh.gov.my/images/technical_report/2020/4_Infographic_Booklet_NHMS_2019_-_English.pdf> [Accessed 9 April 2023].

Nakayama, Y., Yamaguchi, H., Einaga, N. and Esumi, M., 2016. Pitfalls of DNA quantification using DNA-binding fluorescent dyes and suggested solutions. *PloS one*, 11(3), p.e0150528.

National Heart, Lung and Blood Institute (NHLBI), 2022. *What Is metabolic syndrome?* [online] Available at: <https://www.nhlbi.nih.gov/health/metabolic-syndrome> [Accessed 26 February 2023].

National Heart, Lung, and Blood Institute (NHLBI), 2023. *Assessing Your Weight and Health Risk*. [online] Available at: <https://www.nhlbi.nih.gov/health/educational/lose_wt/risk.htm#:~:text=Measuring%20waist%20circumference%20helps%20screen,disease%20and%20type%202%20diabetes> [Accessed 26 February 2023].

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), 2022. *Diabetes tests & diagnosis.* [online] Available at: <https://www.niddk.nih.gov/health-information/diabetes/overview/tests-diagnosis> [Accessed 26 February 2023].

National Institutes of Health (NIH), 2019. *National Health & Morbidity Survey 2019: Non-Communicable Diseases, Healthcare Demand and Healthcare Literacy*. [online] Available at: <https://iptk.moh.gov.my/images/technical_report/2020/4_Infographic_Booklet_NHMS_2019_-_English.pdf> [Accessed 26 February 2023].

National Library of Medicine (NLM), 2016. *HNF1A gene.* [online] Available at: <https://medlineplus.gov/genetics/gene/hnf1a/#conditions> [Accessed 25 December 2022].

National Library of Medicine (NLM), 2019. *Maturity-onset diabetes of the young.* [online] Available at: <https://medlineplus.gov/genetics/condition/maturity-onset-diabetes-of-the-young/> [Accessed 25 December 2022].

National Library of Medicine (NLM), 2022a. R*s2975760*. [online] Available at: https://www.ncbi.nlm.nih.gov/snp/rs2975760 [Accessed 25 December 2022].

National Library of Medicine (NLM), 2022b. *Rs735396*. [online] Available at: https://www.ncbi.nlm.nih.gov/snp/rs735396 [Accessed 25 December 2022].

Obradovic, J., Jurisic, V., Tosic, N., Mrdjanovic, J., Perin, B., Pavlovic, S. and Djordjevic, N., 2013. Optimization of PCR conditions for amplification of GC-Rich EGFR promoter sequence. *Journal of Clinical Laboratory Analysis*, 27(6), pp.487-493.

Ogden, C.L., Flegal, K.M., Carroll, M.D. and Johnson, C.L., 2002. Prevalence and trends in overweight among US children and adolescents, 1999-2000. *Jama*, 288(14), pp.1728-1732.

Organisation for Economic Co-operation and Development, n.d.. *State of Penang, Malaysia*. [online] Available at: https://www.oecd.org/education/imhe/47506877.pdf [Accessed 26 February 2023].

Park, S.I., Suh, J., Lee, H.S., Song, K., Choi, Y., Oh, J.S., Choi, H.S., Kwon, A., Kim, H.S., Kim, J.H. and Chae, H.W., 2021. Ten-year trends of metabolic syndrome prevalence and nutrient intake among Korean children and adolescents: a population-based study. *Yonsei Medical Journal*, 62(4), p.344.

Pejabat Daerah/Tanah Kuala Selangor, 2019. *Portal Rasmi PDT Kuala Selangor Demografi Penduduk Daerah Kuala Selangor*. [online] Available at: <https://kualaselangor.selangor.gov.my/kualaselangor.php/pages/view/104?mid=212> [Accessed 25 April 2023].

Pihlajamäki, J., Salmenniemi, U., Vänttinen, M., Ruotsalainen, E., Kuusisto, J., Vauhkonen, I., Kainulainen, S., Ng, M.C.Y., Cox, N.J., Bell, G.I. and Laakso, M., 2006. Common polymorphisms of calpain-10 are associated with abdominal obesity in subjects at high risk of type 2 diabetes. *Diabetologia*, 49(7), pp. 1560-1566.

Pradeepa, R., Anjana, R.M., Joshi, S.R., Bhansali, A., Deepa, M., Joshi, P.P., Dhandania, V.K., Madhu, S.V., Rao, P.V., Geetha, L. and Subashini, R., 2015. Prevalence of generalized & abdominal obesity in urban & rural India-the ICMR-INDIAB Study *The Indian Journal of Medical Research*, 142(2), p. 139.

Prajapat, R. and Bhattacharya, I., 2016. In Silico Structure Analysis of Type 2 Diabetes Associated Cysteine Protease Calpain-10 (CAPN10). *Advances in Diabetes and Metabolism*, 4(2), pp. 32–43.

Qian, H., Deng, X., Huang, Z.-W., Wei, J., Ding, C.-H., Feng, R.-X., Zeng, X., Chen, Y.-X., Ding, J., Qiu, L., Hu, Z.-L., Zhang, X., Wang, H.-Y., Zhang, J.-P. and Xie, W.-F., 2015. An HNF1α-regulated feedback circuit modulates hepatic fibrogenesis via the crosstalk between hepatocytes and hepatic stellate cells. *Cell Research*, 25(8), pp. 930–945.

Ramli, A.S., Daher, A.M., Noor Khan Nor-Ashikin, M., Mat-Nasir, N., Keat Ng, K., Miskan, M., Ambigga, K.S., Ariffin, F., Yasin Mazapuspavina, M., Abdul-Razak, S. and Abdul-Hamid, H., 2013. JIS definition identified more Malaysian adults with metabolic syndrome compared to the NCEP-ATP III and IDF criteria. *BioMed Research International*, *2013*.

Romao, I. and Roth, J., 2008. Genetic and environmental interactions in obesity and type 2 diabetes. *Journal of the American Dietetic Association*, 108(4), pp. S24-S28.

Ryrsø, C.K., Dungu, A.M., Hegelund, M.H., Jensen, A.V., Sejdic, A., Faurholt-Jepsen, D., Krogh-Madsen, R. and Lindegaard, B., 2022. Body composition, physical capacity, and immuno-metabolic profile in community-acquired pneumonia caused by COVID-19, influenza, and bacteria: a prospective cohort study. *International Journal of Obesity*, 46(4), pp. 817-824.

Saez, M.E., Gonzalez-Sanchez, J.L., Ramirez-Lorca, R., Martinez-Larrad, M.T., Zabena, C., Gonzalez, A., Moron, F.J., Ruiz, A. and Serrano-Rios, M., 2008. The CAPN10 gene is associated with insulin resistance phenotypes in the Spanish population. *PLoS One*, 3(8), p. e2953.

Samadoulougou, S., Diallo, M., Cissé, K., Ngwasiri, C., Aminde, L.N. and Kirakoya-Samadoulogou, F., 2022. High Urban-Rural Inequities of Abdominal Obesity in Malawi: Insights from the 2009 and 2017 Malawi Noncommunicable Disease Risk Factors Surveys. *International Journal of Environmental Research and Public Health*, 19(19), p. 11863.

Santoso, M., Fahmy, A., Tsani, A., Purwanti, R., Fithra Dieny, F. and Fitranti, D., 2022. Neck Circumference and Waist to Hip Ratio Related to Fasting Blood Glucose Levels in Security Officers. *Malaysian Journal of Medicine and Health Sciences*, 18(SUPP12), pp. 73-78.

Sapra A, Bhandari P., 2022. *Diabetes Mellitus*. [online] Available at: <https://www.ncbi.nlm.nih.gov/books/NBK551501/> [Accessed 26 February 2023].

Shafiee, M.N.I., Abd Rahman, S.Z., Zubir, S.N.S., Hamadan, H.A., Salim, N.S., Tan, E., Baruji, M.E. and Zein, R.M., 2022. Prevalence of Abdominal Obesity Among Malaysian Workers in Peninsular Malaysia. *International Journal of Allied Health Sciences*, 6(1), pp. 2549-2554.

Shastry, B.S., 2007. SNPs in disease gene mapping, medicinal drug development and evolution. *Journal of Human Genetics*, 52, pp. 871-880.

Shen, C., Zhou, Z., Lai, S., Tao, X., Zhao, D., Dong, W., Li, D., Lan, X. and Gao, J., 2019. Urban-rural-specific trend in prevalence of general and central obesity, and association with hypertension in Chinese adults, aged 18–65 years. *BMC Public Health*, 19(1), pp. 1-8.

Shimokata, H., Muller, D.C., Fleg, J.L., Sorkin, J., Ziemba, A.W. and Andres, R., 1991. Age as independent determinant of glucose tolerance. *Diabetes*, 40(1), pp. 44-51.

Sims, E.K., Mirmira, R.G. and Evans-Molina, C., 2020. The Role of Beta Cell Dysfunction in Early Type 1 Diabetes. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 27(4), p.215.

Song, Y., Niu, T., Manson, J.E., Kwiatkowski, D.J. and Liu, S., 2004. Are Variants in the CAPN10 Gene Related to Risk of Type 2 Diabetes? A Quantitative Assessment of Population and Family-Based Association Studies. *The American Journal of Human Genetics*, 74(2), pp. 208–222.

Sun, Y., Liu, P., Zhao, Y., Jia, L., He, Y., Xue, S.A., Zheng, X., Wang, Z., Wang, N. and Chen, J., 2014. Characteristics of TCM constitutions of adult Chinese women in Hong Kong and identification of related influencing factors: a cross-sectional survey. *Journal of Translational Medicine*, 12(1), pp. 1-11.

Tan, A.K., Dunn, R.A. and Yen, S.T., 2011. Ethnic disparities in metabolic syndrome in Malaysia: an analysis by risk factors. *Metabolic Syndrome and Related Disorders*, 9(6), pp.441-451.

Tan, V.M.H., Lee, Y.S., Venkataraman, K., Khoo, E.Y.H., Tai, E.S., Chong, Y.S., Gluckman, P., Leow, M.K.S. and Khoo, C.M., 2015. Ethnic differences in insulin sensitivity and beta-cell function among Asian men. *Nutrition & Diabetes*, 5(7), p. e173.

Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.F., Montagner, A. and Gourdy, P., 2020. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia*, 63(3), pp. 453-461.

Tsai, C.-I., Su, Y.-C., Lin, S.-Y., Lee, I-Te., Lee, C.-H. and Li, T.-C., 2014. Reduced Health-Related Quality of Life in Body Constitutions of Yin-Xu, and Yang-Xu, Stasis in Patients with Type 2 Diabetes: Taichung Diabetic Body Constitution Study. *Evidence-Based Complementary and Alternative Medicine*, 2014, pp. 1–10.

Turner, M.D., Cassell, P.G. and Hitman, G.A., 2005. Calpain-10: from genome search to function. *Diabetes/Metabolism Research and Reviews*, 21(6), pp. 505-514.

Veghari, G., Sedaghat, M., Joshaghani, H., Banihashem, S., Moharloei, P., Angizeh, A., Tazik, E., Moghaddami, A., Hajian-Tilaki, K. and ZahedPasha, Y., 2014. The association of fasting blood glucose (FBG) and waist circumference in northern adults in Iran: a population based study. *Journal of Diabetes & Metabolic Disorders*, 13(2), pp. 1-6.

Venkataraman, K., Kao, S.L., Thai, A.C., Salim, A., Lee, J.J.M., Heng, D., Tai, E.S. and Khoo, E.Y.H., 2012. Ethnicity modifies the relation between fasting plasma glucose and HbA1c in Indians, Malays and Chinese. *Diabetic Medicine*, 29(7), pp. 911-917.

Vinayagamoorthy, N., Hu, H.J., Yim, S.H., Jung, S.H., Jo, J., Jee, S.H. and Chung, Y.J., 2014. New variants including ARG1 polymorphisms associated with C-reactive protein levels identified by genome-wide association and pathway analysis. *PloS one*, 9(4), p. e95866.

Vittal, B.G., Praveen, G. and Deepak, P., 2010. A study of body mass index in healthy individuals and its relationship with Fasting blood sugar. *Journal of Clinical and Diagnostic Research*, 4(6), pp. 3421-3424.

Voges, M.M., Giabbiconi, C.M., Schöne, B., Waldorf, M., Hartmann, A.S. and Vocks, S., 2019. Gender differences in body evaluation: Do men show more self-serving double standards than women?. *Frontiers in Psychology*, 10, p. 544.

Wang, C.R., Hu, C., Zhang, R., Fang, Q.C., Ma, X., Jia, W. and Xiang, K., 2007. Association of a common haplotype of hepatocyte nuclear factor 1alpha with type 2 diabetes in Chinese population. *Biomedical and Environmental Sciences*, 20(1), p. 41.

