ASSOCIATION OF TRADITIONAL CHINESE MEDCINE (TCM) BODY CONSTITUTION, FASTING BLOOD GLUCOSE AND WAIST CIRCUMFERENCE WITH *CAPN10* (rs2975760) AND *HNF1A* (rs735396)

TAN SHU ROU

BACHELOR OF SCIENCE (HONS)

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By

TAN SHU ROU

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ABSTRACT

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Tan Shu Rou

Metabolic syndrome (MetS) is a condition characterized by elevated blood glucose levels and increased waist circumferences, leading to type 2 diabetes mellitus (T2DM) and abdominal obesity. Traditional Chinese Medicine (TCM) categorizes individuals into different body constitutions, offering insights into disease prevention and diagnosis. Genetic variations such as single nucleotide polymorphisms (SNPs) in Calpain-10 (*CAPN10*) and Hepatocyte Nuclear Factor 1 Alpha (*HNF1A*) genes have been implicated in T2DM pathogenesis. This study contributes to a deeper understanding of the complex interplay between genetic variants, metabolic health and TCM body constitutions. A total of 80 eligible subjects were recruited and demographic factors (gender, age and ethnicity) were considered. Interview-based TCM body constitution assessment was conducted, venous blood samples were collected for DNA analysis (genotyping) and fasting blood glucose (FBG) levels and waist circumference were measured. IBM SPSS Statistical 26.0 was used for data analysis. This study found 1.25% and 24.20% prevalence of diabetes and abdominal obesity, respectively. Minor allelic frequency (MAF) for rs2975760 and rs735396 were

greater than 0.05, indicating their significance in the study population. A weak positive correlation was found between FBG levels and waist circumference, reflecting the impact of abdominal obesity on T2DM. Gender-based differences in FBG levels and waist circumferences were observed, possibly due to hormonal influences. FBG levels and waist circumferences differed significantly across five age groups, while no significant differences in FBG levels among ethnicities. There was no significant association between TCM body constitutions and genetic variations, suggesting the challenge of assessing dynamic nature of body constitutions. Rs2975760 (*CAPN10*) showed no significant differences in relation to FBG levels and waist circumference. The mutant CC allele of rs735396 (*HNF1A*) was associated with significantly lower FBG levels, but not for waist circumference. Further research involving larger and more diverse samples is recommended to fully understand the interactions between these variables.

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My project could not have been accomplished without the guidance and support from my senior, who consistently provided assistance throughout this study.

Lastly, thanks to my beloved family members for their unlimited love, support and understanding during this period.

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 it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

TAN SHU ROU

APPROVAL SHEET

This project report entitled "**ASSOCIATION OF TRADITIONAL CHINESE MEDICINE (TCM) BODY CONSTITUTION, FASTING BLOOD GLUCOSE AND WAIST CIRCUMFERENCE WITH *CAPN10* (rs2975760) AND *HNF1A* (rs735396)**" was prepared by TAN SHU ROU 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: 26 April 2024

Supervisor

Department of Allied Health Sciences

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: 26 April 2024

PERMISSION SHEET

It is hereby certified that **TAN SHU ROU** (ID No: **20ADB02256**) has completed this final year project report entitled "**ASSOCIATION OF TRADITIONAL CHINESE MEDICINE (TCM) BODY CONSTITUTION, FASTING BLOOD GLUCOSE AND WAIST CIRCUMFERENCE WITH *CAPN10* (rs2975760) AND *HNF1A* (rs735396)**" under supervision of Dr. Teh Lai Kuan from the Department of Allied Health Science, Faculty of Science.

I hereby give the 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,

(TAN SHU ROU)

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LIST OF ABBREVATIONS

ARMS	Amplification refractory mutation system
BC	Body constitution
BMI	Body mass index
bp	Base pair
CAPN10	Calpain-10
CI	Confident interval
ddH_2O	Autoclaved distilled water
EDTA	Ethylenediaminetetraacetic acid
ER	Estrogen receptor
FBG	Fasting blood glucose
F_o	Outer forward primer
F_I	Inner forward primer
GLUT	Glucose transporter
HNF1A	Hepatic Nuclear Factor 1-Alpha
IGR	Impaired glucose regulation
MAF	Minor allelic frequency
MAP	Mitogen activated protein kinase
MetS	Metabolic syndrome
MODY	Maturity-onset diabetes of the young
NCBI	National Center for Biotechnology Information
NTC	Non template control
NLM	National Library of Medicine
PCR	Polymerase chain reaction

PI3K	Phosphatidylinositol 3-kinase
R_o	Outer reverse primer
R_I	Inner reverse primer
rpm	Revolutions per minute
SNP	Single nucleotide polymorphism
TAE	Tris acetate EDTA
TCM	Traditional Chinese Medicine
T2DM	Type 2 diabetes mellitus
x g	Relative centrifugal force

CHAPTER 1

INTRODUCTION

1.1 Research Background

Metabolic syndrome (MetS) is characterized by a cluster of interconnected risk factors that increase the likelihood of developing type 2 diabetes mellitus, abdominal obesity and cardiovascular disease. These factors include elevation of fasting blood glucose levels, increased waist circumferences, hypertension, and dyslipidemia (Wan, et al., 2023). In year 2021, the prevalence of metabolic syndrome among Malaysian adults ranges from 25 to 40% was found predominantly for those elderly individuals (Manaf, et al., 2021).

Traditional Chinese Medicine's concept in dividing people into different body constitution had brought an insight to prevent metabolic syndrome. This concept plays an important role in clinical diagnosis and treatment based on body constitution in reflecting their susceptibility to certain diseases. To proactively ward off illnesses and maintain a healthy regimen, it becomes vitally important for the public to maintain a neutral body constitution. TCM posits that every individual possesses distinct structural, physiological, and psychological characteristics, with men leaning more towards reliance on Qi (vital energy) and women more towards on blood (Vorian, 2016).

TCM highlights the importance to maintain and restore the Yin-Yang equilibrium within an individual's body, thereby preventing the development of diseases. Imbalances in Yin and Yang often signify the occurrence of illness in

an individual. The establishment of TCM body constitution is focusing on the five viscera and five fundamental substances. Dysfunction in the viscera may lead to disruption in the regulation of fundamental substances in giving rise to imbalanced body constitution (Yap, et al., 2022).

TCM classifies body constitutions into nine types through determination of both hereditary and acquired factors throughout an individual's life (Yap, et al., 2022). These constitutions include neutral, qi-deficiency, Yang-deficiency, Yin-deficiency, blood-stasis, phlegm-dampness, damp-heat, qi-depression, and special-constitution (Vorian, 2016). A neutral constitution is considered balanced and indicative of overall health, while the others represent unbalanced constitutions that render an individual more susceptible to certain diseases (You, et al, 2017). TCM body constitution has been identified as a potential indicator for the risk of developing diabetes mellitus, a condition characterized by elevation of blood glucose level. You, et al. (2017) revealed that individual with phlegm-dampness, damp-heat, and qi-deficiency constitutions were commonly found among subjects with impaired glucose regulation (IGR) and are at higher risk in developing diabetes. Most of the earlier studies was conducted at China. Hence, this had brought us an interest to study the body constitutions among our population.

According to the World Health Organization (2023), diabetes is a common health issue globally and it affects approximately 422 million people worldwide. The prevalence of diabetes is constantly increasing over the past few decades, especially T2DM. Although the onset of T2DM should typically occur in

adulthood, but there has been a significant increase among younger individuals, due to the rising rates of obesity. In type 2 diabetic patient, the insulin production is continued, but the body's cells become irresponsive to it, which subsequently leading to a condition known as insulin resistance. Over the time, it will cause insulin deficiency when the demand for insulin surpasses the pancreas's production capacity (Diabetes Research Connection, 2023). According to Ministry of Health Malaysia (2020), the reported prevalence of abdominal obesity stands at 52.6%. This condition is closely linked to T2DM due to the excessive accumulation of adipose tissue at the abdominal region. It can induce chronic inflammation in body cells, reducing their sensitivity to insulin and thereby increasing the risk of diabetes (Ko, et al., 2006)

T2DM is a multifactorial disease which can be caused by lifestyle factor and genetic factors. Shoily, et al. (2021) highlighted the impact of genetic variations, such as single nucleotide polymorphisms (SNPs) on gene expression in contributing to the development of high blood glucose level. Variations in the Calpain-10 (*CAPN10*) and Hepatic Nuclear Factor 1 Alpha (*HNF1A*) genes have been associated with an increased risk of abdominal obesity and T2DM in affecting pancreatic β-cells development and insulin gene expression (Evans, et al., 2001; Tabatabaiefar, et al., 2019). The comprehensive integration of TCM concepts with biomedical understanding remains an area that requires further exploration. Therefore, this study aims to investigate the association of TCM body constitutions in relation to metabolic syndrome with polymorphisms in *CAPN10* and *HNF1A* genes.

1.2 Significance of Study

This study provides a better insight into personalized health strategies by using TCM body constitutions to understand the uniqueness of an individual. Identification of TCM body constitution would serve as a potential indicator for diabetes risk which in turn offering early risk assessment. It may help in targeted prevention and intervention strategies. This research explores the genetic basis of diabetes and abdominal obesity, providing valuable insights into the complex interaction between genetic factors, metabolic health and TCM body constitutions.

1.3 Objectives of Study

The general objective in this study is:

To investigate the association of TCM body constitution in relation to fasting blood glucose level, waist circumference and its regulating genes, *CAPN10* (rs2975760) and *HNF1A* (rs735396).

The specific objectives in this study are:

- To determine the prevalence of diabetes and abdominal obesity among the study population.
- To explore the relationship between fasting blood glucose level and waist circumference.
- To compare the fasting blood glucose level and waist circumference with demographic factors (gender, age and ethnicity).
- To evaluate the specific TCM body constitution (phlegm dampness, damp heat and qi deficiency) in relation to fasting blood glucose level.

- To study the association between TCM body constitution with rs2975760 and rs735396.
- To compare the fasting blood glucose level and waist circumference with genetic variations (rs2975760 and rs725296).

CHAPTER 2

LITERATURE REVIEW

2.1 Metabolic Syndrome

Metabolic syndrome (MetS) is established when an individual exhibits three or more of the following conditions: abdominal obesity which is excessive abdominal fat accumulation; elevated fasting blood glucose levels that contribute to vascular damage and clot formation; persistent high blood pressure which may damage the cardiovascular system; heightened blood triglycerides levels that increase the risk of heart disease; and low high-density lipoprotein cholesterol. Collectively, these factors pose a significant health risk to the public. The concept of metabolic syndrome allows clinical assessment to be done on the identification of individuals at an increased risk of T2DM or cardiovascular disease (Huang, 2009; National Heart, Lung, and Blood Institute, 2022).

2.1.1 Epidemiology of Metabolic Syndrome

According to Lim and Cheah (2016), the prevalence of MetS in Malaysia can be assessed based on the risk factors, including diabetes, obesity, hypertension and hypercholesterolemia. The prevalence of MetS in elderly was found greater than 40% and females were generally exhibited a higher prevalence than males. Indians had the highest rates among ethnic groups while urban areas demonstrated higher prevalence of MetS compared to rural areas. They further suggested that the prevalence of MetS was lower among healthy individual but higher among diabetic and obese patients.

2.2 Diabetes mellitus

Diabetes mellitus is part of the MetS and its name was from the Greek word "diabetes", defining pass through, while the Latin word "mellitus," which signifies sweet. The term was first coined by Apollonius of Memphis, who observed the sweet nature of urine in individuals. Diabetes mellitus is a metabolic disorder characterized by inappropriately elevated blood glucose levels in bloodstream and urine. It is categorized into various types of diabetes, including type 1 diabetes mellitus, type 2 diabetes mellitus, maturity-onset diabetes of the young (MODY), gestational diabetes and so forth (Sapra and Bhandari, 2022).

2.2.1 Prevalence of Diabetes Mellitus

Diabetes remains as a prevalent chronic disease globally. The earlier reported data highlighted that diabetes mellitus affects 1 in 11 adults, with 90% of cases being T2DM. The onset of T2DM typically occurs later in life, but there is a growing prevalence in younger populations due to increased obesity rates among adolescents. The International Diabetes Federation estimates a global increase in diabetes mellitus with 415 to 642 million cases by 2040 (Sapra and Bhandari, 2022).