Wang, Y., Rimm, E.B., Stampfer, M.J., Willett, W.C. and Hu, F.B., 2005. Comparison of abdominal adiposity and overall obesity in predicting risk of type 2 diabetes among men. *The American Journal of Clinical Nutrition*, 81(3), pp.555-563.

Willenberg, T., Schumacher, A., Amann-Vesti, B., Jacomella, V., Thalhammer, C., Diehm, N., Baumgartner, I. and Husmann, M., 2010. Impact of obesity on venous hemodynamics of the lower limbs. *Journal of Vascular Surgery*, 52(3), pp. 664-668.

Wilmet, G., Verlinde, R., Vandevoorde, J., Carnol, L. and Devroey, D., 2017. Correlation between Body Mass Index and abdominal circumference in Belgian adults: a cross-sectional study. *Romanian Journal of Internal Medicine*, 55(1), pp. 28-35.

World Health Organization (WHO), 2022. *Diabetes.* [online] Available at: <https://www.who.int/news-room/fact-sheets/detail/diabetes> [Accessed 18 December 2022].

Xu, L., Zhao, W., Wang, D. and Ma, X., 2018. Chinese medicine in the battle against obesity and metabolic diseases. *Frontiers in Physiology*, 9, p. 850.

Yan, S.-T., Li, C.-L., Tian, H., Li, J., Pei, Y., Liu, Y., Gong, Y.-P., Fang, F.-S. and Sun, B.-R., 2014. Association of calpain-10 rs2975760 polymorphism with type 2 diabetes mellitus: a meta-analysis. *International Journal of Clinical and Experimental Medicine*, 7(10), pp. 3800–7.

Yap, S.Y., Foo, C.N., Lim, Y.M., Ng, F.L., Mohd-Sidik, S., Tang, P.Y., Najar Singh, J.K. and Pheh, K.S., 2021. Traditional Chinese medicine body constitutions and psychological determinants of depression among university students in Malaysia: a pilot study. *International Journal of Environmental Research and Public Health*, 18(10), p. 5366.

Yau M, Maclaren NK, Sperling MA., 2021. *Etiology and Pathogenesis of Diabetes Mellitus in Children and Adolescents*. [online] Available at: < https://www.ncbi.nlm.nih.gov/books/NBK498653/> [Accessed 26 February 2023].

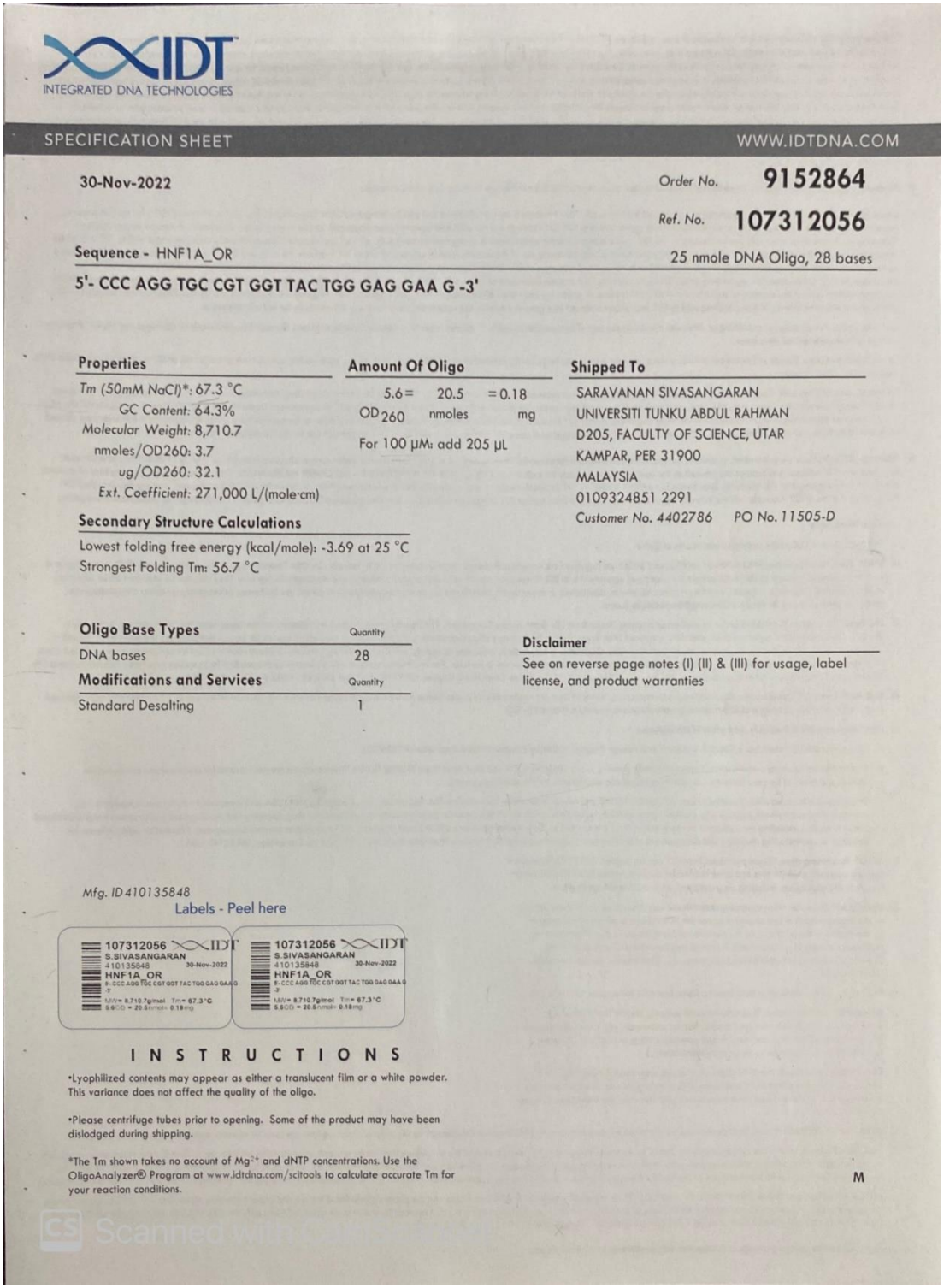
You, H., Zhang, T., Feng, W. and Gai, Y., 2017. Association of TCM body constitution with insulin resistance and risk of diabetes in impaired glucose regulation patients. *BMC Complementary and Alternative Medicine*, 17(1), pp. 1-10.

Zhang, X., Shi, C., Wei, L., Sun, F. and Ji, L., 2019. The association between the rs2975760 and rs3792267 single nucleotide polymorphisms of calpain 10 (CAPN10) and gestational diabetes mellitus. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, 25, pp. 5137.

Zhao, Q., Ding, L., Yang, Y., Sun, J., Wang, M., Li, X. and Liu, M., 2022. Clinical Characteristics of Patients With HNF1-alpha MODY: A Literature Review and Retrospective Chart Review. *Frontiers in Endocrinology*, 13.

Zheng, H., Pu, S., Zhang, Y., Fan, Y. and Yang, J., 2021. The Association between the rs312457 Genotype of the SLC16a13 Gene and Diabetes Mellitus in a Chinese Population. *Computational and Mathematical Methods in Medicine*, 2021.

APPENDIX A



IDT
INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

30-Nov-2022

Order No. 9152864

Ref. No. 107312056

Sequence - HNF1A_OR 25 nmole DNA Oligo, 28 bases

5'- CCC AGG TGC CGT GGT TAC TGG GAG GAA G -3'

Properties

Tm (50mM NaCl):* 67.3 °C
GC Content: 64.3%
Molecular Weight: 8,710.7
nmoles/OD260: 3.7
ug/OD260: 32.1
Ext. Coefficient: 271,000 L/(mole·cm)

Amount Of Oligo

5.6 = 20.5 nmoles = 0.18 mg
OD_{260}

For 100 µM: add 205 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -3.69 at 25 °C
Strongest Folding Tm: 56.7 °C

Oligo Base Types	Quantity
DNA bases	28
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135848

Labels - Peel here

107312056 IDT
S.SIVASANGARAN
410135848 30-Nov-2022
HNF1A_OR
5'-CCC AGG TGC CGT GGT TAC TGG GAG GAA G-3'
MW= 8,710.7g/mol Tm= 67.3°C
5.6OD = 20.5nmol= 0.18mg

107312056 IDT
S.SIVASANGARAN
410135848 30-Nov-2022
HNF1A_OR
5'-CCC AGG TGC CGT GGT TAC TGG GAG GAA G-3'
MW= 8,710.7g/mol Tm= 67.3°C
5.6OD = 20.5nmol= 0.18mg

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312057**

Sequence - HNF1A_OF

25 nmole DNA Oligo, 28 bases

5'- CTA CCT CGG CAT CTC ACC GGG GCT TCT C -3'

Properties

Tm (50mM NaCl):* 66.8 °C
GC Content: 64.3%
Molecular Weight: 8,452.5
nmoles/OD260: 4.1
ug/OD260: 35.0
Ext. Coefficient: 241,500 L/(mole·cm)

Amount Of Oligo

4.9 = OD_{260} 20.4 nmoles = 0.17 mg

For 100 µM: add 204 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -3.23 at 25 °C
Strongest Folding Tm: 54.9 °C

Oligo Base Types	Quantity
DNA bases	28
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135810

Labels - Peel here

107312057 IDT
S.SIVASANGARAN
410135810 30-Nov-2022
HNF1A_OF
5'-CTA CCT CGG CAT CTC ACC GGG GCT TCT C-3'
MW= 8,452.5g/mol Tm= 66.8°C
4.9OD = 20.4nmole 0.17mg

107312057 IDT
S.SIVASANGARAN
410135810 30-Nov-2022
HNF1A_OF
5'-CTA CCT CGG CAT CTC ACC GGG GCT TCT C-3'
MW= 8,452.5g/mol Tm= 66.8°C
4.9OD = 20.4nmole 0.17mg

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312058**

Sequence - HNF1A_IR

25 nmole DNA Oligo, 29 bases

5'- GGA CAC TGC AGA GGC AAA CAA GGC TGA TG -3'

Properties

Tm (50mM NaCl):* 64.7 °C
GC Content: 55.2%
Molecular Weight: 9,009.9
nmoles/OD260: 3.4
ug/OD260: 30.8
Ext. Coefficient: 292,500 L/(mole·cm)

Amount Of Oligo

7.3 = OD_{260} 24.9 nmoles = 0.22 mg

For 100 µM: add 249 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -1.44 at 25 °C
Strongest Folding Tm: 35.7 °C

Oligo Base Types	Quantity
DNA bases	29
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135847

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET

WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312059**

Sequence - HNF1A_IF

25 nmole DNA Oligo, 27 bases

5'- GTG GGT GTG GGT GCC TGG TGG GTG TCT -3'

Properties

Tm (50mM NaCl):* 69.6 °C
GC Content: 66.7%
Molecular Weight: 8,481.5
nmoles/OD260: 4.0
ug/OD260: 33.9
Ext. Coefficient: 250,200 L/(mole·cm)

Amount Of Oligo

5.9 = OD_{260} 23.6 nmoles = 0.20 mg

For 100 µM: add 236 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -0.74 at 25 °C
Strongest Folding Tm: 34.7 °C

Oligo Base Types	Quantity
DNA bases	27
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135860

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312060**

Sequence - CAPN10_OR 25 nmole DNA Oligo, 28 bases

5'- TCA CCA TGG GAG TGA GCC TCT GGC ATT G -3'

Properties

Tm (50mM NaCl):* 65.5 °C
GC Content: 57.1%
Molecular Weight: 8,620.6
nmoles/OD260: 3.8
ug/OD260: 32.8
Ext. Coefficient: 262,600 L/(mole·cm)

Amount Of Oligo

8.1 = 30.7 = 0.26
OD_{260} nmoles mg

For 100 µM: add 307 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -2.29 at 25 °C
Strongest Folding Tm: 39.6 °C

Oligo Base Types	Quantity
DNA bases	28
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135846

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET

WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312061**

Sequence - CAPN10_OF

25 nmole DNA Oligo, 28 bases

5'- AAG GCA ACT GGA CTG ACA GGC AGG CAG G -3'

Properties

Tm (50mM NaCl):* 67.5 °C
GC Content: 60.7%
Molecular Weight: 8,721.7
nmoles/OD260: 3.5
ug/OD260: 30.8
Ext. Coefficient: 282,800 L/(mole·cm)

Amount Of Oligo

6.7 = 23.5 = 0.21
OD_{260} nmoles mg

For 100 µM: add 235 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -2.86 at 25 °C
Strongest Folding Tm: 46.5 °C