According to Akhtar, et al. (2022), Malaysia has the highest diabetes rate in the Western Pacific region and is among the highest globally, incurring an annual cost of approximately 600 million US dollars. The prevalence of diabetes surged from 11.2% in 2011 to 18.3% in 2019, with 3.6 million Malaysian adults aged 18 and above having diabetes mellitus. By 2025, it is predicted that 7 million

Malaysian adults aged 18 and older will be diagnosed with diabetes, with a prevalence of 31.3%. This escalating trend is attributed by the factors such as population growth, aging, urbanization, and the increasing rate of obesity can be resulted by physical inactivity and unhealthy dietary habits.

2.2.2 Pathogenesis of Diabetes Mellitus

The islets of Langerhans in pancreas consist of insulin-producing beta cells and glucagon-secreting alpha cells. These cells dynamically adjust their hormone secretions in response to the glucose environment, striving to maintain a delicate balance. Disruption of this balance due to the absence of insulin or impaired insulin action can lead to high blood glucose levels (Sapra and Bhandari, 2022). Insulin is a hormone in response to elevated blood glucose levels and plays a crucial role in regulating glucose utilization across various tissues. In skeletal muscle and adipose tissue, insulin facilitates glucose uptake by translocating glucose transporter to the cell surface. Simultaneously in the liver, insulin promotes glycogen synthesis while inhibiting glycogenolysis and gluconeogenesis, effectively curbing those excess glucose to be released into the bloodstream. Insulin has antilipolytic effect by preventing fats breakdown to increase glucose uptake and thus reducing the circulating glucose in bloodstream and facilitating the storage of glucose in the form of glycogen (Huang, 2009). Insulin resistance occurs when cells exhibit a reduced response to insulin which leads to sustained high blood glucose levels. T2DM develops when there is inadequate insulin production to compensate for the resistance. The factors such as genetics, adiposity, and fitness can contribute to its complexity. The disruption of signaling pathways involved in insulin function, such as phosphoinositide 3-

kinase (PI3K) and mitogen-activated protein (MAP) kinase can affect glucose uptake and contribute to vascular abnormalities, potentially leading to conditions like atherosclerosis (Huang, 2009; Sapra and Bhandari, 2022).

Type 1 diabetes mellitus is characterized by autoimmune destruction of beta cells in the pancreas, leading to the absence or extremely low levels of insulin. Conversely, T2DM has a more gradual onset by involving an imbalance between insulin levels and insulin sensitivity. A common factor in T2DM is insulin resistance which often develops due to obesity and aging. Genetic factor plays an important role for both types of diabetes. However, T2DM exhibits a more intricate interplay between genetics and lifestyle. Various genetic loci have been identified with impacts on pathways related to pancreatic development, insulin synthesis, and regulation of insulin resistance (Sapra and Bhandari, 2022).

Fasting blood glucose (FBG) test is a commonly used method for diabetes screening. A fasting blood sample is obtained through finger pricking after fasting for at least 8 hours and measured using glucometer. A FBG level below 5.6 mmol/L is considered as normal, while the range between 5.6 to 6.9 mmol/L is categorized as prediabetes. If the measurement is 7 mmol/L or higher, it indicates the presence of T2DM (Mayo Clinic, 2020).

2.3 Abdominal Obesity

Abdominal obesity is characterized by an accumulation of excessive adipose tissues around the abdominal region and posing a significant risk for diseases development. It is closely associated with metabolic syndrome, particularly in

increasing fasting blood glucose level. According to Papaetis, et al. (2015), presence of excessive adipose tissues triggers the production of adipocyte cytokines, thereby fostering insulin resistance. Lipid metabolites deposited in the abdominal region can disrupt the insulin signalling pathway, contributing to the onset of diabetes (Papaetis, et al., 2015). Abdominal obesity does not necessarily occur in those obese individuals with high body mass index (BMI). It can be found in individuals with normal BMI but metabolically obese due to the high deposition of visceral fat at the abdominal region (Grover and Misra, 2023). The etiology of abdominal obesity is complex and multifactorial, involving genetic predispositions, lifestyle behaviours and environmental influences. It could be varied across demographic factors including gender, age and ethnicity (Olinto, et al., 2017).

2.4 Calpain-10 (*CAPN10*) and T2DM

There are a couple of studies that found relationships between *CAPN10* variants and T2DM due to changes in insulin action and secretion (Turner, et al., 2005). The calpain-10 gene (*CAPN10*) is located on chromosome 2q37.3 and it encodes for calpain-10 protein. This protein is the calcium-dependent intracellular cysteine protease to limit proteolysis and involves cell motility and signal transduction pathways. One of the polymorphisms in *CAPN10* is SNP-44 (rs2975760) which is localized in the intronic region to alter gene expression or the alternative splicing mechanism and play a role in the pathogenesis of T2DM. The calpain-10 protein is found to have involvement in pancreatic beta cell function. In a normal condition, calpain-10 plays a role in the exocytosis pathway to increase insulin secretion in a calcium-dependent manner. It acts as

a sensor to induce the calcium currents for secreting insulin during the detection of glucose. Calpain-10 protein is crucial for insulin-stimulated glucose uptake by aiding the externalization of glucose transporters (GLUTs) induced by insulin. This is essential to facilitate the glucose uptake into the body cells for further glucose metabolism and the mechanism is as shown in **Figure 2.1**. However, variants or defects in *CAPN10* can reduce glucose uptake and affect the translocation of GLUT4 to the plasma membrane of adipocytes and muscle cells through the reorganization of the actin filament. With that being said, this variant may potentially use as a biomarker for T2DM (Khan, et al., 2014; Pánico, et al., 2014).

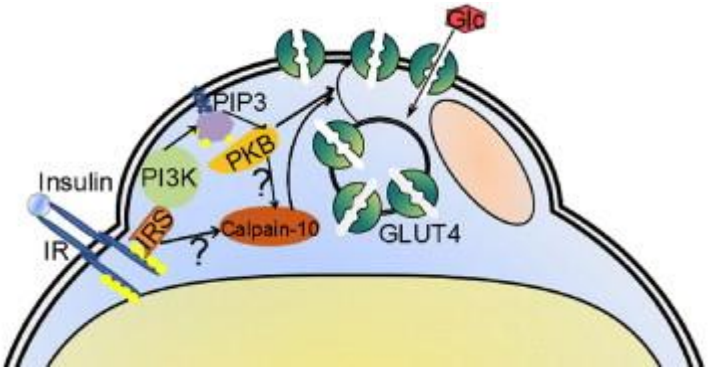

Figure 2.1: The mechanism of calpain-10 to facilitate glucose uptake (Pánico, et al., 2014).

2.5 Hepatocyte Nuclear Factor-1 Alpha (*HNF1A*) and T2DM

The *HNF1A* gene is located at chromosome 12q24.3 and encodes for hepatocyte nuclear factor-1 alpha protein (HNF-1α). HNF-1α possess dimerization domain to interact with another protein to form a dimer that acts as a transcription factor. It can regulate the activity of genes to produce pancreatic beta cells which play roles in insulin secretion. The mutation in *HNF1A* may alter HNF-1α protein

production and affects its function to form dimers. The changes in the HNF-1α protein prevent it from interacting with DNA by blocking its entry into the cell nucleus or preventing its binding to DNA for regulating gene activity. This causes an impairment of the beta cell's function, which reduces its ability to produce insulin in response to glucose in the bloodstream. The *HNF1A* mutation will also reduce insulin production by altering the metabolism of glucose in beta cells by reducing glucose transporter 2 and glucose uptake. Hence, it would decrease glycolysis in beta cells (MedlinePlus, 2020; Miyachi, et al., 2022). HNF-1α is also a tumor suppressor in controlling the genes that regulate cell growth and survival. It can prevent tumor formation by stopping uncontrollable cell growth and division (MedlinePlus, 2020). Knockdown of this gene will increase proliferation and decrease apoptosis, which leads to abnormal cell growth.

Single nucleotide polymorphism in *HNF1A* such as rs725296 have been found to be associated with the occurrence of T2DM by affecting glucose metabolism (Miyachi, et al., 2022). According to Dallali, et al. (2022), diabetes has been associated with abdominal obesity and a high waist circumference is significantly linked to the rs735396 variant in *HNF1A.* The previous study found that individuals carrying two copies of the rs735396 minor allele had higher waist circumference compared to those with heterozygous genotypes. These findings suggest that rs735396 may indicate a genetic susceptibility to obesity (Dallali, et al., 2022).

2.6 Traditional Chinese Medicine Body Constitutions (TCMBC)

TCMBC can be varied from person to person. It is classified into nine types of body constitutions which are neutral, qi-deficiency, Yang-deficiency, Yin-deficiency, blood-stasis, phlegm-dampness, damp-heat, qi-depression, and special-constitution (Vorian, 2016). Neutral type is balanced body constitution, indicating a healthy state, while the rest are imbalanced body constitutions which expose an individual to certain diseases (You, et al., 2017). Individuals with a neutral body constitution typically exhibit a stronger physique, stable emotional well-being, and feel optimistic. They have glossy skin and hair, bright eyes, proper senses of smell and taste, red and moist lips, less frequent fatigue, regular eating and sleeping patterns, healthy bowel and urinary habits (Vorian, 2016).

Individuals with qi-deficiency body constitution often have flabby muscles and tend to be introverted. They normally exhibit a feeble voice, shortness of breath and tiredness. They are more susceptibility to colds and flu, sweating, and may have teeth marks along the edge of the tongue, indicating a weaker immune system and longer recovery times from illnesses. Individuals with Yang-deficiency always complaint of icy hands and feet, a sense of coldness in the stomach and are sensitive to low temperatures and noises. They feel discomfort after eating cold foods, tiredness, and may have a pale and bulky tongue. They are not feeling well in windy, cold, and humid conditions and are susceptible to puffiness, diarrhea, and too much throat secretion. Individuals with Yin-deficiency tend to be extroverted and have slim appearance. In contrast to Yang-deficiency, they have warm palms, dry mouth, dry nose, constipation, and enjoy cold drinks. They feel uncomfortable in hot and dry environments and are prone

to coughing, having seminal emissions, and insomnia (Qi, et al., 2021; Vorian, 2016). Individuals with phlegm-dampness body constitution are typically overweight. They tend to have an oily face, a sticky or sweet taste in the mouth, and excessive secretion of fluid in the throat. Sweating, chest congestion, a preference for sweet and greasy foods and a thick tongue coating are common characteristics (Vorian, 2016).

Individuals with damp-heat body constitution may have either normal or slim bodies size. They typically exhibit oily face, acne breakouts, a bitter taste in the mouth, fatigue, an incomplete feeling after urination or dry feces, yellow urine and a yellow, greasy coating on the tongue. They often feel uncomfortable in hot and humid conditions and are prone to skin and urinary problems (Vorian, 2016; Yuan, et al., 2021). Individuals with blood-stasis often experience forgetfulness and impatience. Common characteristics of these individuals include dull complexion, facial patches, dark-red lips, dark circles, rough skin, unknown bruise and varicose veins. They are uncomfortable in cold conditions and are prone to bleeding, painful illness, and abnormal growths (Vorian, 2016). Individuals with qi-depression are typically have a thin physique and are emotionally unstable. Depression, anxiety, frequent sighing, and heart palpitations are commonly seen in this group of people. They may struggle to adapt to stressful situations and are prone to insomnia, depression, anxiety disorders, and breast lumps (Liu, et al., 2017; Vorian, 2016). Individuals with inherited special body constitution usually have an inborn weakness and are sensitive to drugs, foods, smells, and allergens. Nasal congestion, sneezing, running nose, wheezing, itchiness, and the presence of purple spots under the

skin are common indicators. They are prone to drug allergies, hay fever, and asthma. Combination of body constitutions are common such as yin deficiency and damp heat, qi deficiency and dampness, qi stagnation and blood stasis which may increase the difficulty in diagnosis (Vorian, 2016).

2.7 Association between TCMBC and T2DM

TCMBC assessment may aid in identifying the predisposition to impaired glucose regulation, indicating higher risk of T2DM. Phlegm-dampness, damp-heat, and qi-deficiency were found to be the three most common unbalanced body constitutions among those with impaired glucose regulation, showing a significantly elevated risk of diabetes (You, et al., 2017). Symptom observed in diabetic patient like inadequate energy supply from glucose is commonly seen in individuals with qi-deficiency. Qi-deficiency in the spleen and stomach is identified as a significant pathogenesis of obesity, further escalating the risk of diabetes. Phlegm-damp body constitution is linked to various lifestyle diseases, including metabolic syndromes and diabetes. Unhealthy lifestyle behaviors, such as the consumption of fatty foods, sugary foods and engaging in less physical activity are associated with this constitution (Niu and Ren, 2023; You, et al., 2017). In TCM, spleen plays a crucial role in the transportation and transformation of nutrition by converting food and water. A damp-heat body constitution can result from impaired spleen function and abnormal water metabolism. Symptom of edema, which is a common indicator of diabetes, are frequently found in those with damp-heat body constitution due to the accumulation of sodium in intracellular fluid (You, et al., 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals, Reagents, and Instruments

The chemical, reagents, and instruments used throughout this project has been 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 Qiagen, The Netherlands
DNA Loading Dye	1st BASE, Singapore
EDTA	Orioner Hightech Sdn Bhd, Malaysia
Gel-Red Stain	Nippon Genetics Europe GmbH, Germany
Master Mix	GeneDireX, United States
Primers	Integrated DNA technologies, United States
PrimeWay Gel Extraction/PCR Purification Kit	1st BASE, Singapore
Tris Base Powder	Thermo Fisher Scientific, United States
Tris-EDTA (TE) Buffer	1st BASE, Singapore

Table 3.2: Instruments used in this study.

Instruments / consumables	Manufacturer
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 Imager Viewer	Biorad, United States
Gel Tank	Major Science, United States
Glucometer	Controur Plus, Germany
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	Biometra, Germany
Pipette P10	DLAB Scientific Co., Ltd., United Kingdom
Pipette P200	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
UV Transilluminator	GeneDireX, United States
Vortex	AITbiotech, Singapore
Water Bath Incubator	Memmert, Schwabach

3.1.1 Tris Acetate EDTA (TAE) Buffer Preparation

The 50 X TAE buffer was prepared by dissolving 242 g of Tris Base powder in 600 mL of distilled water, stirring the mixture with a magnetic stirrer until complete dissolution. Subsequently, 100 mL of 0.5 M EDTA and 57.1 mL of acetic acid were added to the solution. Finally, distilled water was added to reach a final volume of 1000 mL.

3.2 Experimental Design

The experimental flow chart for this study is presented in **Figure 3.1.**

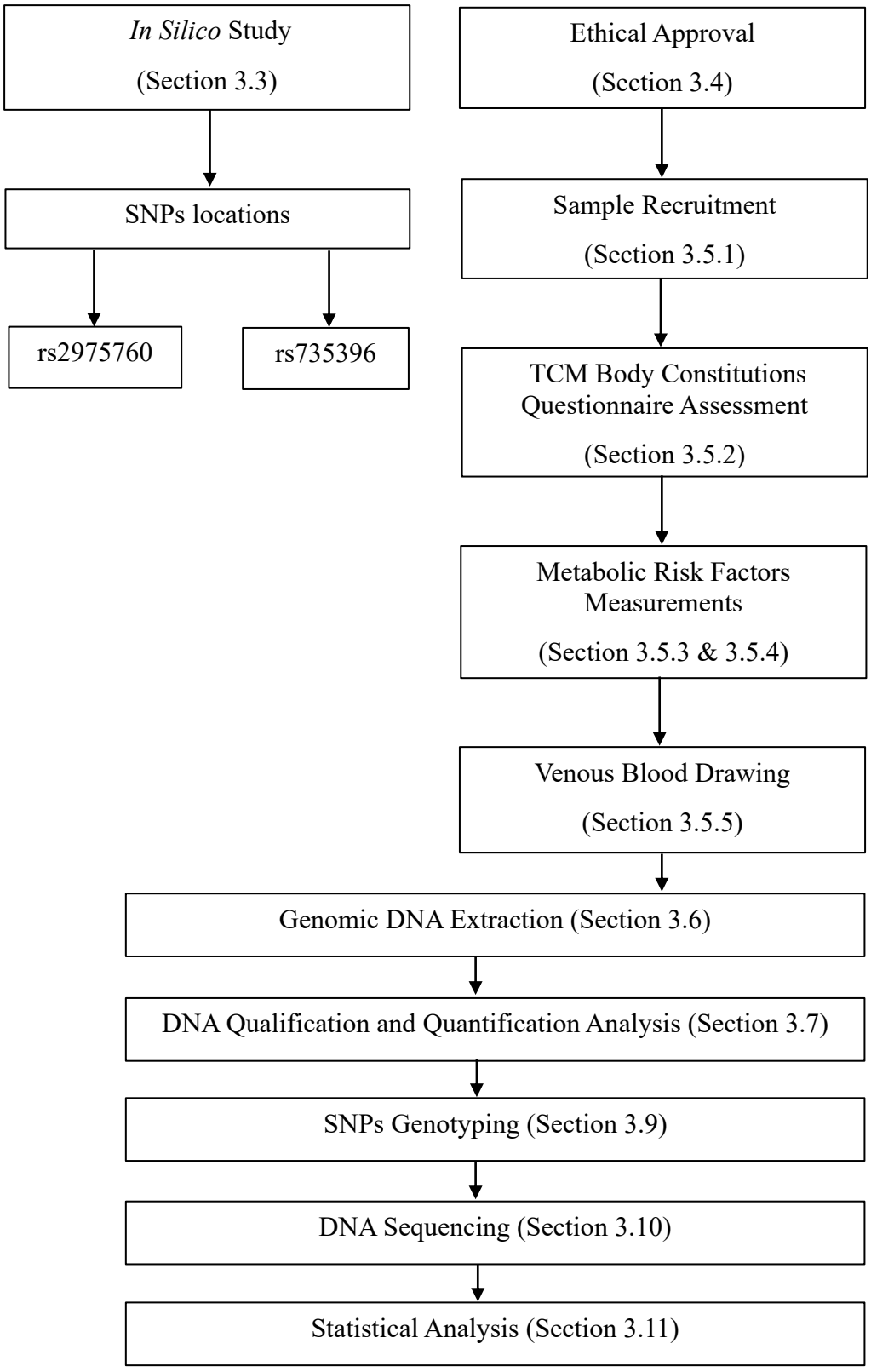

Figure 3.1: Experimental flow chart for this study.

3.3 In Silico Study

The information of SNPs was obtained from National Center for Biotechnology Information (NCBI) SNP database. For rs2975760 (*CAPN10*), the MAF of the C allele was 0.151. For rs735396 (*HNF1A*), the MAF of the T allele was 0.4887 in the Asian population. These SNPs are considered significantly important and can be studied as the MAF values exceed 0.05 (National Library of Medicine, 2022a; National Library of Medicine, 2022b)

3.4 Ethical Approval

Before commencing the research, ethical approval was obtained from UTAR Scientific and Ethical Review Committee (SERC) due to the involvement of human samples. The corresponding ethical approval is documented in **Appendix A.**

3.5 Sample Size Calculation

The sample size was determined based on the reported prevalence of Metabolic Syndrome in the Malaysian population, which ranges from 25 to 40% (Manaf, et al., 2021). Based on 90% confidence interval (CI), the calculated sample size was 60. The final sample size was increased to 72 by considering the 20% drop-off rate. The calculation was presented as below:

90% Confidence Interval:

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

$$n = \frac{1.65^2 \times 0.33 \times (1-0.33)}{0.1^2}$$

$$n = 60$$

Additional 20% drop off rate:

$$n = 60 \times 120\%$$

$$n = 72$$

n = sample size

Z = statistical level for 90% confidence interval

p = expected prevalence of MetS

d = 10% allowable error

3.5.1 Sample Recruitment

Convenient sampling was employed, and participants were voluntarily recruited after providing thorough informed consent. Sampling was done at several locations, including the UTAR Kampar campus and Tabib Cina Teh Hun Phing in Ipoh, aiming to collect 80 samples across diverse age ranges. The inclusion and exclusion criteria in participant selection were as follows:

Inclusion criteria:

- 18 years old and above
- Fasting for 8 to 12 hours
- Willing to be venipuncture and finger pricked

Exclusion criteria:

- Below 18 years old
- Pregnancy
- Not willing to be venipuncture and finger-pricked

3.5.2 TCM Body Constitutions Assessment

TCM Body Constitution Questionnaire was originally developed, and some modifications were implemented to simplify the research process. A notable adjustment was the changing of scoring pattern to ease the calculation process as shown in **Figure 3.2** and **Figure 3.3**. TCMBC questionnaire comprised demographic inquiries and approximately 60 TCMBC questions, tailored to the nine types of body constitutions. The assessment was conducted in person, and it was interview-based, aimed at minimizing the incidence of two contradictory body constitutions appearing in the results. Subjects were requested to recall the frequency of certain conditions experienced within the past month. Based on a 5-point Likert-type scale scoring system, individuals were categorized into their respective TCMBC types.

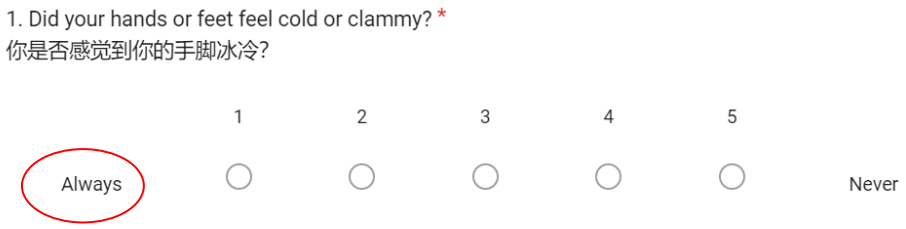

Figure 3.2: Scoring pattern before modification.

Figure 3.3: Scoring pattern after modification.

3.5.3 Fasting Blood Glucose Level Measurements

Fasting blood glucose was measured using a finger pricking method. An alcohol swab was used to clean the fingertip to avoid contamination. A lancet was used to prick the side of the fingertip, and the first drop of blood was wiped off. The second drop of blood was then squeezed and applied to the edge of the inserted test strip in the Contour Plus glucometer for measurement. The displayed reading was recording.

3.5.4 Anthropometric Measurements

According to Casadei and Kiel (2022), anthropometric measurements offer a non-invasive method of quantifying various parameters like waist circumference. The subjects were instructed to stand upright while a tape was positioned around the midpoint and above the hipbones. The tape was placed horizontally around the waist and was kept snug but not overly tight to avoid excessive compression of the skin (Center for Disease Control and Prevention, 2022).

3.5.5 Venous Blood Drawing

Venous blood samples were drawn through venipuncture conducted by supervisor. A total of 6 mL volume of venous blood was obtained and equally distributed into two ethylenediaminetetraacetate (EDTA) vacutainers to prevent coagulation. These blood samples were then stored in a 4 °C chiller to prevent degradation and reserved for further analysis.

3.6 Genomic DNA Extraction

Both Favorgen and Qiagen DNA extraction kits were used to extract the DNA, with different protocols applied. The blood samples were centrifuged at 3,000 rpm for 5 minutes at room temperature to separate them into three layers. The upper clear layer comprised the plasma. The intermediate layer consisted of the the buffy coat, which was concentrated with leukocyte, while the bottom layer contained packed red cells. A total of 200 µL buffy coat was isolated from the blood samples and transferred into a 1.5 mL microcentrifuge tube.

3.6.1 Favorgen DNA Extraction Mini Kit Protocol

A volume of 20 µL of Proteinase K and 200 µL of FABG buffer were added to the samples and thoroughly mixed using a vortex. The samples were then incubated in a water bath at 60 ºC for 15 minutes. Every 5 minutes, the tubes were briefly removed for vortexing to enhance cell lysis. After a brief spin down, 200 µL of 96% ethanol was added, pulse-vortexed for 10 seconds, and spun down to remove any residual drops inside the lid. The resulting mixtures were transferred to FABG mini column with collection tube and centrifuge at 6,000 x g for 1 minute. Following this step, 400 µL of W1 buffer was added and centrifuged at 17,000 x g for 30 seconds. The filtrate was removed, and 750 µL of wash buffer was added, centrifuged under the same settings, and the filtrate was discarded again. To remove excess fluid, the columns were centrifuged at 17,000 x g for 3 minutes. The collection tubes were replaced with new microcentrifuge tubes, and 50 µL elution buffer was added. The columns were incubated at room temperature for 30 minutes and then centrifuged at 17,000 x g for 1 minutes. An additional 50 µL of elution buffer was added, followed by

another 30 minutes incubation, and centrifugation at 17,000 x g for 1 minute (Favorgen, n.d.). Finally, the extraction DNA was prepared for further analysis.