Oligo Base Types	Quantity
DNA bases	28
Modifications and Services	Quantity
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135845

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET

WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312062**

Sequence - CAPN10_IR — 25 nmole DNA Oligo, 29 bases

5'- TTA GCC TCA CCT TCA AAC GCC TTA CTG CG -3'

Properties

Tm (50mM NaCl):* 63.7 °C
GC Content: 51.7%
Molecular Weight: 8,748.7
nmoles/OD260: 3.9
ug/OD260: 33.8
Ext. Coefficient: 258,600 L/(mole·cm)

Amount Of Oligo

8.0 = 31 = 0.27
OD_{260} nmoles mg

For 100 µM: add 310 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -2.29 at 25 °C
Strongest Folding Tm: 50.5 °C

Oligo Base Types	Quantity
DNA bases	29
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410137659

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

INTEGRATED DNA TECHNOLOGIES

SPECIFICATION SHEET WWW.IDTDNA.COM

30-Nov-2022

Order No. **9152864**

Ref. No. **107312063**

Sequence - CAPN10_IF 25 nmole DNA Oligo, 26 bases

5'- GAC TGC AGG GCG CTC ACG CTT GCG GT -3'

Properties

Tm (50mM NaCl):* 70.7 °C
GC Content: 69.2%
Molecular Weight: 8,004.2
nmoles/OD260: 4.3
ug/OD260: 34.1
Ext. Coefficient: 234,700 L/(mole·cm)

Amount Of Oligo

5.7 = 24.3 = 0.19
OD_{260} nmoles mg

For 100 µM: add 243 µL

Shipped To

SARAVANAN SIVASANGARAN
UNIVERSITI TUNKU ABDUL RAHMAN
D205, FACULTY OF SCIENCE, UTAR
KAMPAR, PER 31900
MALAYSIA
0109324851 2291
Customer No. 4402786 *PO No. 11505-D*

Secondary Structure Calculations

Lowest folding free energy (kcal/mole): -4.42 at 25 °C
Strongest Folding Tm: 49.8 °C

Oligo Base Types	Quantity
DNA bases	26
Modifications and Services	**Quantity**
Standard Desalting	1

Disclaimer

See on reverse page notes (I) (II) & (III) for usage, label license, and product warranties

Mfg. ID 410135859

Labels - Peel here

INSTRUCTIONS

•Lyophilized contents may appear as either a translucent film or a white powder. This variance does not affect the quality of the oligo.

•Please centrifuge tubes prior to opening. Some of the product may have been dislodged during shipping.

*The Tm shown takes no account of Mg^{2+} and dNTP concentrations. Use the OligoAnalyzer® Program at www.idtdna.com/scitools to calculate accurate Tm for your reaction conditions.

M

APPENDIX B

Sample ID	Gender	Age	Ethnicity	Place of Origin	COVID-19 History	BMI
001	Female	22	Chinese	Perak	No	19.00
002	Female	21	Chinese	Johor	No	27.44
003	Female	21	Chinese	Johor	No	30.73
004	Male	39	Chinese	Penang	No	31.94
005	Female	21	Chinese	Labuan	No	17.38
006	Male	22	Chinese	Perak	No	22.17
007	Female	21	Chinese	KL	No	21.52
008	Male	20	Chinese	Perak	No	22.02
009	Female	21	Chinese	Melaka	No	20.67
010	Female	21	Chinese	Melaka	No	27.68
011	Female	22	Chinese	Kuala Lumpur	No	22.61
012	Female	20	Chinese	Selangor	No	29.64
013	Female	21	Chinese	Selangor	Yes	17.40
014	Female	20	Chinese	Negeri Sembilan	No	22.38
015	Male	21	Chinese	Selangor	No	23.58
016	Female	20	Chinese	Penang	No	17.91
017	Female	20	Chinese	Kuala Lumpur	No	18.79
018	Female	20	Chinese	Penang	No	17.55
019	Female	20	Chinese	Melaka	No	26.94
020	Male	21	Chinese	Ipoh	No	21.82
021	Female	20	Chinese	Perak	No	22.88
022	Female	34	Malay	Pahang	No	24.04
023	Male	21	Chinese	Johor	No	18.64
024	Male	21	Chinese	Perak	No	19.93
025	Male	21	Chinese	Kuala Lumpur	No	17.00
026	Female	21	Chinese-Filipinos	Labuan	No	20.66
027	Female	22	Chinese	Negeri Sembilan	No	18.69
028	Male	24	Chinese	Melaka	No	18.55
029	Female	21	Chinese	Johor	No	19.26
030	Female	32	Malay	Johor	No	19.31
031	Male	21	Chinese	Terengganu	No	19.20
032	Female	21	Chinese	Pahang	No	18.24
033	Male	22	Chinese	Selangor	No	22.07
034	Male	20	Chinese	Negeri Sembilan	No	28.60
035	Male	50	Chinese	Ipoh	No	32.11
036	Male	40	Chinese	Perak	Yes	25.44

037	Male	70	Chinese	Perak	No	27.18
038	Male	65	Chinese	Perak	No	31.84
039	Female	62	Chinese	Perak	No	21.10
040	Male	43	Chinese	Perak	No	23.51
041	Male	61	Chinese	Perak	No	22.84
042	Male	59	Chinese	Perak	No	25.41
043	Male	37	Chinese	Perak	No	25.79
044	Female	58	Chinese	Perak	No	26.08
045	Male	58	Chinese	Perak	No	30.68
046	Male	60	Chinese	Perak	No	30.30
047	Female	19	Chinese	Perak	No	34.83
048	Female	86	Chinese	Perak	No	20.49
049	Female	45	Chinese	Perak	No	28.23
050	Male	46	Chinese	Perak	No	29.48
051	Male	42	Chinese	Perak	No	31.14
052	Male	55	Chinese	Perak	No	26.19
053	Female	36	Chinese	Perak	No	24.34
054	Male	38	Chinese	Perak	No	21.47
055	Male	44	Chinese	Perak	No	23.72
056	Female	50	Chinese	Perak	No	23.48
057	Female	36	Chinese	Perak	No	21.10
058	Female	42	Chinese	Perak	No	20.58
059	Male	63	Chinese	Perak	No	22.47
060	Female	30	Chinese	Perak	Yes	18.59
061	Male	35	Chinese	Perak	No	18.31
062	Male	29	Chinese	Perak	No	17.93
063	Female	28	Chinese	Perak	No	17.22
064	Male	39	Chinese	Perak	No	23.04
065	Female	51	Chinese	Perak	No	24.88
066	Male	51	Chinese	Perak	No	27.27
067	Female	25	Chinese	Perak	Yes	19.92
068	Male	37	Chinese	Perak	No	25.74
069	Male	58	Chinese	Perak	No	21.12
070	Male	20	Chinese	Selangor	No	20.25
071	Female	22	Chinese	Penang	No	17.96
072	Female	25	Malay	Perak	No	31.95
073	Female	19	Chinese	Johor	No	21.08
074	Female	22	Chinese	Penang	No	22.31
075	Female	21	Chinese	Sabah	No	20.72
076	Female	21	Chinese	Perak	No	17.26
077	Male	55	Chinese	Perak	No	19.64
078	Male	21	Chinese	Kuala Lumpur	No	19.65
079	Male	21	Chinese	Perak	No	21.42
080	Female	21	Indian	Selangor	No	31.25

081	Male	48	Chinese	Perak	No	22.82
082	Female	39	Chinese	Perak	Yes	18.89
083	Male	56	Chinese	Perak	No	27.61
084	Female	21	Chinese	Penang	No	19.38
085	Male	40	Chinese	Perak	No	22.84
086	Female	55	Chinese	Perak	No	21.08
087	Male	39	Chinese	Perak	No	29.46
088	Female	39	Chinese	Perak	No	31.53
089	Male	36	Chinese	Perak	No	34.24
090	Female	37	Chinese	Perak	No	25.53
091	Male	37	Chinese	Perak	No	27.33
092	Male	52	Chinese	Perak	No	19.90
093	Male	37	Chinese	Perak	No	27.22
094	Male	50	Chinese	Perak	No	27.13
095	Female	23	Chinese	Perak	No	17.48
96	Male	76	Chinese	Perak	No	22.65
097	Female	57	Chinese	Perak	No	18.42
098	Female	50	Chinese	Perak	No	18.76
099	Female	53	Chinese	Perak	No	21.02
100	Male	29	Chinese	Perak	No	28.15
101	Male	21	Chinese	Perak	No	17.79
102	Male	49	Chinese	Perak	No	22.45

APPENDIX C

Sample ID	Waist circumference (cm)	Blood glucose (mmol/L)
001	63	4.6
002	81	5.2
003	82	4.9
004	106	4.3
005	70	4.7
006	82	5.2
007	81	4.2
008	74	5.3
009	69	5.0
010	88	5.3
011	75	6.5
012	88	5.2
013	65	5.1
014	72	4.9
015	81	4.9
016	63	4.4
017	63	4.8
018	65	5.4
019	79	4.7
020	73	4.5
021	80	5.3
022	85	3.3
023	71	6.6
024	73	6.0
025	60	5.1
026	69	5.0
027	65	5.4
028	70	4.3
029	74	5.3
030	67	5.1
031	70.5	5.1
032	64	4.4
033	70	4.4
034	84	5.1
035	93	5.6
036	79	5.9
037	94	5.6

038	111	5.8
039	82	4.9
040	89	5.2
041	81	6.9
042	94	5.9
043	89	5.9
044	86	5.6
045	108.5	5.5
046	89	6.9
047	110	5.1
048	82	7.3
049	66	5.0
050	98	5.2
051	103	5.7
052	93	5.2
053	84	4.9
054	77	5.3
055	93	5.7
056	76	5.2
057	74	6.3
058	65	5.3
059	82	5.4
060	67	4.6
061	68	5.1
062	69	4.9
063	65	4.1
064	79	5.2
065	74.5	4.9
066	100	6.1
067	71	4.7
068	89	5.2
069	81	5.6
070	81	4.8
071	64	4.8
072	86.5	5.2
073	70	5.2
074	69	5.0
075	79	5.9
076	62	4.8
077	76	5.4
078	84	4.7
079	83	4.6
080	83	4.8
081	81	5.8

082	74	5.4
083	108	5.8
084	66	5.4
085	89	5.3
086	72	5.8
087	110	5.6
088	90	5.8
089	106	5.9
090	90	5.6
091	102	6.1
092	83	6.1
093	95	5.8
094	99	6.1
095	61	4.8
96	93	6.3
097	70	5.5
098	72	5.3
099	70	5.3
100	92	4.9
101	69	4.9
102	86	8.3

APPENDIX D

Sample ID	Concentration (ng/µL)	Purity (A_{260}/A_{280})
001	148.2	1.86
002	72.5	1.84
003	47.2	1.64
004	49.4	1.81
005	41.8	1.8
006	76.6	1.84
007	33.6	2.45
008	27.4	1.86
009	83.9	1.85
010	63.9	1.99
011	61.8	0.68
012	42.9	1.77
013	57.9	1.52
014	113.7	1.87
015	39.8	1.79
016	138.7	1.88
017	23.8	1.75
018	23.1	1.64
019	26.5	1.75
020	42.8	1.76
021	79.8	1.85
022	39.3	1.79
023	52.3	1.82
024	36.8	1.78
025	59.6	1.53
026	51	1.82
027	26.7	1.78
028	44.4	1.81
029	30.1	1.79
030	31.6	1.78
031	60.8	1.82
032	36.7	1.74
033	35.8	1.79
034	36.9	1.82
035	64.5	1.87

036	55.6	1.88
037	58.6	1.39
038	43.5	1.84
039	79.1	1.61
040	35.9	1.9
041	38.6	1.89
042	37.3	1.82
043	57.7	1.8
044	21.9	1.87
045	32.2	1.78
046	27.2	1.95
047	40.4	1.89
048	325.2	1.53
049	64.2	1.87
050	18.8	1.8
051	87.3	1.84
052	25.6	1.88
053	25.6	1.8
054	26.9	1.58
055	59.7	1.87
056	31.7	1.76
057	74.3	1.89
058	62.5	1.88
059	17.2	1.81
060	30.4	1.67
061	42.3	1.87
062	23	1.67
063	99.9	1.49
064	19.9	1.88
065	38.2	1.87
066	58	1.54
067	110.6	2.09
068	66.1	1.86
069	33.6	1.87
070	20.8	1.9
071	33.6	1.8
072	25	1.78
073	52	1.82
074	28.9	1.81
075	20.3	1.81
076	51.5	1.85