3.6.2 Qiagen DNA Extraction Kit Protocol

A total of 20 µL Qiagen protease (or proteinase K) and 200 µL of AL buffer were added to the samples, followed by pulse-vortexing for 15 seconds to get a homogeneous solution. The sample was incubated at 56 ºC for 10 minutes, with brief interruptions every 5 minutes for vortexing to enhance cell lysis. After a brief spin down, 200 µL of 96% ethanol was added, pulse-vortexed for 15 seconds, and spun down to remove any residual drops inside the lid. The resulting mixtures were transferred to the column with collection tube and centrifuge at 17,000 x g for 1 minute. Following this step, 500 µL of AW1 buffer was added and centrifuged at 6000 x g for 1 minute. The filtrate was removed, and 500 µL of AW2 buffer was added, followed by centrifugation at full speed (17,000 x g) for 3 minutes. The filtrate was discarded again, and the columns were centrifuged at 17,000 x g for 1 minutes to remove excess fluid. The collection tubes were replaced with new microcentrifuge tubes, and 50 µL AE buffer was added. The columns were then incubated at room temperature for 30 minutes and centrifuged at 17,000 x g for 1 minutes. An additional 50 µL of elution buffer was added, followed by another 30 minutes incubation, and centrifugation at 17,000 x g for 1 minute. Finally, the extraction DNA was prepared for further analysis (Qiagen, 2023).

3.7 DNA Quantification and Qualification Analysis

3.7.1 Nanodrop

The concentration and the purity of the extracted DNA were assessed using a nanodrop. Initially, 1 µL of blanking solution (elution buffer) was pipetted and transferred onto the pedestal for calibration. Subsequently, the blanking solution was replaced with DNA samples, and their concentration and purity were recorded. DNA sample with concentration greater than 25 ng/µL were stored for further analysis. The A260/A280 ratio was calculated to discern the DNA purity from potential protein contamination, aiming for a favorable range between 1.80 to 2.00. Additionally, thecA260/A230 ratio was calculated to determine the purity of the sample from salts and other contaminants which can absorb at 230 nm (Bitesize Bio, 2022).

3.7.2 Genomic DNA Gel Electrophoresis

Genomic DNA Gel Electrophoresis was conducted to assess DNA integrity. For a small gel, 20 mL of 1 X TAE buffer was measured and mixed with 0.4 g of agarose powder in a conical flask. The mixture was heated in a microwave until the powder fully dissolved in the solution. 0.5 µL of GelRed was added into the mixture, which was then poured into a gel tray equipped with a gel comb and covered using aluminum foil. The gel was left at room temperature until it solidified. In contrast, a larger gel was prepared using 30 mL of 1 X TAE buffer and 0.6 g of agarose powder. Once solidified, the gel was placed into a gel tray and fully submerged in 1 X TAE buffer. Subsequently, a mixture of 4 µL loading dye and 2 µL diluted DNA (working DNA) was loaded into the wells. Afterwards, a power supply of 90 V was applied to the tank and allowed to run for 30 minutes.

Post-electrophoresis, the gel was examined using a gel imager to evaluate the quantity of DNA bands present. DNA samples which formed only one band and no fragment present would be stored for further analysis.

3.8 Working DNA Preparation

The working DNA was prepared by diluting the extracted stock DNA (M_1) using autoclaved distilled water, achieving a final concentration of 25 ng/µL (M_2) with a volume of 30 µL (V_2). The formula used to calculate the necessary volumes for the stock DNA (V_1) and the autoclaved distilled water needed was as follows:

- $M_1 V_1 = M_2 V_2$
- Autoclaved distilled water needed = 30 µL - V_1

M_1: Concentration of stock DNA M_2: Concentration of working DNA

V_1: Volume of stock DNA V_2: Volume of working DNA

3.9 SNPs Genotyping (rs2975760 and rs735396)

Tetra-primer amplification-refractory mutation system (AMRS) PCR was used to detect SNP by using sequence-specific PCR primers which only allow test DNA (*CAPN10* and *HNF1A*) to be amplified when the target allele for rs2975760 and rs735396 present in the sample (Medrano and De Oliveira, 2014). Two set of primers were used, including outer forward and reverse primers (non-allele-specific primers) and inner forward and reverse primers (allele-specific primers). The longer fragments produced by outer primers served as PCR products which act as internal controls, while the shorter fragments produced by the inner primers reflect the respective genotype. For example, inner forward

primers designed to complement the T allele can only amplify the DNA in the presence of the T allele. This amplification is initiated simultaneously with the outer reverse primer. Similarly, the inner reverse primer which designed complementary to the C allele can only amplify DNA in the presence of the C allele. The amplification was initiated concurrently with the outer forward primer.

3.9.1 Primer Sequence

Two sets of primers designed to target rs2975760 and rs735396 were presented in **Table 3.3** and **Table 3.4** respectively.

Table 3.3: Primer sequence, target, and amplicon size for rs2975760.

Primer sequence 5' → 3'	Amplification	Amplicon Size (bp)
Inner forward primer (T allele): GACTGCAGGGCGCTCACGCTTGCG GT	Wild type IF-OR	200
Inner reverse primer (C allele): TTAGCCTCACCTTCAAACGCCTTAC TGCG	Mutant OF - IR	274
Outer forward primer (5' – 3') AAGGCAACTGGACTGACAGGCAG GCAGG Outer reverse primer (5' – 3') TCACCATGGGAGTGAGCCTCTGGC ATTG	Internal Control OF - OR	419

*Designed primer by senior

Table 3.4: Primer sequence, target, and amplicon size for rs735396.

Primer sequence 5' → 3'	Amplification	Amplicon Size (bp)
Forward inner primer (T allele): GTGGGTGTGGGTGCCTGGTGGGTGTCT	Wild type IF-OR	193
Reverse inner primer (C allele): GGACACTGCAGAGGCAAACAAGGCTGATG	Mutant OF - IR	263
Forward outer primer (5' – 3') CTACCTCGGCATCTCACCGGGGCTTCTC	Internal Control	400
Reverse outer primer (5' – 3') CCCAGGTGCCGTGGTTACTGGGAGGAAG	OF - OR	

*Designed primer by senior

3.9.2 Primer Mix Preparation

Primer mixes for both SNPs were prepared based on the recipes shown in **Table 3.5** and **Table 3.6**, respectively.

Table 3.5: Primer mix preparation for rs2975760.

Primers	Initial Concentration (µM)	Final Concentration (µM)	Volume (µL)
F_o	100	4	2.0
R_o	100	4	2.0
F_I	100	4	2.0
R_I	100	4	2.0
ddH_2O	-	-	42.0
Total			50.0

Table 3.6: Primer mix preparation for rs735396.

Primers	Initial Concentration (µM)	Final Concentration (µM)	Volume (µL)
F_o	100	4	2.0
R_o	100	4	2.0
F_I	100	4	2.0
R_I	100	4	2.0
ddH_2O	-	-	42.0
Total			50.0

3.9.3 PCR Mix Preparation

A final volume of 10 µL of PCR mix was prepared based on the **Table 3.7** and **Table 3.8**, which consisted of master mix, primer mix, working DNA and ddH_2O.

Table 3.7: PCR mix preparation for rs2975760.

Reagents	Initial Concentration	Final Concentration	Volume (µL)
PCR Master Mix Buffer	2X	1X	5.0
Primer Mix	-	-	1.0
Working DNA	25 ng/µL	50 ng/µL	2.0
ddH_2O	-	-	2.0
Total	-	-	10.0

Table 3.8: PCR mix preparation for rs735396.

Reagents	Initial Concentration	Final Concentration	Volume (µL)
PCR Master Mix Buffer	2X	1X	5.0
Primer Mix	-	-	0.8
Working DNA	25 ng/µL	50 ng/µL	2.0
ddH_2O	-	-	2.2
Total	-	-	10.0

3.9.4 PCR Cycling Conditions

The Biometra thermal cycler PCR machine was used to conduct PCR with the cycling conditions as shown in the **Table 3.9** and **Table 3.10**. The cycling conditions for both SNPs were similar, except for annealing temperature.

Table 3.9: PCR cycling conditions 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

*Optimized protocol from senior

Table 3.10: PCR cycling conditions 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	1 minute	1

*Optimized protocol from senior

3.9.5 PCR Products Gel Electrophoresis

PCR products were separated by running agarose gel electrophoresis. The first lane and second lane were loaded with 1 µL of 50 bp DNA ladder and non-template control (NTC) respectively. The subsequent lanes were loaded with three positive controls (TT, TC and CC). The remaining wells were loaded with the PCR products. Before loading each well, 2 µL of 1 X DNA loading dye was mixed with 8 µL of PCR products. Once the gel was loaded, a power supply with a setting of 90 volts was connected to the gel tank and run for 35 minutes for rs2975760, while it was set to 85 volts and run for 35 minutes for rs735396. Subsequently, the gel was examined under a gel imager. The gel image was labeled, and band size was interpreted by comparing it with the ladder.

Genotypes were identified based on the amplicon size listed in the **Table 3.11** and **Table 3.12**.

Table 3.11: Amplicon size for the genotypes of rs2975760.

rs2975760	Genotype	Amplicon Size (bp)
Wild type	TT	200
Mutant	CC	274
Heterozygous	TC	200 & 274
Internal control	-	419

Table 3.12: Amplicon size for the genotypes of rs735396.

rs735396	Genotype	Amplicon Size (bp)
Wild type	TT	193
Mutant	CC	263
Heterozygous	TC	193 & 263
Internal control	-	400

3.10 DNA Sequencing

Two samples for rs2975760 and one sample for rs735396 were randomly selected for DNA sequencing. Prior sending out for sequencing, PCR reactions were carried out for each sample by using outer forward and reversed primer. Following PCR, the agarose gel electrophoresis was conducted to visualize the bands. A total of 2 µL 1 X loading dye and 5 µL of samples were loaded into the well. After confirming the presence of desired band, PCR purification was conducted to purify the PCR product. Gel purification was performed if multiple bands were observed.

PrimeWay Gel Extraction/PCR Purification Kit was used for purifying. A total of 90 µL PCR product was transferred into a 1.5 mL centrifuge tube, followed by an addition of 450 µL BD buffer. The mixtures were then transferred into the column and centrifuged at 11,000 x g for 30 seconds. Afterwards, the filtrate was discarded. A total of 750 µL wash buffer was added and centrifuged for another 30 seconds at 11,000 x g. After discarding the filtrate, an additional spin for 3 minutes at 17,000 x g was performed to dry the column. The column was transferred into a 1.5 mL centrifuge tube and 40 µL elution buffer was added to the center of column and incubated for 1 minute at room temperature. Finally, the columns were centrifuged for 1 minutes at 17,000 x g to obtain the purified PCR products (First Base, n.d.)

For gel purification, the gel was placed on a UV transilluminator, and the desired section of the gel was excised using a scalpel blade with holder. The cut gels were transferred into a 1.5 mL centrifuge tube, and 500 µL BD buffer was added. The mixtures were then incubated for 10 minutes in 55 °C water bath before cooling it to room temperature. Afterwards, the gel mixture was transferred into the column and the remaining steps mentioned in the PCR purification part were repeated (First Base, n.d.).

After obtaining the purified PCR product, the concentration and purity were analyzed using a nanodrop to ensure a concentration of more than 5 ng/µL and an A260/280 ratio between 1.80 to 2.00. The labeled purified samples were then sealed with parafilm and sent for automated Sanger sequencing at First Base Company, along with the respective outer forward primers. These primers were

prepared by mixing 2 µL outer primer with 18 µL ddH_2O. The results were interpreted using DNA Baser software.

3.11 Statistical Analysis

IBM SPSS Statistics 26 software was used for data analysis. The Pearson Correlation test was used to investigate the relationship between fasting blood glucose level and waist circumference. Besides, Kolmogorov-Smirnov test was performed on waist circumference and fasting blood glucose level to determine the normality. A non-parametric test was conducted to compare categorical data (demographic data, TCM body constitution, genotype) with continuous data (fasting blood glucose level and waist circumference). Both the Mann-Whitney U test and Kruskal-Wallis test were used for two independent groups and three or more independent groups, respectively. Fisher's Exact test was performed to assess the association between two categorical data, TCM body constitutions and the genotypes for both SNPs.

CHAPTER 4

RESULTS

4.1 Prevalence of Diabetes and Abdominal Obesity

A total of 80 subjects who fulfilled the inclusion and exclusion criteria were recruited to meet the calculated sample size of 72 and achieved a 90% confidence interval with 20% drop off rate. Fasting blood glucose (FBG) levels were measured to evaluate the diabetic status as follow WHO guideline. An individual was diagnosed as diabetes mellitus when the FBG levels more than 7 mmol/L, while an individual was determined as normal when the FBG levels lesser than 5.6 mmol/L. Pre-diabetes status was determined if the FBG levels between 5.6 to 6.9 mmol/L. Out of 80 subjects, only one was diagnosed with diabetes, resulting in a prevalence of diabetes at 1.25%, while the prevalence of pre-diabetes was 21.25%. Abdominal obesity was also assessed in this study to investigate the relationship with diabetes mellitus. The prevalence of abdominal obesity was found to be 24.40%, with men occupying 8.33% and women 16.07%. **Appendix B** shows the FBG levels and waist circumference measurements for all the studied subjects.

4.2 Genomic DNA Analysis

DNA was isolated from the collected blood samples using both Favorgen and Qiagen DNA Extraction Mini Kits. The concentration and purity of the isolated DNA were determined using nanodrop and genomic gel electrophoresis. **Appendix C** shows the purity and concentration for each extracted DNA. The average genomic DNA concentration was 138 ng/µL, while the average DNA

purity (A260/A280 ratio) was 1.86. **Figure 4.1** depicts a representative genomic gel image obtained after running gel electrophoresis. Pure DNA samples were shown as each lanes formed only one band.

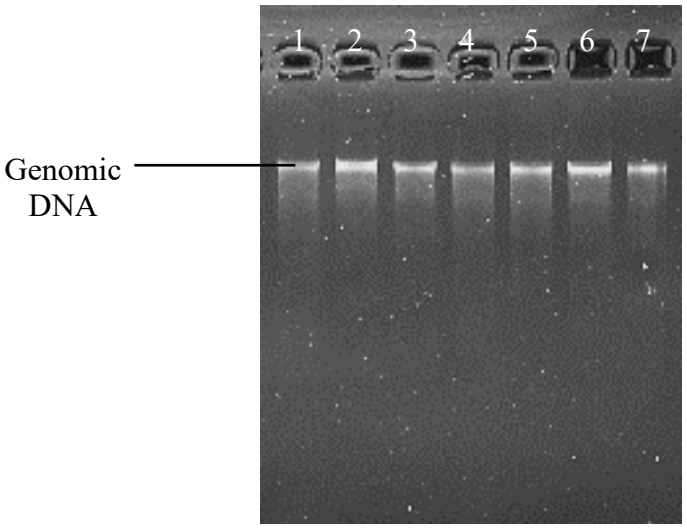

Figure 4.1: Representative genomic gel electrophoresis for seven genomic DNA samples. Lane 1: sample 022; Lane 2: sample 024; Lane 3: sample 026; Lane 4: sample 027; Lane 5: sample 028; Lane 6: sample 029; Lane 7: sample 030.

4.3 Genotyping of rs2975760 (*CAPN10*) and rs735396 (*HNF1A*)

A working DNA concentration of 25 ng/µL was prepared for tetra-primer AMRS PCR genotyping of rs2972760 and rs735396. **Figure 4.2** illustrates the representative gel image for the genotyping of rs2975760. A 50 bp DNA ladder was loaded in parallel with PCR samples to determine the amplicon size (lane 1). A non-template control, serving as a negative control was included to ensure no contamination occurred. Positive controls for genotypes TT, TC and CC were run in parallel as shown in lanes 3, 4 and 5. An internal control was amplified at 419 bp while a wildtype T allele and a mutant C allele were amplified at 200 bp and 274 bp respectively. Lane 17 showed a band at 274 bp, indicating a

homozygous mutant CC genotype while lanes 9, 10 and 13 showed two bands at 200 bp and 274 b, denoting a heterozygous TC genotype. The remaining lanes showed a band at 200 bp, indicating a homozygous wildtype TT genotype.

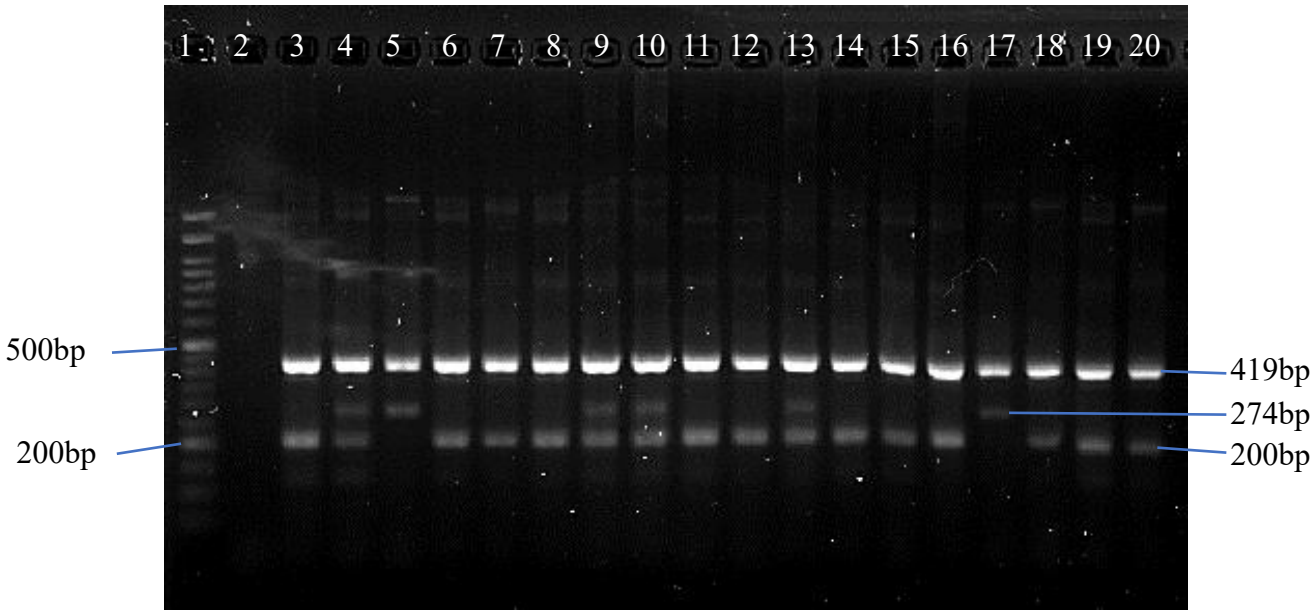

Figure 4.2: Representative gel image for rs2975760 genotyping. Lane 1: 50 bp DNA ladder; Lane 2: NTC; Lane 3: TT ; Lane 4: TC; Lane 5: CC; Lane 6: sample 081; Lane 7: sample 082; Lane 8: sample 084; Lane 9: sample 085; Lane 10: sample 086; Lane 11: sample 087; Lane 12: sample 088; Lane 13: sample 090; Lane 14: sample 091; Lane 15: sample 093; Lane 16: sample 094; Lane 17: sample 095; Lane 18: sample 096; Lane 19: sample 101: Lane 20: sample 099.

Figure 4.3 demonstrates a representative genotyping gel image for rs735396. Gel electrophoresis was run in parallel with a 50 bp DNA ladder (lane 1), a non-template control (lane2) and three positive controls representing TT, TC and CC genotypes (lane 3, 4 and 5). Lane 2 showed no bands formed, indicating the PCR was run without contamination. An internal control with a 400 bp amplicon size was shown in all the lanes. The wildtype T allele and mutant C allele were detected at 193 bp and 263 bp respectively. A single band at 193 bp as seen in lane 6, suggesting a homozygous wildtype TT genotype. Meanwhile, a band at

263 bp as in lane 8, indicating a homozygous mutant CC genotype. Lanes 7 and 9 were genotyped as heterozygous TC genotypes with the presence of two bands at 193 and 273 bp. All obtained genotypes were tabulated in **Appendix D** for both SNPs.

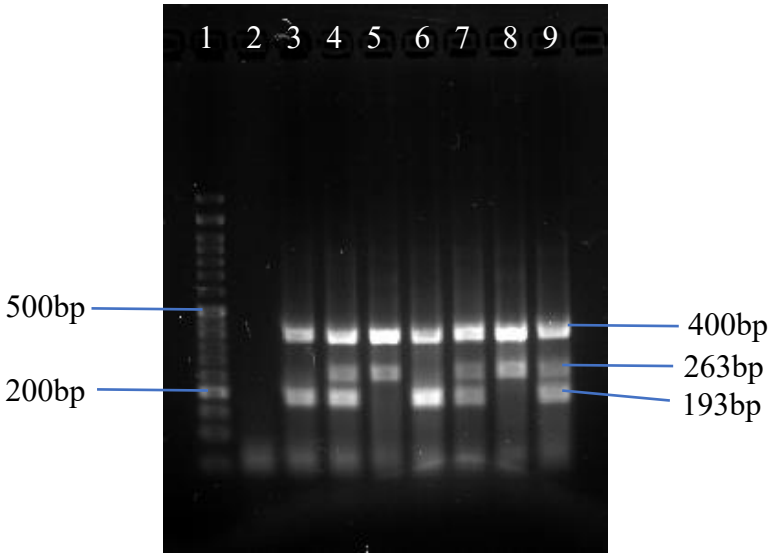

Figure 4.3: Representative gel image for rs735396 genotyping. Lane 1: 50 bp DNA ladder; Lane 2: NTC; Lane 3: TT; Lane 4: TC; Lane 5: CC; Lane 6: sample 009; Lane 7: sample 010; Lane 8: sample 027; Lane 9: sample: 028.

4.4 DNA Sequencing

Samples were randomly selected for both SNPs and the purified PCR samples were forwarded to the Fist Base Company for DNA sequencing to validate the genotyping results. Sequencing results were analyzed using DNA Baser and the chromatograms obtained are presented in **Figure 4.5, Figure 4.6** and **Figure 4.8**. For rs2975760, sample 070 indicated a homozygous wildtype TT genotype by showing a single peak of T nucleotide. Sample 071 showed a heterozygous TC genotype with two peaks of T and C nucleotides observed. The consistency with the genotyping result validated the accuracy of tetra-primer AMRS PCR

genotyping. Regarding rs735396, a single peak of T nucleotide was observed in sample 009, revealing a homozygous wildtype TT genotype. This was in agreement with the Tetra-Primer AMRS PCR genotyping result. Further SNPs sequences validation was performed by retrieving details from the NCBI SNPs database, as depicted in **Figure 4.4** and **Figure 4.7.** Upon comparing the chromatograms to the SNPs database, both SNPs were accurately targeted with the presence of specific alleles confirming their correctness.

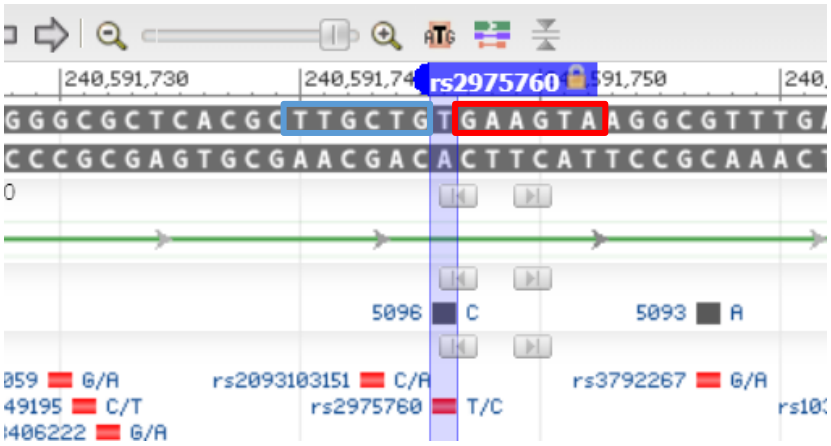

Figure 4.4: Nucleotide sequencing from NCBI SNPs database for rs2975760 (National Library of Medicine, 2022a).