077	40.7	1.19
078	32.8	1.92
079	34.6	1.91
080	70.3	1.88
081	24.6	1.88
082	46.1	1.88
083	30.6	1.86
084	35.6	1.9
085	18	1.93
086	44.9	1.9
087	39.5	1.82
088	25.1	1.73
089	33.2	1.79
090	39	1.84
091	17.5	1.96
092	31.8	1.63
093	26.8	1.74
094	48	1.81
095	57.4	1.82
96	50.5	1.86
097	109.1	1.79
098	196.2	1.86
099	137.4	1.96
100	121	1.84
101	69.2	1.85
102	69.8	1.78

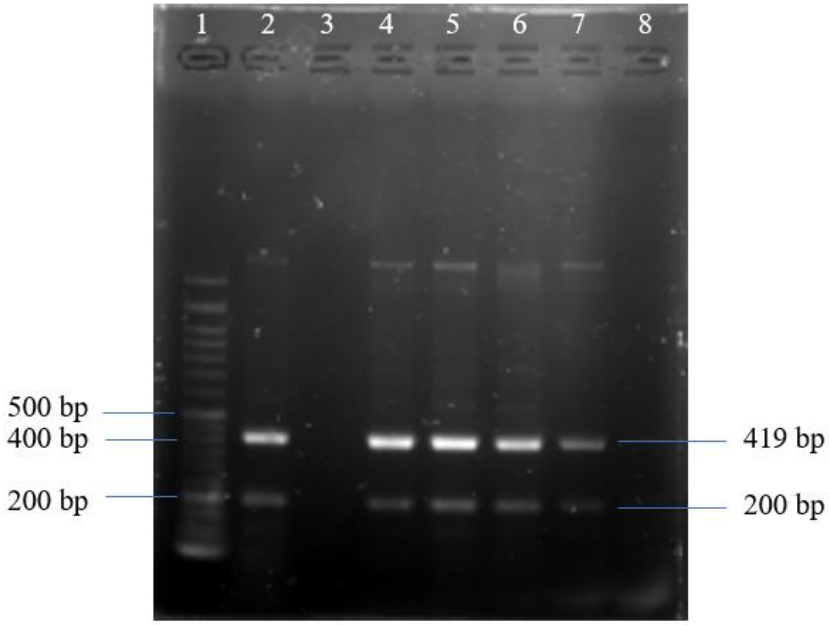
APPENDIX E

Sample ID	rs735396 (T>C)	rs2975760 (T>C)
001	TT	TT
002	CC	CC
003	CC	TT
004	CC	TT
005	TT	TT
006	TT	TT
007	TC	TT
008	TC	TC
009	TC	TT
010	CC	TT
011	TC	TC
012	TC	TT
013	TC	TT
014	CC	TT
015	TC	TT
016	TT	TT
017	TC	TT
018	TC	TT
019	CC	TT
020	CC	TT
021	TC	TT
022	TC	TT
023	TC	TT
024	TC	CC
025	TC	TT
026	TC	TT
027	TC	TT
028	TC	TT
029	TC	TT
030	TT	TT
031	CC	TT
032	CC	TT
033	CC	TT
034	CC	TT
035	CC	TT

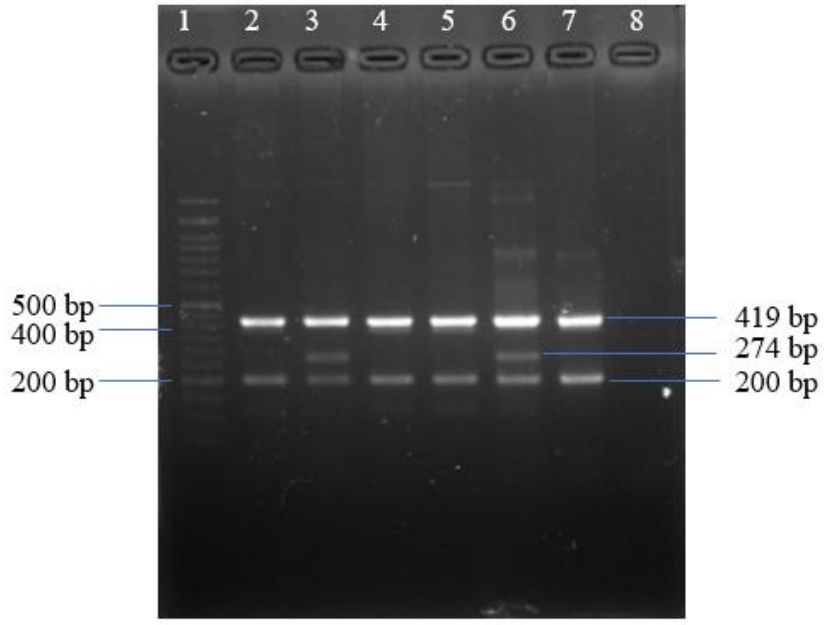
036	TC	TC
037	TC	TT
038	TC	TT
039	TC	TC
040	TC	TT
041	TT	TC
042	TC	TT
043	TC	TT
044	TC	TT
045	TC	TT
046	CC	TT
047	TC	TT
048	TT	TC
049	CC	TT
050	TT	TT
051	CC	TT
052	TC	TT
053	TC	TT
054	CC	TT
055	TC	TT
056	TC	TT
057	TC	TT
058	TC	TT
059	TC	TT
060	CC	TT
061	TC	TT
062	TC	TT
063	TT	TT
064	CC	TT
065	TC	TT
066	TT	TT
067	TC	TC
068	TC	TT
069	TC	TT
070	TC	TT
071	TC	TC
072	TC	TT
073	TT	TT
074	TT	TT
075	TC	TT
076	TC	TT

077	TC	TT
078	TC	TT
079	TC	TT
080	CC	TT
081	TT	CC
082	TC	TT
083	TT	TT
084	TT	TC
085	TT	TT
086	TT	TT
087	TT	TT
088	TT	TT
089	TT	TT
090	TT	TC
091	TT	TT
092	TC	TT
093	TC	TT
094	TC	TT
095	TC	TT
96	TC	TT
097	CC	TT
098	TT	TT
099	CC	TT
100	CC	TT
101	TC	TT
102	TC	TT

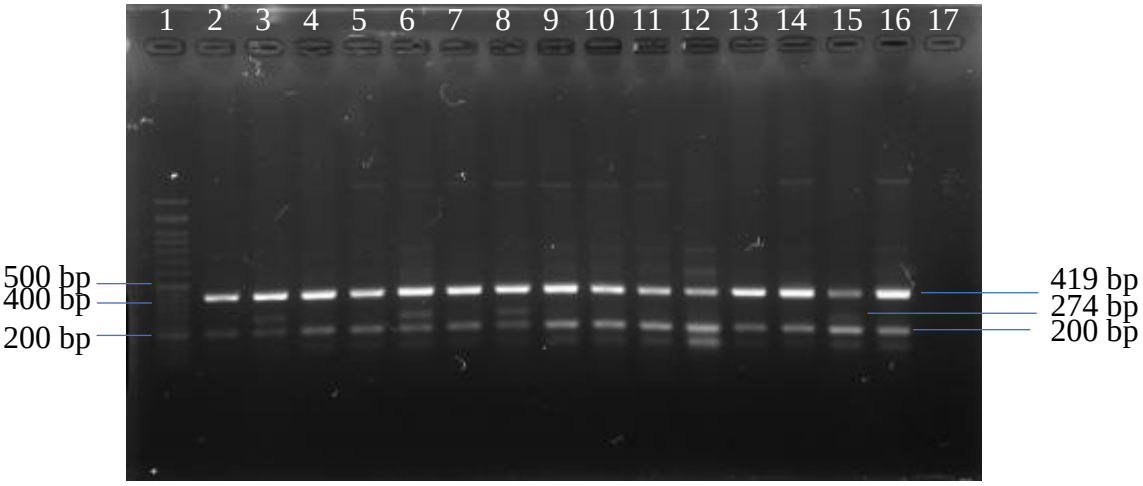
APPENDIX F



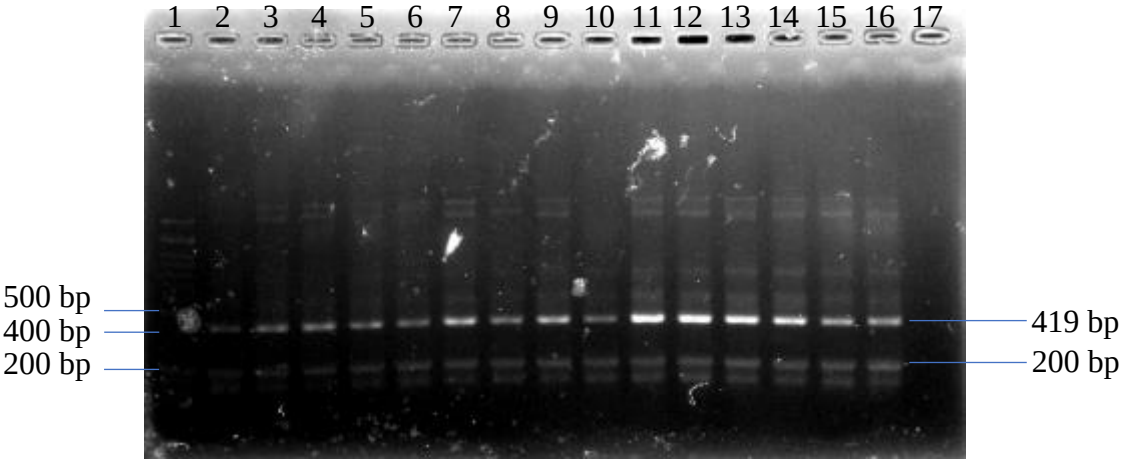
Lane 1: 50 bp ladder; Lane 2: sample 001; Lane 3: sample 002; Lane 4: sample 003; Lane 5: sample 004; Lane 6: sample 005; Lane 7: sample 006; Lane 8: Non-template control (NTC)



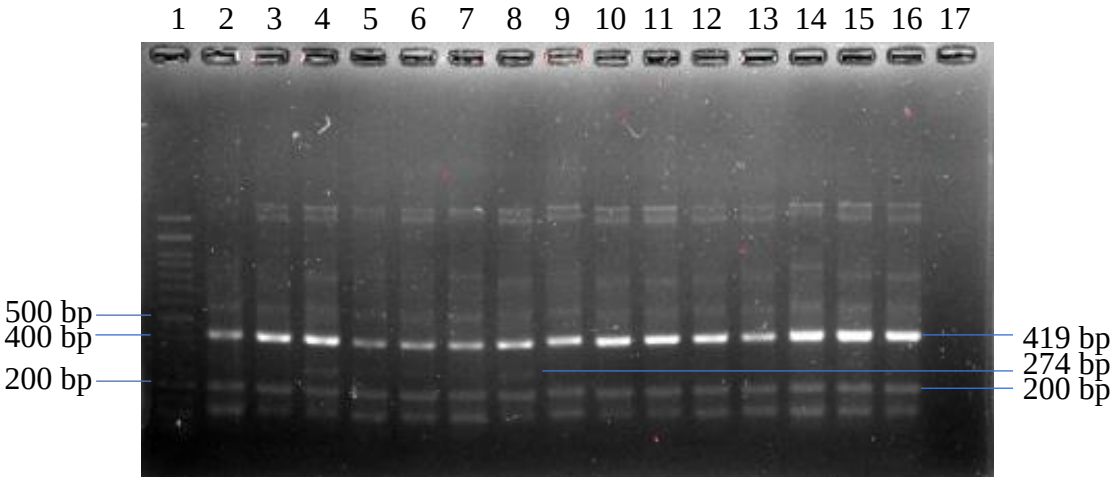
Lane 1: 50 bp ladder; Lane 2: sample 007; Lane 3: sample 008; Lane 4: sample 009; Lane 5: sample 010; Lane 6: sample 011; Lane 7: sample 012; Lane 8: Non-template control (NTC)