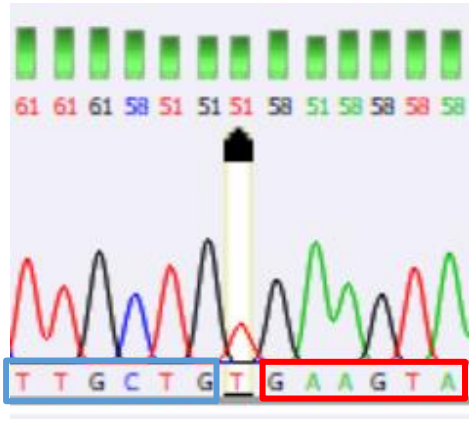

Figure 4.5: DNA sequencing result for sample 070, showing a single peak for T nucleotide.

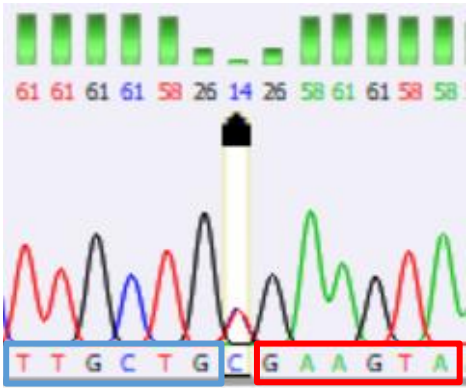

Figure 4.6: DNA sequencing result for sample 071, showing two overlapping peaks for T and C nucleotides.

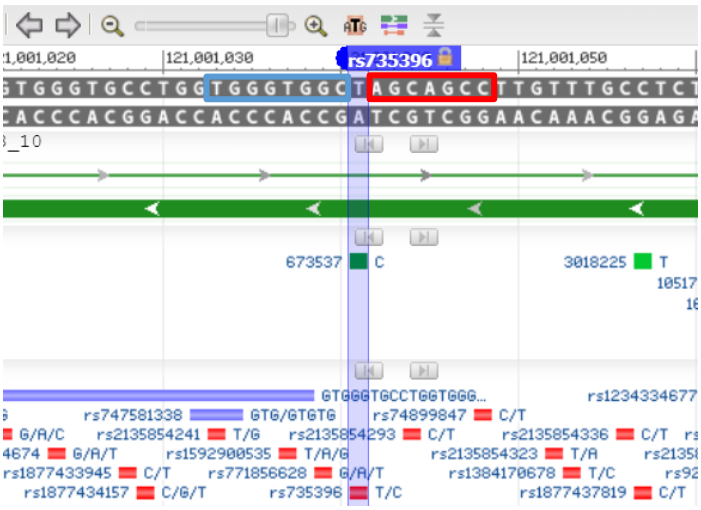

Figure 4.7: Nucleotide sequencing from NCBI SNPs database for rs73539 (National Library of Medicine, 2022b).

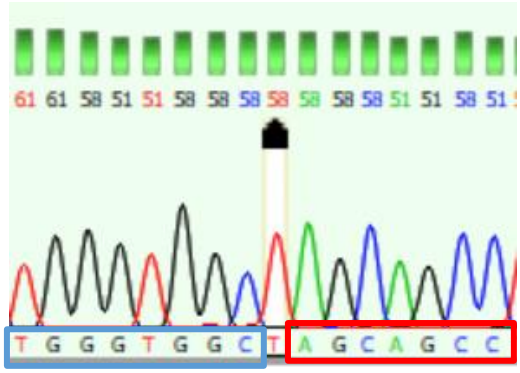

Figure 4.8: DNA sequencing result for sample 009, showing a single peak for T nucleotide.

4.5 Genotypic and Allelic Frequency for rs2975760 and rs735396

The genotypic and allelic frequencies for both SNPs are tabulated in **Table 4.1**. For rs2975760, the homozygous wildtype TT genotype had the highest genotypic frequency at 64%, followed by the heterozygous TC genotype at 16.25%, while the homozygous mutant CC genotype possessed the lowest genotypic frequency at 3.75%. For rs735396, the heterozygous TC genotype exhibited the highest genotypic frequency at 39%, followed by the homozygous wildtype TT genotype at 27% and the homozygous mutant CC genotype had the lowest percentage at 14%. The MAF for rs2975760 was 0.12 while the MAF for rs735396 was 0.42. Both polymorphisms were considered as significant SNPs with the MAF values were greater than 0.05.

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

Gene Variants	Genotypic Frequency, N (%)			Allelic Frequency	
	TT	TC	CC	T (Major)	C (Minor)
rs2975760 (T>C) n=80	64 (80)	13 (16.25)	3 (3.75)	0.88	0.12
rs735396 (T>C) n=80	27 (33.75)	39 (48.75)	14 (17.50)	0.58	0.42

4.6 Relationship between Fasting Blood Glucose Level and Waist Circumference

Pearson Correlation test was used to determine the relationship between fasting blood glucose level with waist circumference. A weak positive correlation was observed between fasting blood glucose level and waist circumference (R^2 =

0.012; p = 0. 337) as shown in **Table 4.2**. **Figure 4.9** shows the scatter plot along with its respective best fit line.

Table 4.2: Pearson correlation analysis between FBG level with waist circumference.

		Waist Circumference
FBG	**Correlation Coefficient**	0.109
	P-value	0.337

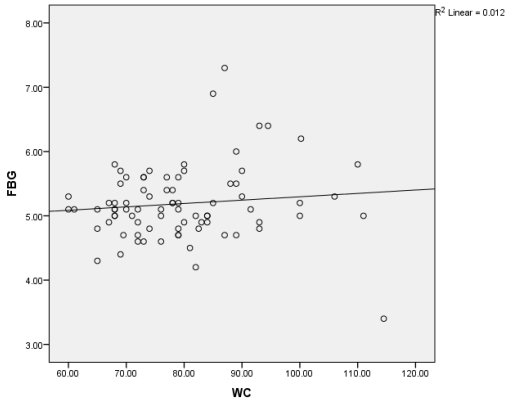

Figure 4.9: Scatter plot of FBG levels against waist circumferences.

4.7 Normality Test for Fasting Blood Glucose Levels and Waist Circumferences

The Kolmogorov-Smirnov normality test was used to assess the distributions of FBG levels and waist circumference. Both FBG levels and waist circumference were not normally distributed with p-values less than 0.10 as shown in **Table 4.3**. Hence, non-parametric test was used for further analysis.

Table 4.3: Kolmogorov-Smirnov normality tests on FBG level and waist circumference.

	Kolmogorov-Smirnov	
	df	**p-value**
Fasting Blood Glucose Level	80	0.026
Waist Circumference	80	0.000

df = degree of freedom

4.8 Fasting Blood Glucose Level and Waist Circumference across Various Demographic Factors

Demographic factors including gender, age and ethnicity were compared between FBG levels and waist circumference. This was to determine the factors that related to high FBG level and high waist circumference.

4.8.1 Gender

In this study, the subjects consisted of 70% females and 30% males. The Mann Withey U test was used to compare FBG levels between different genders and significant differences were found. The mean rank for FBG levels among females was 37.52 which was lower than males at 47.46. For waist circumference, the Kruskal Wallis test was used instead. No significant differences were found between waist circumferences in different genders. Females had a mean rank of 39.17 which was lower than males at 43.6. The data are presented in **Table 4.4**.

4.8.2 Age

All 80 subjects were categorized into 5 age groups. Among these groups, the predominant age range was from 19 to 28 with the percentage at 45%, followed

by the age groups of 39 to 48 at 15%, 29 to 38 at 13.75%, 49 to 58 at 11.25% and >59 at 15%. The Kruskal-Wallis test was used and both FBG levels and waist circumferences were found significantly different across different age groups. For FBG levels, the age group of >59 showed the highest mean rank at 56.92, with mean ranks decreasing progressively as the age range decreased where the age groups of 49 to 58 at 50.56, 39 to 48 at 40.83, 29 to 38 at 36.50 and 19 to 28 at 33.63. Moreover, the highest mean rank for waist circumferences was 55.89 for the age range of 49 to 58, followed by >59 at 51.83, 29 to 38 at 48.18, 39 to 48 at 44.29 and lastly the age group of 19 to 28 had the lowest mean rank at 29.26.

4.8.3 Ethnicity

The ethnicities were divided into two groups which were Chinese as the predominant group at 88.75% and non-Chinese at 11.25% which consisted of Malay, Indian and Bumiputera. The Mann-Whitney U test was used, and no significant differences were found between FBG levels and waist circumferences across various ethnic groups. For FBG level, the Chinese group showed a higher mean rank value of 41.15 as compared to the non-Chinese group at 35.39. For waist circumference, the non-Chinese group showed a higher mean rank value at 50.22 than the Chinese group at 39.27.

Table 4.4: Non-parametric analysis of FBG levels and waist circumferences across various demographic factors.

	N	WC mean (cm) ± SD	Mean Rank	P-value	FBG level mean(mmol/L) ± SD	Mean Rank	P-value
Gender[a]							
Female	56	78.57 ± 10.51	39.17	0.434	5.13 ± 0.48	37.52	**0.079***
Male	24	81.49 ± 14.46	43.60		5.32 ± 0.73	47.46	
Age[b]							
19 – 28	36	74.28 ± 9.39	29.26	**0.002***	5.02 ± 0.38	33.62	**0.024***
29 – 38	22	81.18 ± 7.77	48.18		5.19 ± 0.65	36.50	
39 – 48	23	81.96 ± 13.32	44.29		5.17 ± 0.39	40.83	
49 – 58	9	87.63 ± 12.95	55.89		5.31 ± 0.89	50.56	
>59	12	84.70 ± 13.59	51.83		5.62 ± 0.67	56.92	
Ethnicity[a]							
Chinese	71	79.03 ± 12.06	39.27	0.182	5.19 ± 0.55	41.15	0.482
Non-Chinese**	9	82.72 ± 9.56	50.22		5.21 ± 0.72	35.39	

*Significant data with p-value lesser than 0.1

**Non-Chinese group include Malay, Indian and Bumiputera.

[a] Mann-Whitney U test was used for two independent groups.

[b] Kruskal-Wallis test was used for three or more independent groups.

4.9 Phlegm-dampness, Qi-deficiency and Damp-heat with Fasting Blood Glucose Levels

The Mann-Whitney U test was used to compare the differences in FBG levels among subjects with presence or absence of phlegm dampness, qi deficiency and damp heat body constitution. The obtained p-value of 0.664 revealed there was no significant differences between these body constitutions with FBG levels. However, the group with presence of these three body constitutions achieved a higher mean rank of 42.75 compared to the group without. **Table 4.5** shows the data analysis from Mann-Whitney U test.

Table 4.5: Mann-Whitney U test analysis of FBG levels in relation to phlegm dampness, qi deficiency and damp heat.

Phlegm-dampness, Qi-deficiency, Damp-heat	N	FBG levels mean (mmol/L) ± SD	Mean rank	P-value
Absence	64	5.18 ± 0.59	39.94	0.664
Presence	16	5.20 ± 0.48	42.75	

4.10 Association between TCM Body Constitution with rs2965760 and rs735396

Fisher's Exact test was conducted to assess the association of TCM body constitutions with both SNPs. No association were found between TCM body constitutions with both rs2975760 (p = 0.502) and rs735396 (p=0. 675) as the p-values were greater than 0.1. **Table 4.6** shows the data from Fisher's Exact test.

4.11 rs2975760 and rs735396 with Fasting Blood Glucose Level and Waist Circumference

The genotyping results for rs2975760 and rs735396 were categorized into three genotypes which were homozygous wild type TT, homozygous mutant CC and heterozygous TC. There were no significant differences found between genotypes of rs2975760 with both FBG levels ($p = 0.823$) and waist circumference ($p=0.137$). For rs735396, significant differences were found between the genotypes with FBG levels ($p = 0.060$) while no for waist circumference ($p = 0.214$). For rs2975760, the mean rank of TC genotypes for waist circumferences achieved the highest value at 51.31, while CC genotypes showed a slightly lower mean rank than TC genotypes at 48.67. TT genotypes had the lowest mean rank at 37.93. It was found that the mean rank of FBG level for CC genotypes achieved the highest value at 47.50, followed by TC genotypes at 42.12 and TT genotypes at 39.84. For rs735396, the mean rank for TC genotypes showed the highest value at 45.14, followed by TT genotypes at 36.65 and CC genotypes at 35. The mean rank of FBG level for TT genotypes was the highest (48.78), followed by TC genotypes at 37.53 and CC genotypes at 32.82. **Table 4.7** shows the Kruskal-Wallis analysis data for both FBG levels and waist circumferences with genetic variants.

Table 4.6: Fisher's Exact test analysis of TCM body constitution with rs2975760 and rs735390.

Variables		TCM body constitution* (N = 80)				df	P-value
		Neutral	1 unbalanced body constitution	2 unbalanced body constitutions	≥3 unbalanced body constitutions		
Variant rs2975760	**TT**	32	19	7	6	6	0.502
	TC	8	3	1	1		
	CC	1	0	1	1		
Variant rs735396	**TT**	14	9	3	1	6	0.675
	TC	21	8	4	6		
	CC	6	5	2	1		

*TCM body constitutions include neutral, Yang-deficiency, Yin-deficiency, qi-deficiency, qi-depression, phlegm-dampness, damp-heat, blood-stasis, and special-constitution

Table 4.7: Kruskal-Wallis test analysis of FBG levels and waist circumferences in relation to genetic variants (rs2975760 and rs735396).

	N	Waist circumferences mean (cm) ± SD	Mean rank	P-values	FBG levels mean (mmol/L) ± SD	Mean rank	P-value
rs2975760							
TT	64	77.76 ±10.22	37.92	0.137	5.19 ± 0.55	39.84	0.823
TC	13	87.58 ± 16.81	51.31		5.15 ± 0.73	42.12	
CC	3	80.33 ± 1.53	48.67		5.30 ± 0.44	47.50	
rs735396							
TT	27	77.23 ± 9.73	36.65	0.214	5.36 ± 0.52	48.78	**0.060***
TC	39	81.54 ± 12.57	45.14		5.15 ± 0.59	37.53	
CC	14	77.89 ± 13.06	35.00		4.95 ± 0.54	32.82	

***** Significant data with p-value lesser than 0.1

CHAPTER 5

DISCUSSION

5.1 Prevalence of Diabetes and Abdominal Obesity

The prevalence of diabetes among the study population was 1.25% with the mean fasting blood glucose level at 5.19 mmol/L ± 0.57. However, this was significantly lower than the expected value of 18.5% as reported in the national survey report (Akhtar, et al., 2022). This may be attributed to the sampling bias in which the predominant age range in the study population was from 19 to 28 years, constituting 45% of the sample. Younger populations would generally exhibit better health conditions compared to the elderly. Aging process would gradually impair insulin secretion and resistance, thereby increasing the risk of developing diabetes (Mordarska and Zawada, 2017). Moreover, the prevalence of prediabetes was found to be 21.25%, notably higher than the reported figure of 11.62% in the study by Akhtar, et al. (2022). This suggests the importance of screening for prediabetes to evaluate the risk of developing diabetes.

The prevalence of abdominal obesity was determined by evaluating waist circumference measurements. This study showed a prevalence of 24.40% which was lower than the reported prevalence by MOH at 52.6% (Ministry of Health Malaysia (MOH), 2020). The discrepancy could be due to the limited sampling sites where most of the subjects were recruited at university with better academic level. Hence, they would have better health awareness and attitudes towards body image. Increased in health awareness and a positive attitude towards body

image allow the population to adopt healthier lifestyles, thereby reducing the risk of diseases such as abdominal obesity and diabetes (Yazdani, et al., 2018).

5.2 Genotyping and Allelic Frequency for rs2975760 and rs735396

Tetra-primer amplification refractory mutation system polymerase chain reaction (ARMS PCR) was used to genotype rs2975760 and rs735396. It is a simple and economical method, but prior optimization steps and the primers design are needed. Two outer primers were designed as non-allele-specific primers to serve as internal control. Two inner primers were designed as allele-specific primers to reveal the SNPs' genotypes. A mismatch was introduced at the 3' end of each inner primer to target different genotypes. For rs2975760 and rs735396, the 3' ends of the inner forward and reverse primers were designed to target wild type T allele (3' end – A allele) and mutant C allele (3' end – G allele), respectively. The specific primer that complemented to the sequence (A – T and G - C) would allow the amplifications, generating the respective amplicons. Consequently, the genotypes could be distinguished by different amplicon fragments (Medrano and De Oliveira, 2014).

For rs2975760, the major allelic frequency for T allele and minor allelic frequency for C allele were found to be 0.88 and 0.12, respectively. The result was consistent with the allelic frequencies as reported in the SNP database for the Asian population, where the major T allelic frequency was 0.85 and minor C allelic frequency was 0.15 (National Library of Medicine (NLM), 2022a). For rs735396 the major allelic frequency for T allele was 0.58, while the minor allelic frequency for C allele was 0.42. However, inconsistency was noted when

comparing with the NLM database in which the database reported the major allelic frequency as C allele (0.51) while the minor allelic frequency as T allele (0.49) in Asian population (NLM, 2022b). This inconsistency could be explained by population differences and ethnic diversity where 80 study subjects with majority being Chinese cannot fully represent the entire Asian population, which includes diverse ethnicities such as Filipino, Indonesian, Korean, Japanese and others. This is supported by Kliman (2008) and suggested that sample size can reduce random sampling error.

5.3 Relationship between Fasting Blood Glucose Level with Waist Circumference

In this study, there was a weak positive relationship between FBG levels and waist circumference, indicating that FBG levels were in relation with abdominal obesity. This was supported by Klein, et al. (2022) in revealing that the prevalence of T2DM increased linearly with increasing abdominal obesity. Excessive body fat was shown to have adverse effects on the pathogenesis of T2DM. It was found that obesity-induced alteration in adipose tissue biology was the main factor in contributing to insulin resistance and pancreatic β cell dysfunction. These alterations may lead to adipose tissue fibrosis, inflammation and the production of exosomes that cause the insulin resistance. A study conducted among Indian population further supports this positive relationship when showing a significant positive correlation between FBG levels and waist circumference. Among the obese subjects, the prevalence of prediabetes and diabetes were found to be 34.2% and 13.6% respectively (Telles, et al., 2018).

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

5.4.1 Gender

Our study demonstrated significant differences between FBG levels in different genders where females showed lower FBG level than males. These findings were supported by the previous studies in which females tend to have higher insulin sensitivity as compared to males, resulting in more efficient glucose uptake by cells, particularly skeletal muscle. Hormonal influences such as estrogen provide further insight into these findings. Estrogen increases the glucose uptake in various tissues and enhances insulin sensitivity due to its anti-inflammatory effect, thereby contributing to lower FBG levels in women (Kautzky-Willer, et al., 2015; Mauvais-Jarvis, 2018)

There were no significant differences found between waist circumferences in different genders which could be due to the skewed gender distribution in the sample, with female constituting 70% of the participants. However, from the mean rank value, females showed lower waist circumference measurements than males. This was consistent as reported by Kee, et al. (2008) which showed a mean waist circumference of 84.0 cm for males and 80.3 cm for female, suggesting the gender-based differences in fat distribution. Differences in sex hormones may contribute to variations in fat distribution. Females with higher level of estrogen tend to store more adipose tissue in the subcutaneous layer and femoral region, contributing to a pear-shaped body, while males with higher testosterone levels are prone to have apple-shaped body due to the accumulation of abdominal fat, leading to higher waist circumference. They noted that

estrogen production may affect the fat distribution through its effects on receptor distribution. In female, higher estrogen receptor (ER) α relative to Erβ in adipose tissue in abdominal region, resulting in a lower fat accumulation. Conversely, males express lower ERα levels in this region, making them more prone to central abdominal fat accumulation (Lumish, et al., 2020).

5.4.2 Age

FBG levels showed significant differences across five age groups with the oldest age group had the highest FBG levels. This finding aligned with the previous study by Ko, et al. (2006) which demonstrated a significant positive correlation between FBG levels and age. In younger individuals, the body cells are highly sensitive to insulin in regulating blood glucose levels. However, as individuals age, body cells become less responsive to insulin, resulting in prolong retention of glucose and fat in the bloodstream, and thus increase the blood glucose levels. Apart from that, pancreatic insulin production decreases in elderly individuals, resulting in prolonged elevation of blood glucose levels as there is insufficient facilitation of glucose uptake into body cells for energy production or storage. Consequently, blood glucose levels remain high for longer periods, increasing the risk of developing diabetes among the elderly.

For waist circumferences, significant differences across five age groups were observed, with aged groups showed the higher waist circumference, indicating waist circumference tends to increase with age. This was in concordance as according to the Australian Bureau of Statistics (2023) in which elderly with age 65 to 74 years were more likely to have higher waist circumferences, while

younger individuals in the age group of 18 to 24 years had lower waist circumference measurements. Aging is associated with a gradual decline in muscle mass and an increase in fat mass, particularly visceral fat. This age-related shift in body composition leads to an increase in waist circumference. Hormonal changes, such as a reduction in estrogen and testosterone levels in females and male respectively, also contribute to the changes in fat distribution and eventually leading to increased abdominal fat and waist circumference as they age. Additionally, older individuals tend to have a sedentary lifestyle and experience a decrease in physical activity, further contributes to an increase in waist circumference (Stevens, et al., 2009).

5.4.3 Ethnicity

The study showed no significant differences in FBG levels across various ethnic groups, but the mean rank values revealed that Chinese group had higher FBG levels. This finding contradicted with the previous studies which could be due to the imbalance distribution of ethnicities, with Chinese comprising the highest percentage at 88.75%. Venkataraman, et al. (2012) reported that Indians had the highest FBG level at a mean value of 5.24 mmol/L, followed by Malays at 5.10 mmol/L and Chinese had the lowest FBG level at 4.84 mmol/L. Asian Indians typically have a greater prevalence of insulin resistance and lower insulin sensitivity compared to other ethnicity, making them more susceptible to diabetes (Tan, et al., 2015; Yeo, et al. 2006). Lower insulin sensitivity will decrease the responsiveness of body cells towards insulin, leading to unregulated blood glucose level and thus increasing the risk of developing diabetes (Cleveland Clinic, 2021).

There were no significant differences observed in waist circumferences among various ethnic groups, but non-Chinese groups showed higher waist circumference measurements. Previous studies claimed that Indians are more prone to abdominal obesity as they tend to store excess fat predominantly in the abdominal region, leading to increased waist circumference. The authors suggested this propensity among Indians could be attributed to lower levels of physical activity and lesser consumption of fibrous foods such as fruits and vegetable, and thus leading to higher waist circumference (Tan, Dunn and Yen, 2011). The dietary preference for Chinese population is relatively healthier where they tend to have low-fat, low-sugar and low-salt foods. This unique food culture contributes to lower waist circumferences in Chinese populations (Teh, et al., 2023) According to Gujral, et al. (2013), individuals of South Asian descent such as Indians, are genetically predisposed to insulin resistance in glucose metabolism. When more insulin is produced due to irresponsive body cells, the inhibition of fat breakdown increases as well as the antilipolytic effect of insulin remains preserved, leading to high fat accumulation in abdominal region (Kahn and Flier, 2000).

5.5 Phlegm-dampness, Qi-deficiency and Damp-heat in relation to Fasting Blood Glucose Levels

The analysis revealed no significant differences in FBG levels between subjects with and without phlegm-dampness, qi-deficiency and damp-heat constitutions. However, the group with the presence of these three body constitutions presented with higher FBG levels in agreement with the previous study conducted by You,

et al. (2017). Phlegm-dampness, damp-heat and qi-deficiency constitutions were commonly observed among impaired glucose regulation patients, with tendencies toward higher FBG levels. The authors claimed that phlegm-dampness can disrupt metabolic pathways, impairing glucose metabolism and thereby raising the FBG levels. Internal accumulation of dampness and heat may trigger inflammation that affects the insulin sensitivity and glucose regulation and eventually leading to elevated FBG level. Qi-deficiency was implicated in altering glucose metabolism, further contributing to higher FBG levels and increasing the risk of diabetes. Overall, these findings underscore the role of TCM body constitutions in influencing FBG levels. The potential mechanisms of phlegm-dampness, damp-heat and qi-deficiency constitution need to be highlighted as it may impact glucose metabolism and increase the risk in developing diabetes.