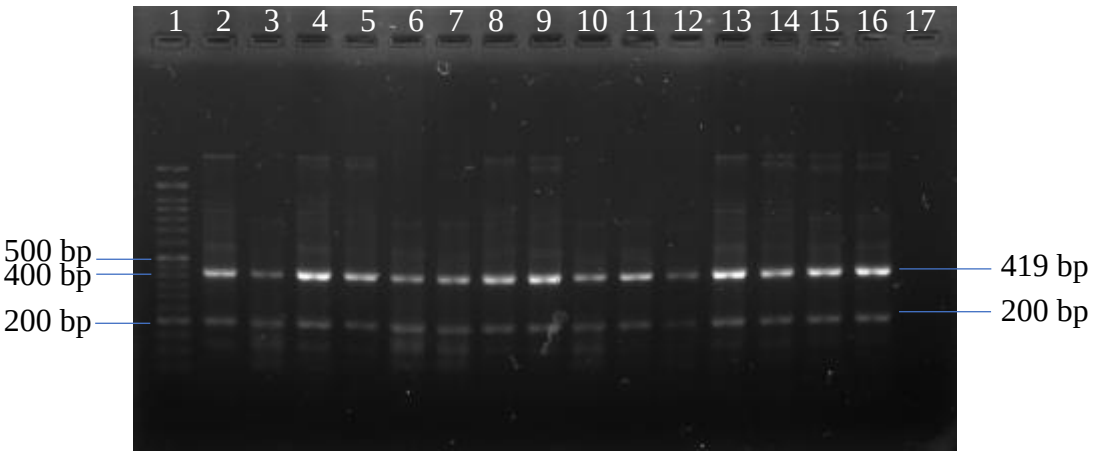
Lane 1: 50 bp ladder; Lane 2: sample 035; Lane 3: sample 036; Lane 4: sample 037; Lane 5: sample 038; Lane 6: sample 039; Lane 7: sample 040; Lane 8: sample 041; Lane 9: sample 042; Lane 10: sample 043; Lane 11: sample 044; Lane 12: sample 045; Lane 13: sample 046; Lane 14: sample 047; Lane 15: sample 048; Lane 16: sample 049; Lane 17: Non-template control (NTC)



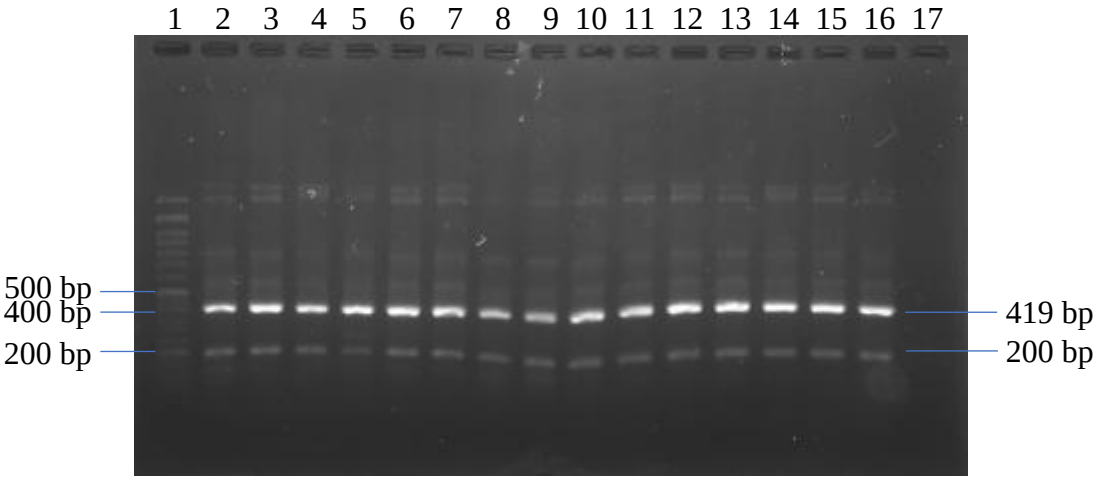
Lane 1: 50 bp ladder; Lane 2: sample 050; Lane 3: sample 051; Lane 4: sample 052; Lane 5: sample 053; Lane 6: sample 054; Lane 7: sample 055; Lane 8: sample 056; Lane 9: sample 057; Lane 10: sample 058; Lane 11: sample 059; Lane 12: sample 060; Lane 13: sample 061; Lane 14: sample 062; Lane 15: sample 063; Lane 16: sample 064; Lane 17: Non-template control (NTC)



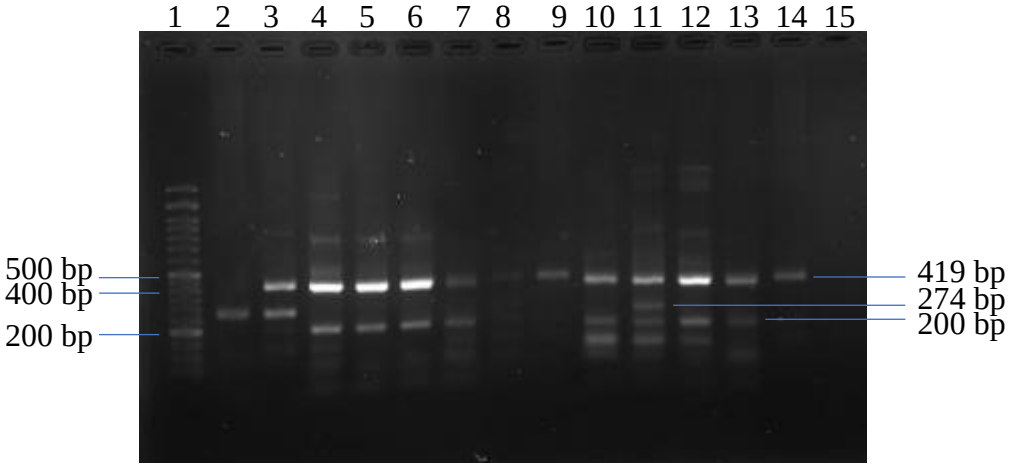
Lane 1: 50 bp ladder; Lane 2: sample 065; Lane 3: sample 066; Lane 4: sample 067; Lane 5: sample 068; Lane 6: sample 069; Lane 7: sample 070; Lane 8: sample 071; Lane 9: sample 072; Lane 10: sample 073; Lane 11: sample 074; Lane 12: sample 075; Lane 13: sample 076; Lane 14: sample 078; Lane 15: sample 079; Lane 16: sample 080; Lane 17: Non-template control (NTC)



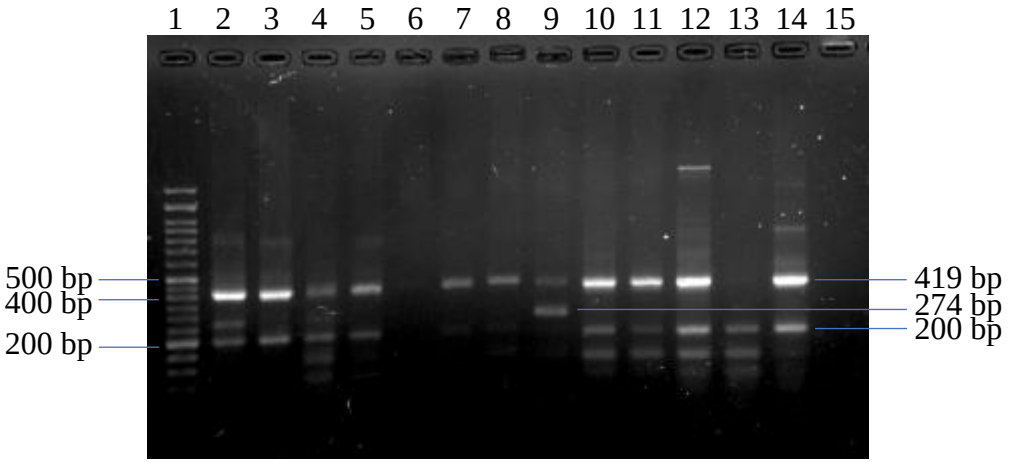
Lane 1: 50 bp ladder; Lane 2: sample 096; Lane 3: sample 097; Lane 4: sample 098; Lane 5: sample 099; Lane 6: sample 100; Lane 7: sample 101; Lane 8: sample 102; Lane 9: sample 013; Lane 10: sample 014; Lane 11: sample 015; Lane 12: sample 016; Lane 13: sample 017; Lane 14: sample 018; Lane 15: sample 019; Lane 16: sample 020; Lane 17: Non-template control (NTC)



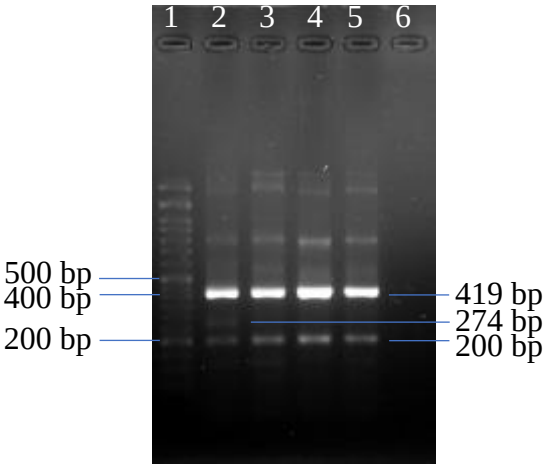
Lane 1: 50 bp ladder; Lane 2: sample 021; Lane 3: sample 022; Lane 4: sample 023; Lane 5: sample 024; Lane 6: sample 025; Lane 7: sample 026; Lane 8: sample 027; Lane 9: sample 028; Lane 10: sample 029; Lane 11: sample 030; Lane 12: sample 031; Lane 13: sample 032; Lane 14: sample 033; Lane 15: sample 034; Lane 16: sample 077; Lane 17: Non-template control (NTC)



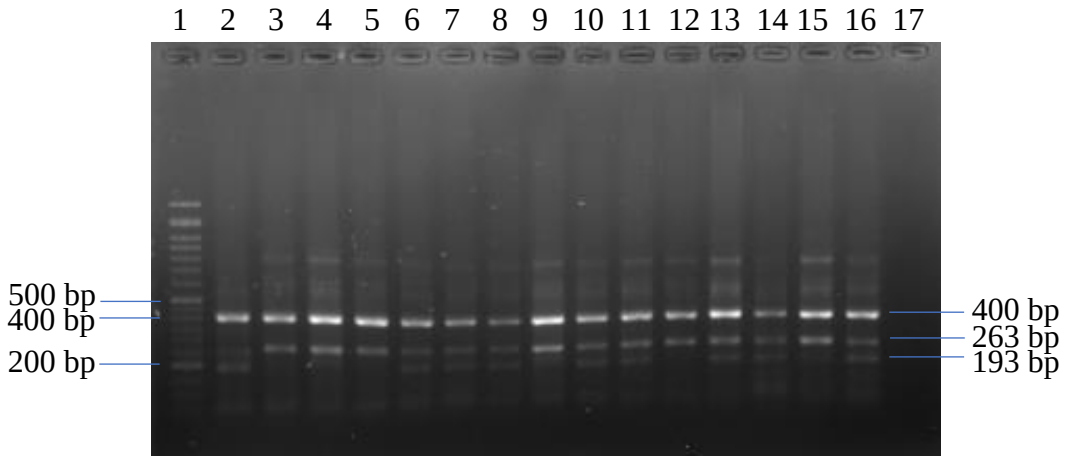
Lane 1: 50 bp ladder; Lane 2: sample 002; Lane 3: sample 024; Lane 4: sample 050; Lane 5: sample 051; Lane 6: sample 052; Lane 7: sample 053; Lane 8: sample 067; Lane 9: sample 068; Lane 10: sample 069; Lane 11: sample 071; Lane 12: sample 072; Lane 13: sample 081; Lane 14: sample 082; Lane 15: Non-template control (NTC)



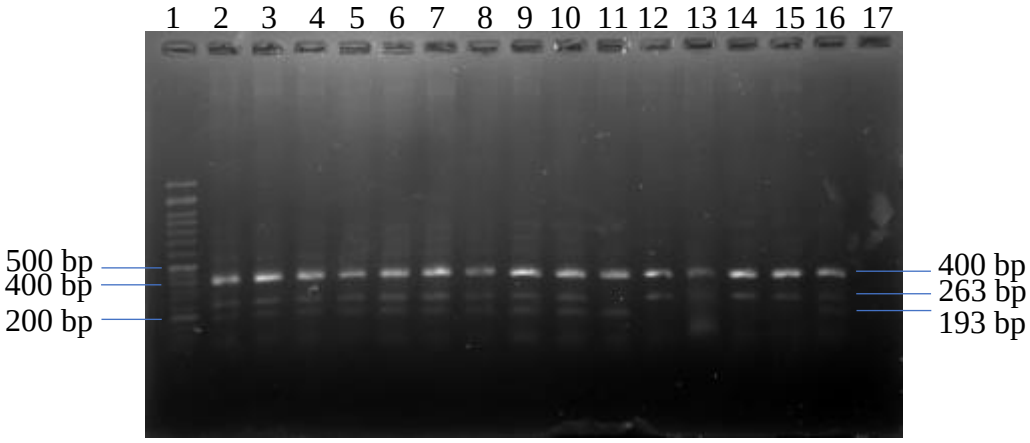
Lane 1: 50 bp ladder; Lane 2: sample 090; Lane 3: sample 091; Lane 4: sample 092; Lane 5: sample 093; Lane 6: sample 094; Lane 7: sample 095; Lane 8: sample 083; Lane 9: sample 084; Lane 10: sample 085; Lane 11: sample 086; Lane 12: sample 087; Lane 13: sample 088; Lane 14: sample 089; Lane 15: Non-template control (NTC)



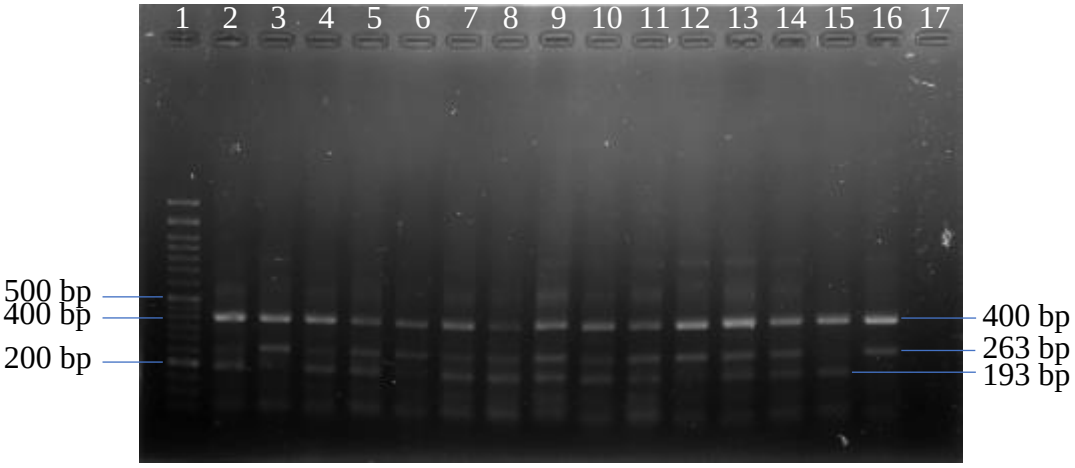
Lane 1: 50 bp ladder; Lane 2: sample 067; Lane 3: sample 068; Lane 4: sample 082; Lane 5: sample 094; Lane 6: Non-template control (NTC)



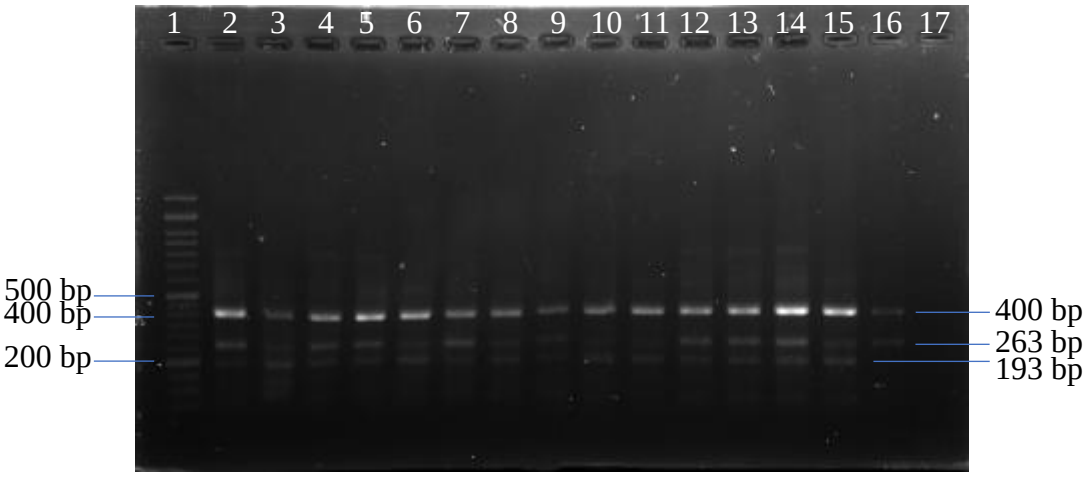
Lane 1: 50 bp ladder; Lane 2: sample 001; Lane 3: sample 002; Lane 4: sample 003; Lane 5: sample 004; Lane 6: sample 007; Lane 7: sample 008; Lane 8: sample 009; Lane 9: sample 010; Lane 10: sample 011; Lane 11: sample 012; Lane 12: sample 032; Lane 13: sample 082; Lane 14: sample 092; Lane 15: sample 093; Lane 16: sample 094; Lane 17: Non-template control (NTC)



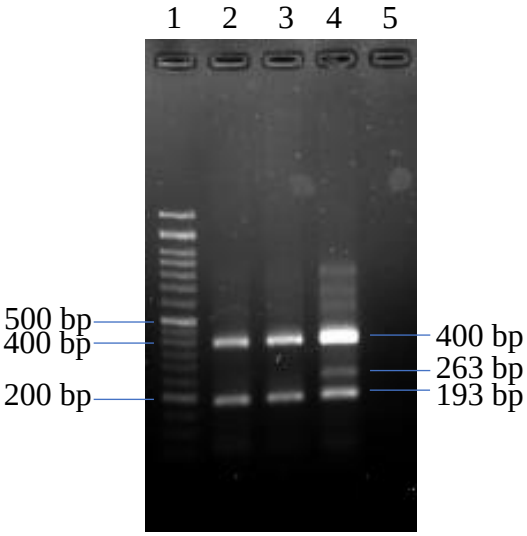
Lane 1: 50 bp ladder; Lane 2: sample 021; Lane 3: sample 022; Lane 4: sample 023; Lane 5: sample 024; Lane 6: sample 025; Lane 7: sample 026; Lane 8: sample 027; Lane 9: sample 028; Lane 10: sample 029; Lane 11: sample 030; Lane 12: sample 031; Lane 13: sample 032; Lane 14: sample 033; Lane 15: sample 034; Lane 16: sample 077; Lane 17: Non-template control (NTC)



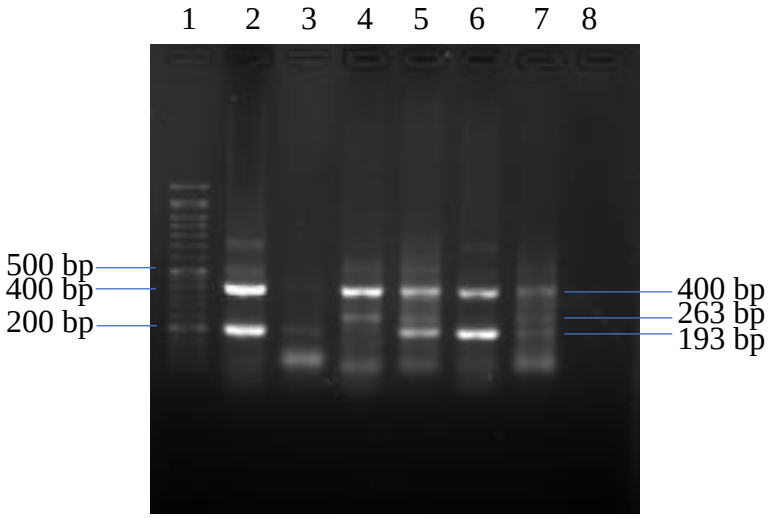
Lane 1: 50 bp ladder; Lane 2: sample 050; Lane 3: sample 051; Lane 4: sample 052; Lane 5: sample 053; Lane 6: sample 054; Lane 7: sample 055; Lane 8: sample 056; Lane 9: sample 057; Lane 10: sample 058; Lane 11: sample 059; Lane 12: sample 060; Lane 13: sample 061; Lane 14: sample 062; Lane 15: sample 063; Lane 16: sample 064; Lane 17: Non-template control (NTC)



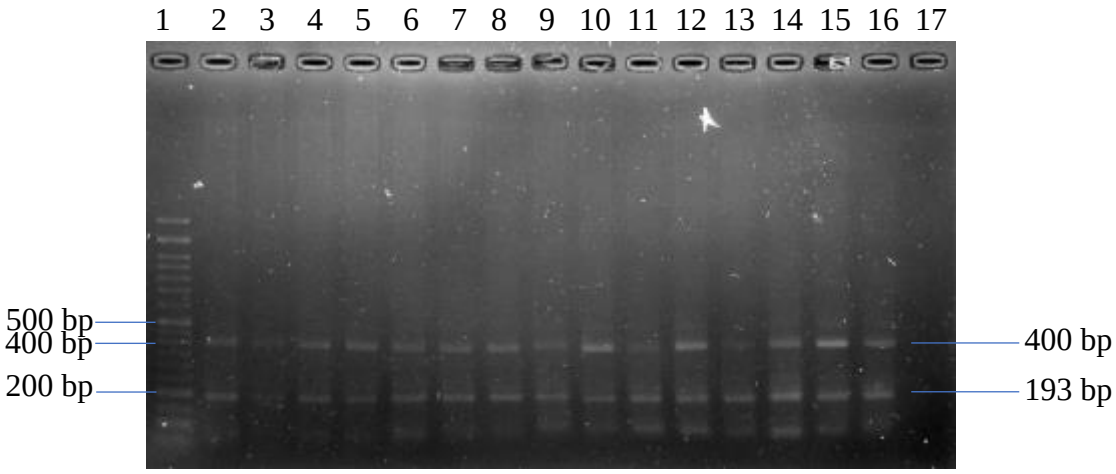
Lane 1: 50 bp ladder; Lane 2: sample 065; Lane 3: sample 066; Lane 4: sample 067; Lane 5: sample 068; Lane 6: sample 069; Lane 7: sample 070; Lane 8: sample 071; Lane 9: sample 072; Lane 10: sample 073; Lane 11: sample 074; Lane 12: sample 075; Lane 13: sample 076; Lane 14: sample 078; Lane 15: sample 079; Lane 16: sample 080; Lane 17: Non-template control (NTC)



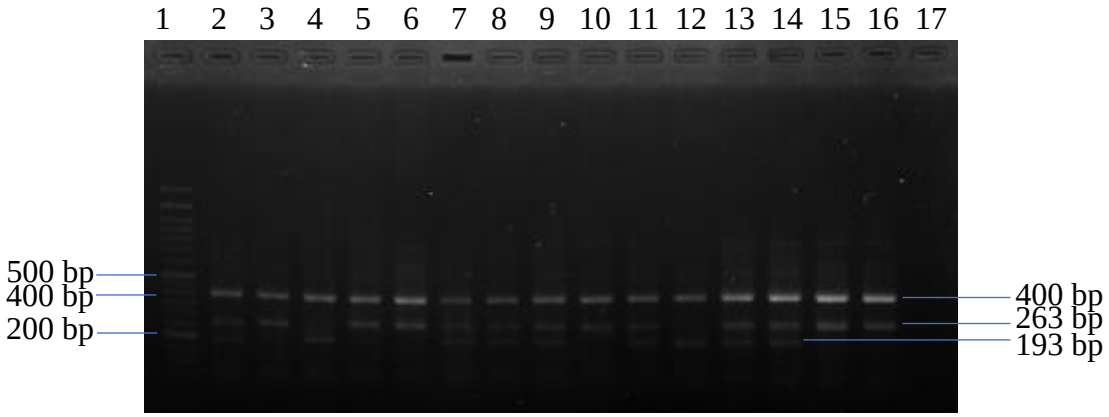
Lane 1: 50 bp ladder; Lane 2: sample 050; Lane 3: sample 090; Lane 4: sample 095; Lane 5: Non-template control (NTC)



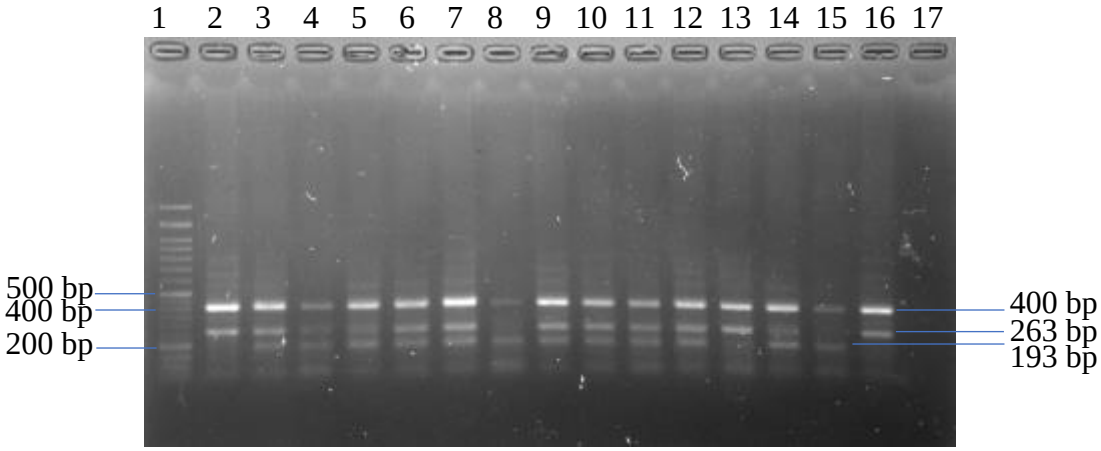
Lane 1: 50 bp ladder; Lane 2: sample 001 Lane 3: sample 048; Lane 4: sample 049; Lane 5: sample 065; Lane 6: sample 066; Lane 7: sample 093; Lane 8: Non-template control (NTC)



Lane 1: 50 bp ladder; Lane 2: sample 081; Lane 3: sample 082; Lane 4: sample 083; Lane 5: sample 084; Lane 6: sample 085; Lane 7: sample 086; Lane 8: sample 087; Lane 9: sample 088; Lane 10: sample 089; Lane 11: sample 090; Lane 12: sample 091; Lane 13: sample 092; Lane 14: sample 093; Lane 15: sample 094; Lane 16: sample 095; Lane 17: Non-template control (NTC)



Lane 1: 50 bp ladder; Lane 2: sample 096; Lane 3: sample 097; Lane 4: sample 098; Lane 5: sample 099; Lane 6: sample 100; Lane 7: sample 101; Lane 8: sample 102; Lane 9: sample 013; Lane 10: sample 014; Lane 11: sample 015; Lane 12: sample 016; Lane 13: sample 017; Lane 14: sample 018; Lane 15: sample 019; Lane 16: sample 020; Lane 17: Non-template control (NTC)



Lane 1: 50 bp ladder; Lane 2: sample 035; Lane 3: sample 036; Lane 4: sample 037; Lane 5: sample 038; Lane 6: sample 039; Lane 7: sample 040; Lane 8: sample 041; Lane 9: sample 042; Lane 10: sample 043; Lane 11: sample 044; Lane 12: sample 045; Lane 13: sample 046; Lane 14: sample 047; Lane 15: sample 048; Lane 16: sample 049; Lane 17: Non-template control (NTC)

APPENDIX G

Sample ID	Yang Xu	Yin Xu	Qi Xu	Tan Shi	Shi Re	Xue Yu	Te Bing	Qi Yu	Ping He	BC type
001	46	13	14	16	0	18	39	21	59	Yang Xu
002	36	50	64	69	42	25	29	64	53	Yin Xu, Qi Xu, Tan Shi, Shi Re, Qi Yu
003	39	9	14	3	8	7	18	18	72	Ping He
004	4	31	32	28	17	7	14	0	78	Ping He
005	43	41	64	6	17	4	0	18	66	Yang Xu, Yin Xu, Qi Xu
006	64	63	50	19	21	29	43	29	53	Yang Xu, Yin Xu, Qi Xu, Te Bing
007	46	28	46	28	42	39	46	32	63	Yang Xu, Shi Re, Te Bing, Qi Xu
008	14	6	39	16	25	25	25	32	59	Qi Yu
009	14	34	32	6	17	29	11	32	56	Yin Xu
010	7	19	29	28	42	21	32	46	66	Shi Re, Qi Yu
011	29	53	29	31	38	46	54	39	53	Xue Yu, Te Bing
012	36	6	18	6	13	7	29	11	69	Ping He
013	29	13	11	3	13	18	25	43	69	Qi Yu
014	25	22	7	6	13	7	11	7	78	Ping He
015	4	9	7	0	8	7	0	0	84	Ping He
016	7	13	32	19	33	25	18	14	66	Ping He

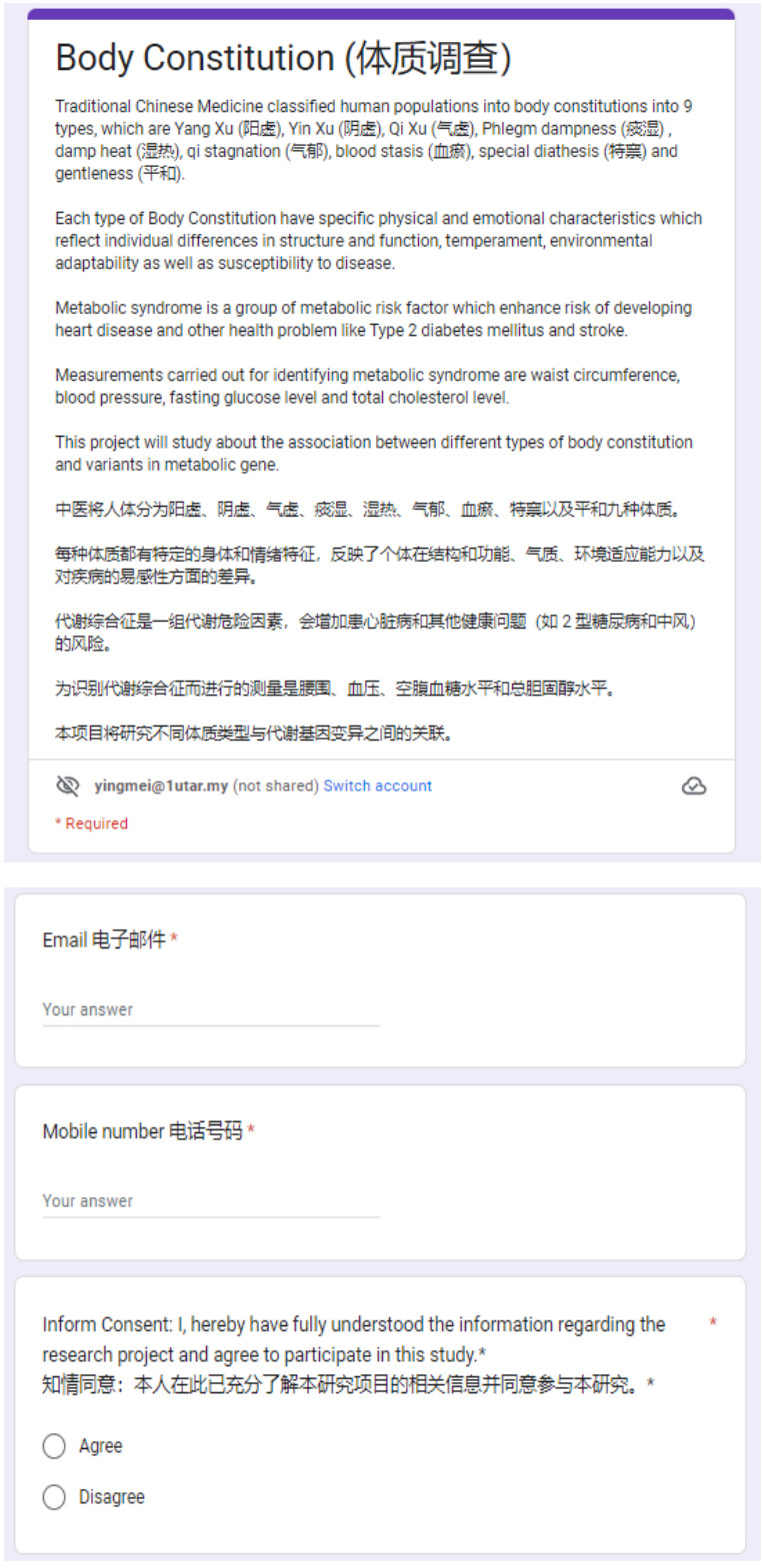
017	7	38	32	44	17	43	18	18	66	Tan Shi, Xue Yu
018	25	41	25	31	33	25	57	25	66	Yin Xu, Te Bing
019	4	16	11	16	29	14	0	18	72	Ping He
020	32	19	32	13	29	18	7	43	66	Qi Yu
021	4	22	57	22	29	11	21	39	66	Qi Xu
022	7	9	7	6	4	11	14	7	69	Ping He
023	36	31	36	31	50	36	7	32	50	Shi Re
024	11	13	32	13	21	4	14	21	69	Ping He
025	4	3	14	0	4	11	7	21	75	Ping He
026	46	22	21	28	25	25	4	11	75	Yang Xu
027	46	34	36	22	50	57	57	71	38	Yang Xu, Shi Re, Xue Yu, Te Bing, Qi Yu
028	46	41	21	28	58	32	36	36	50	Yang Xu, Yin Xu, Shi Re
029	43	56	50	41	21	36	25	54	50	Yang Xu, Yin Xu, Qi Xu, Tan Shi, Qi Yu
030	25	25	25	9	21	21	7	14	81	Ping He
031	36	41	50	25	29	25	50	29	44	Yin Xu, Qi Xu, Te Bing
032	75	31	36	22	46	39	54	57	63	Yang Xu, Shi Re, Te Bing, Qi Yu
033	21	75	39	53	50	57	54	68	63	Yin Xu, Tan Shi, Shi Re, Xue Yu, Te Bing, Qi Yu
034	14	6	11	6	13	4	7	7	91	Ping He
035	46	59	64	59	29	61	39	64	38	Yang Xu, Yin Xu, Qi Xu, Tan Shi, Xue Yu, Qi Yu
036	0	0	0	0	0	7	0	0	69	Ping He
037	21	34	50	41	25	50	14	39	59	Qi Xu, Tan Shi, Xue Yu

038	0	0	14	13	8	46	14	0	88	Xue Yu
039	18	34	25	44	13	14	7	29	59	Tan Shi
040	18	13	29	25	25	43	18	11	75	Xue Yu
041	7	19	14	16	21	36	39	0	84	Ping He
042	0	13	21	9	13	18	39	7	75	Ping He
043	4	19	43	28	13	36	7	43	50	Qi Xu, Qi Yu
044	0	31	36	13	29	36	43	29	69	Te Bing
045	0	25	14	0	8	11	0	0	88	Ping He
046	4	31	43	34	29	61	64	7	72	Xue Yu, Te Bing, Qi Xu
047	14	25	43	16	33	25	32	25	63	Qi Xu
048	36	44	32	0	8	36	18	0	53	Yin Xu
049	25	22	32	38	13	46	21	29	56	Xue Yu
050	25	47	32	50	63	36	39	18	50	Yin Xu, Tan Shi, Shi Re
051	25	25	18	22	13	21	14	14	66	Ping He
052	29	50	57	63	42	36	29	57	22	Yin Xu, Qi Xu,Tan Shi, Shi Re, Qi Yu
053	64	56	68	59	58	43	75	75	34	Yang Xu, Yin Xu, Qi Xu, Tan Shi, Shi Re, Xue Yu, Te Bing, Qi Yu
054	21	28	32	28	8	29	29	25	59	Qi Xu
055	4	28	57	31	8	21	0	21	59	Qi Xu
056	21	6	14	13	4	21	21	0	63	Ping He
057	86	28	39	31	42	43	21	21	47	Yang Xu, Shi Re, Xue Yu
058	7	19	32	22	25	18	25	50	53	Qi Yu
059	11	63	54	38	25	25	21	11	50	Yin-Xu, Qi-Xu, Tan-Shi
060	36	44	54	25	42	32	25	29	33	Yin-Xu, Qi-Xu, Shi-Re

061	64	88	43	63	33	50	43	61	25	Yang-Xu, Qi-Xu, Tan-Shi, Shi-Re, Qi-Xu, Te-Bing
062	46	69	50	25	21	46	18	68	0	Yang-Xu, Qi-Xu, Xue-Yu, Qi-Yu
063	29	56	64	28	42	39	21	29	50	Yin-Xu, Shi-Re, Xue-Yu
064	21	78	61	25	21	18	18	14	67	Yin Xu, Qi Xu
065	Excluded	-	-	-	-	-	-	-	-	-
066	Excluded	-	-	-	-	-	-	-	-	-
067	36	66	46	56	46	29	29	25	42	Yin-Xu, Qi-Xu, Tan-Shi, Shi-Re
068	Excluded	-	-	-	-	-	-	-	-	-
069	14	97	71	3	4	11	7	0	50	Yin Xu, Qi Xu
070	21	81	54	0	0	14	50	14	50	Yin Xu, Qi Xu, Te Bing
071	57	56	50	50	38	39	64	36	50	Yang-Xu, Yin-Xu, Qi-Xu, Tan-Shi, Te-Bing
072	29	69	64	6	4	29	29	0	42	Qi-Xu, Yin Xu
073	18	88	71	28	17	21	18	14	33	Yin Xu, Qi Xu
074	7	84	82	9	13	7	7	21	58	Yin Xu, Qi Xu
075	46	59	36	53	50	43	46	46	33	Yang Xu, Yin Xu, Te Bing, Qi Xu, Qi Yu, Tan Shi, Shi Re
076	21	72	57	16	38	29	7	7	67	Yin Xu, Qi Xu
077	4	91	68	16	29	14	25	14	17	Yin Xu, Qi Xu
078	43	50	61	25	50	39	46	43	75	Yang-Xu, Yin-Xu, Qi-Xu, Shi-Re, Qi-Yu, Te-Bing
079	11	84	68	3	21	11	25	14	50	Yin Xu, Qi Xu
080	7	66	61	19	8	25	39	29	58	Yin Xu, Qi Xu

081	100	13	32	88	88	96	86	93	8	Yang-Xu, Tan-Shi, Shi-Re, Xue-Yu, Te-Bing, Qi-Yu
082	57	69	64	22	29	11	32	64	42	Yang-Xu, Yin Xu, Qi-Yu, Qi Xu
083	0	84	75	9	4	25	11	18	17	Yin-Xu, Qi-Xu
084	29	53	46	31	38	14	11	39	50	Yin-Xu, Qi-Xu,
085	11	100	61	13	8	14	11	14	33	Yin Xu, Qi Xu
086	14	94	50	16	17	14	29	18	33	Yin Xu, Qi Xu
087	18	78	54	19	8	4	21	0	100	Yin Xu, Qi Xu
088	0	6	14	3	13	18	7	4	91	Ping He
089	0	34	25	16	13	7	14	0	63	Ping He
090	4	41	50	56	38	18	32	50	66	Tan Shi, Yin Xu, Qi Xu and Qi Yu
091	14	19	36	28	42	29	21	39	78	Shi Re
092	0	0	0	0	0	0	0	0	100	Ping He
093	11	19	29	6	4	11	7	14	75	Ping He
094	7	25	18	19	21	7	7	11	72	Ping He
095	39	56	54	59	46	43	21	43	34	Qi Xu, Yin Xu, Xue Yu, Qi Yu, Shi Re, Tan Shi
96	11	6	32	13	4	54	25	18	44	Xue Yu
097	54	44	50	31	29	50	25	36	41	Yang xu,Yin Xu, Qi Xu and Xue Yu
098	4	6	11	3	4	7	0	0	72	Ping He
099	18	19	11	9	8	21	21	0	69	Ping He
100	21	6	7	3	4	7	25	11	63	Ping He
101	25	9	18	9	8	4	11	4	69	Ping He
102	39	28	54	38	46	36	32	14	66	Qi Xu, Shi Re

APPENDIX H



Body Constitution (体质调查)

Traditional Chinese Medicine classified human populations into body constitutions into 9 types, which are Yang Xu (阳虚), Yin Xu (阴虚), Qi Xu (气虚), Phlegm dampness (痰湿) , damp heat (湿热), qi stagnation (气郁), blood stasis (血瘀), special diathesis (特禀) and gentleness (平和).

Each type of Body Constitution have specific physical and emotional characteristics which reflect individual differences in structure and function, temperament, environmental adaptability as well as susceptibility to disease.

Metabolic syndrome is a group of metabolic risk factor which enhance risk of developing heart disease and other health problem like Type 2 diabetes mellitus and stroke.

Measurements carried out for identifying metabolic syndrome are waist circumference, blood pressure, fasting glucose level and total cholesterol level.

This project will study about the association between different types of body constitution and variants in metabolic gene.

中医将人体分为阳虚、阴虚、气虚、痰湿、湿热、气郁、血瘀、特禀以及平和九种体质。

每种体质都有特定的身体和情绪特征，反映了个体在结构和功能、气质、环境适应能力以及对疾病的易感性方面的差异。

代谢综合征是一组代谢危险因素，会增加患心脏病和其他健康问题（如 2 型糖尿病和中风）的风险。

为识别代谢综合征而进行的测量是腰围、血压、空腹血糖水平和总胆固醇水平。

本项目将研究不同体质类型与代谢基因变异之间的关联。

yingmei@1utar.my (not shared) Switch account

* Required

Email 电子邮件 *

Your answer

Mobile number 电话号码 *

Your answer

Inform Consent: I, hereby have fully understood the information regarding the research project and agree to participate in this study.*
知情同意：本人在此已充分了解本研究项目的相关信息并同意参与本研究。*

Agree

Disagree

Demographic 个人资料

Name 姓名 *

Your answer

Gender 性别 *

- ◯ Male
- ◯ Female

Age 年龄 *

Your answer

Do you tested positive in Covid-19 in near one month? 你是否在近一个月内确诊为新冠肺炎阳性患者?

- ◯ Yes
- ◯ No

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.

(1) Always: >6 times per week
(2) Often: 5-6 times per week
(3) Sometimes: 3-4 times per week
(4) Seldom: 1-2 times per week
(5) Never: 0 time per week

请回忆一下在近一个月内发生的下述特定情况的频率。
(1) 总是：>每周 6 次
(2) 经常：每周5-6次
(3) 有时：每周3-4次
(4)很少：每周1-2次
(5) 从不：每周0次

1. Did your hands or feet feel cold or clammy? *
你是否感觉到你的手脚冰冷?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you feel cold easily in your abdomen, back, lower back or knees? *
你是否能在你的腹部，背后，腰部以及膝盖感觉到冷?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Were you sensitive to cold and tend to wear more clothes than others? *
你是否容易感觉到冷和比其他人要穿更多衣服?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you feel more vulnerable to the cold than others? (winter coldness, air conditioners, fans, etc) *
你觉得你比其他人更容易感冒吗? （冬季寒冷，空调，风扇等）

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you catch colds more easily than others? *
你是否比别人更容易感冒?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did you feel uncomfortable when you drank or ate something cold, or did you avoided to drinking or eating something cold? *
当你喝或吃冷的东西时你是否感到不舒服，或者你是否避免喝或吃冷的东西?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did you easily contract diarrhea when you were exposed to cold or eat (or drink) something cold? *
当你受凉或吃（或喝）冷的东西时，你是否容易腹泻?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did your palm and soles feel hot? *
你的手掌和脚底有没有感觉很热?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did your body and face feel hot? *
你的身体和脸感到热吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did your skin or lips feel dry? *
你感觉你的皮肤或嘴唇干燥吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Were your lips redder than others? *
你的嘴唇比别人红吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you get constipated easily or have dry stool? *
你是否容易便秘或大便干燥?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did your cheeks have a flushing or reddish appearance? *
你的脸颊有潮红或微红的现象吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did your eyes feel dry? *
你觉得眼睛干涩吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

8. Did you often feel thirsty and need to drink water? *
你是否经常感到口渴，需要喝水?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did you get tired easily? *
你容易疲累吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you suffer from shortness of breath? *
你有呼吸急促的问题吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you get palpitations? *
你有心悸吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you get dizziness easily or become giddy when standing up? *
你会容易晕眩或站立时感到晕眩吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you prefer quietness and do not like to talk? *
你喜欢安静和不喜欢说话吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Do you feel weak when talking? (weak and soft sound) *
当你说话时有感觉虚弱吗? （微弱而柔和的声音）

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did you sweat easily when you had a slightly increased physical activity? *
当你的体力活动略有增加时，你是否容易出汗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did you feel chest or stomach stuffiness? *
你有没有感到胸闷或胃闷?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did your body feel heavy or lethargic? *
你的身体是否感到沉重或昏昏欲睡?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Was your stomach/belly flabby? *
你的胃/腹部松弛吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you have an excessively oily forehead and/or T-zone? *
您的额头和/或者 T 区是否过度油腻?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you have upper eyelid swelling? *
你有上眼睑肿胀吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did your mouth feel sticky? *
你有没有觉得嘴巴很粘?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did your tongue have a thick coating? *
你的舌头有厚厚的涂层吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

8. Did you have lots of phlegm, especially in your throat? *
你有很多痰，特别是在你的喉咙里吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did your nose or your face feel greasy, oily or shiny? *
你的鼻子或脸部是否感觉油腻、油腻或有光泽?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you get acne or sores easily? *
你容易长痘痘或疮吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you have bitterness or a strange taste in your mouth? *
你的嘴里有苦味或奇怪的味道吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you have sticky feces with feeling of incomplete defecation? *
你有没有排便不全的粘稠粪便?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did your urethral canal feel hot when you urinated, or did your urine have a darker color? *
小解时是否感觉尿道发烫，或者尿液颜色较深?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. (Only Female) Was your vaginal discharge yellowish?
（仅限女性）你的白带是否呈黄色?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. (Only Male) Was your scrotum always wet?
（仅限男性）你的阴囊总是湿的吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did your skin getting bruise (hemorrhage under the skin) unknowingly? *
你的皮肤是否在不知不觉中出现瘀伤（皮下出血）？

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you have visible capillary/thread veins on your cheeks? *
你的脸颊上有明显的毛细血管/线状静脉吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you feel pain somewhere in your body? *
你有没有感觉到身体某处疼痛?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you have a dark face or get brown spots easily? *
你有没有脸黑或容易长褐色斑点?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you get dark circles under the eyes easily? *
你容易在眼睛下面长黑眼圈吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did you forget things easily? *
你容易忘记事情吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did your lips darker, more blue or purple than usual? *
你的嘴唇比平时更深、更蓝还是更紫?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did you sneeze even when you did not have a cold? *
即使你没有感冒，你也会打喷嚏吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you have a runny or stuffy nose even when you did not have a cold? *
即使你没有感冒，你也有流鼻涕或鼻塞吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you cough due to seasonal change, temperature change or unpleasant odor? *
你是否因季节变化、温度变化或难闻的气味而咳嗽?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Did you have allergies?(E.g. Medicine, food, odors, pollen, pet dander, ordoring seasonal or weather change etc.)? *
你是否过敏？（例如药物、食物、气味、花粉、宠物皮屑、季节性或天气变化等）？

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Are you getting urticaria/rubella easily? *
你容易得荨麻疹/风疹吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did your skin have purpura (purple spot, ecchymosis) due to allergies? *
你的皮肤是否因过敏而出现紫癜（紫斑、瘀斑）？

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did your skin turn red and show traces when you scratched it? *
你的皮肤有没有在你抓挠的时候变红并出现痕迹?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Did you feel gloomy and depressed? *
你有没有感到沮丧和消沉?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Did you feel anxiety and nervous easily? *
你容易感到焦虑和紧张吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you feel sensitive, vulnerable or emotionally upset? *
你是否感到敏感、脆弱或情绪不安?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

4. Were you easily scared or frightened? *
你容易害怕或受惊吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

5. Did you experience distention in the underarm or breast? *
你是否经历过腋下或乳房膨胀?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

6. Did you sigh for no reason? *
你有没有无缘无故的叹息?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

7. Did your throat feel strange (e.g. like something was stuck or there was a lump in your throat)? *
你的喉咙有没有感觉奇怪（例如，好像有什么东西卡住了或喉咙里有肿块）?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

1. Were you energetic? *
你是个有活力的人吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

2. Could you adapt yourself to external natural or social environment change? *
你能适应外部自然或社会环境的变化吗?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

3. Did you suffer from insomnia? *
你是否有失眠的问题?

	1	2	3	4	5	
Always	○	○	○	○	○	Never

APPENDIX I

Re: U/SERC/221/2022

3 November 2022

Dr Teh Lai Kuan
Head, Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti, Bandar Baru Barat
31900 Kampar, Perak.

Dear Dr Teh,

Ethical Approval For Research Project/Protocol

We refer to the application for ethical approval for your students' research projects from Bachelor of Science (Honours) Biomedical Science programme enrolled in course UDDD3108. We are pleased to inform you that the application has been approved under Expedited Review.

The details of the research projects are as follows:

No	Research Title	Student's Name	Supervisor's Name	Approval Validity
1.	Association of Traditional Chinese Medicine (TCM) Body Constitution with Metabolic Syndromes	1. Tan Yoke Hong 2. Lim Joe Sim 3. Ong Chen Mei 4. See Ying Mei	Dr Teh Lai Kuan	3 November 2022 – 2 November 2023

The conduct of this research is subject to the following:

(1) The participants' informed consent be obtained prior to the commencement of the research;

(2) Confidentiality of participants' personal data must be maintained; and

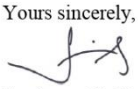
(3) Compliance with procedures set out in related policies of UTAR such as the UTAR Research Ethics and Code of Conduct, Code of Practice for Research Involving Humans and other related policies/guidelines.

(4) Written consent be obtained from the institution(s)/company(ies) in which the physical or/and online survey will be carried out, prior to the commencement of the research.

Should the students collect personal data of participants in their studies, please have the participants sign the attached Personal Data Protection Statement for records.

Thank you.

Yours sincerely,



Professor Ts Dr Faidz bin Abd Rahman
Chairman
UTAR Scientific and Ethical Review Committee

c.c Dean, Faculty of Science
Director, Institute of Postgraduate Studies and Research

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my