5.6 TCM Body Constitution with rs2965760 and rs735396

The study found no association between TCM body constitution and the studied SNPs (rs2975760 and rs735396), suggesting that genetic variations may not exert a substantial influence on TCM body constitutions. The influences of acquired factors such as lifestyle, diet and environments may cause rapid changes in TCM body constitution, contributing to its dynamic nature and weakening the association with genetic variation (Yap, et al., 2021). Previous study by Amos (2010) suggested that genetic variation inherited from parents are not randomly distributed and are typically found at specific location in human genome. However, the rapid changes of TCM body constitutions might mask the influence of relatively stable genetic variations. TCM body constitution

may have transient effects and do not leave a long-lasting influence on an individual's genetic makeup (Propis, 2020).

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

FBG levels showed no significant differences in rs2975760. The major reason could be due to the limited sample size recruited for this study. Previous study had reported a potential association between *CAPN10* polymorphism and T2DM as well as the study conducted by Song, et al. (2004). They suggested a significant association between the mutant C allele in rs297560 and T2DM. The C allele carriers for rs2975760 are at increased risk of developing T2DM. Variants in the *CAPN10* gene, particularly rs2975760 are found to have effects on insulin sensitivity, insulin secretion and glucose metabolism. These dysregulations cause the elevation in FBG levels and thereby exposing individuals to the risk of developing T2DM. In our study population, the group with the mutant CC allele displayed a higher mean rank value, indicating that mutant allele increases the risk of developing high FBG levels. Significant differences were found between rs735396 and FBG levels with the CC allele showed the lowest FBG levels, consistent with the previous studies by Dallali, et al. (2022). They claimed that individual with the TT genotype and TC genotypes showed a slightly higher FBG levels than those with the CC allele. This suggested polymorphism of rs735396 may play a significant role in influencing glucose metabolism due to its impact on pancreatic beta cell function. This could affect glucose metabolism by reducing insulin production, thereby increasing the risk of diabetes (Miyachi, et al., 2022).

Furthermore, no significant differences were found between rs2975760 and waist circumferences. This was consistent with the findings by Jensen, et al. (2006) that showed no significant association between rs2972560 genotypes with waist circumference. This was further supported by Pihlajamaki, et al. (2006) which found that rs2975760 was not associated with abdominal obesity. The study showed no significant differences between rs735396 and waist circumferences, contradicting with the study by Dallali, et al. (2022) that highlighted a significant association between rs735396 and waist circumference, suggesting a potential role of this polymorphism in influencing abdominal obesity. The rs735396 is located in an intronic region of the *HNF1A* gene, known to be involved in transcriptional regulation. The presence of this variation may affect the lipid metabolism and inflammation, contributing to genetic susceptibility to obesity. Morjane, et al. (2017) also revealed the C allele of rs735396 was associated with increased waist circumference, suggesting a potential role of rs735397 in influencing abdominal obesity. The contradictory experimental results with the previous study could be due to bias in the recruited samples and the limited sample size. Further research might be needed to assess the relationship between these two variables.

5.8 Limitations

Several limitations should be considered, including the small sample size and sampling bias. The sample size in this study may not be sufficient to fully capture the variability within the population. Hence, it would potentially affect the reliability of the research findings. A small sample size can reduce statistical

power and decrease the sufficiency of the demographic composition. Sampling bias could lead to inaccurate findings. If the sample predominantly comprises younger individuals, it may not accurately represent the broader population where older age groups are more likely to have diabetes and obesity.

5.9 Future Recommendations and Future Works

Several recommendations can be made to enhance future research efforts. Firstly, it is recommended to increase the sample size to ensure the full capture of the population's variability. Additionally, efforts to minimize sampling bias should be undertaken by recruiting more diverse range of participants, including individuals from different age groups, ethnicities, and backgrounds. Since metabolic factors are associated with gender and age, the sample size should be increased to repeat section 4.11. The significance level is suggested to be 0.05 instead of 0.1 to reduce Type 1 errors (false positives), which occur when an effect is considered significant when it is not truly present. With these improvements, future research can be conducted to overcome the limitations observed in the study and allow for a deeper understanding of the complex interplay between genetic variation, metabolic syndrome and TCM body constitutions.

CHAPTER 6

CONCLUSION

In conclusion, this study revealed a lower prevalence of diabetes (1.25%) and abdominal obesity (24.2%) as compared to national statistics. A weak positive relationship between fasting blood glucose levels and waist circumferences was found. This was consistent with the previous findings in which abdominal obesity was associated with higher risk of developing diabetes. Both studied SNPs (rs2975760 and rs735396) were significantly important in the study population as the MAF values were greater than 0.05. The MAF for rs2975760 was consistent with the reported data in SNP databases for Asian populations, while the MAF for rs735396 contradicted the reported data. This inconsistency could be due to ethnic diversity within the study population where 80 subjects with majority being Chinese cannot fully represent the entire Asian population. DNA sequencing validated the accuracy of genotyping results using tetra-primer AMRS PCR. FBG levels showed significant differences across different gender, age groups and ethnic groups while waist circumferences showed significant differences across different age groups but not for others.

The study further explored the influence of TCM body constitutions on fasting blood glucose levels, indicating a higher mean rank in subjects with phlegm-dampness, damp-heat and qi-deficiency constitutions but they did not differ significantly. There was no association between TCM body constitutions with genetic variations, possibly due to the dynamic nature of TCM body constitutions. FBG levels and waist circumference did not differ significantly

with rs2975760. The rs735396 showed significant difference in FBG levels but not in waist circumference. Despite the limitations in sample size and sampling bias, this study provides valuable insights into the complex interplay between TCM body constitutions, genetic variation and metabolic health among the study population, highlighting the need for larger and more diverse studies to further reveal their relationships.

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APPENDICES

Appendix A

UNIVERSITI TUNKU ABDUL RAHMAN DU012(A)
Wholly owned by UTAR Education Foundation Co. No. 578227-M

Re: U/SERC/330/2023

24 December 2023

Dr Teh Lai Kuan
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 your application which was circulated for consideration of the UTAR Scientific and Ethical Review Committee (SERC). We are pleased to inform that your application for ethical approval of your research project (Undergraduate students' project) involving human subjects has been approved by SERC.

The details of the project are as follows:

Research Title	Traditional Chinese Medicine (TCM) Body Constitution with Anaemia and Metabolic Disorder with Its Regulating Gene
Investigator(s)	Dr Teh Lai Kuan Cheng Kai Ju (UTAR Undergraduate Student) Tan Shu Rou (UTAR Undergraduate Student) Yau Zhi Xuan (UTAR Undergraduate Student)
Research Area	Healthcare
Research Location	UTAR, Kampar Campus; Private Chinese Medicine Clinic
No of Participants	80 participants (Age: 20 -70)
Research Costs	Self-funded
Approval Validity	24 December 2023 - 23 December 2024

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.

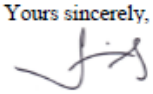
Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

Should you collect personal data of participants in your study, please have the participants in the research signed the attached Personal Data Protection Statement for your records.

The University wishes you all the best in your research.

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

Appendix B

Table A: Fasting blood glucose levels and waist circumferences measurements for 80 study subjects.

Sample ID	FBG (mmol/L)	Waist Circumference (cm)
001	5.8	68.0
002	5.4	78.0
003	5.1	65.0
004	4.4	69.0
005	5.6	73.0
006	5.2	68.0
007	5.6	77.0
009	5.7	90.0
010	5.0	84.0
012	5.3	106.0
014	4.6	73.0
017	5.3	90.0
018	4.7	89.0
020	5.2	100.0
022	5.2	67.0
024	5.4	73.0
026	5.5	69.0
027	4.9	80.0
028	5.2	70.0
029	4.9	72.0
030	6.4	93.0
031	5.2	78.0
033	5.5	89.0
034	5.0	84.0
036	5.0	111.0
037	4.7	79.0
039	4.8	65.0
040	4.7	72.0
041	4.6	72.0
043	4.9	93.0
044	5.1	60.0
045	5.6	73.0
046	5.0	76.0
047	4.8	79.0
049	4.7	79.0
050	4.6	76.0
051	5.1	68.0
052	5.0	68.0
053	5.0	82.0
054	4.9	83.0
056	5.1	79.0
058	6.9	85.0

059	5.2	79.0
060	5.0	71.0
061	4.3	65.0
062	5.1	61.0
063	4.8	74.0
064	5.6	79.0
065	4.9	67.0
066	5.1	68.0
067	5.2	85.0
068	5.6	70.0
069	5.4	77.0
070	5.7	80.0
071	6.4	94.5
072	5.0	100.0
073	6.2	100.2
075	4.9	84.0
076	5.8	110.0
077	3.4	114.5
078	4.7	69.5
079	4.8	82.5
080	5.5	88.0
081	5.0	68.0
082	4.7	87.0
084	5.1	70.0
085	4.5	81.0
086	5.7	69.0
087	4.8	93.0
088	6.0	89.0
090	5.1	72.0
091	5.1	91.5
093	5.3	60.0
094	7.3	87.0
095	5.8	80.0
096	4.2	82.0
098	5.3	74.0
099	5.7	74.0
101	5.2	78.0
103	5.1	76.0

Appendix C

Table B: DNA concentration and purity.

Sample ID	Concentration (ng/µl)	A260/A280 ratio
001	66.80	1.86
002	117.0	1.83
003	92.00	1.80
004	105.2	1.92
005	34.10	1.86
006	64.00	1.86
007	31.40	1.96
009	34.70	1.83
010	27.30	1.85
012	111.3	1.76
014	117.8	1.85
017	25.70	1.84
018	78.90	2.03
020	30.50	1.82
022	173.6	1.97
024	80.50	1.89
026	152.7	1.86
027	139.0	1.89
028	69.50	1.84
029	162.4	1.88
030	123.8	1.79
031	79.70	1.78
033	86.20	1.82
034	58.90	1.81
036	248.6	1.91
037	147.3	1.86
039	142.1	1.86
040	93.40	1.88
041	127.4	1.86
043	63.00	1.79
044	146.3	1.85
045	50.40	1.74
046	34.70	1.8
047	54.10	1.83
049	99.40	1.86
050	210.5	1.95
051	99.00	1.83
052	194.8	2.03
053	538.3	1.86
054	68.50	1.85
056	96.80	1.83
058	92.00	1.84
059	60.50	1.84

060	52.30	1.79
061	62.40	1.85
062	74.70	1.87
063	149.0	1.84
064	113.2	1.83
065	55.20	1.89
066	82.00	1.84
067	137.6	1.85
068	151.7	1.83
069	598.3	1.81
070	586.3	1.89
071	173.3	1.83
072	110.3	1.92
073	95.60	1.80
075	309.2	1.87
076	176.6	1.88
077	369.2	1.84
078	334.7	1.86
079	117.1	1.89
080	208.3	1.80
081	90.60	1.90
082	279.3	1.87
084	171.3	1.89
085	154.0	1.85
086	417.4	1.87
087	230.1	1.89
088	105.8	1.90
090	141.5	1.88
091	160.8	1.90
093	99.30	1.84
094	105.1	1.89
095	35.10	1.84
096	64.30	1.76
098	95.00	1.85
099	173.8	1.84
101	66.60	1.88
103	98.60	1.85

Appendix D

Table C: Genotyping for rs2975760 and rs735396.

Sample ID	rs2975760 (T>C)	rs735396 (T>C)
001	TT	CC
002	TT	TC
003	TT	TT
004	TT	TC
005	TC	TT
006	TT	TT
007	TC	TT
009	TT	TT
010	TT	TC
012	TT	TC
014	TT	CC
017	TT	TC
018	TC	TC
020	TT	TC
022	TC	CC
024	TT	TC
026	TT	CC
027	TT	CC
028	TT	TT
029	TT	TT
030	TT	TC
031	TT	TT
033	TT	TC
034	TT	TT
036	TC	TC
037	TT	TT
039	TT	TC
040	TT	TC
041	TT	TC
043	TC	TC
044	TT	TC
045	TT	TC
046	TT	CC
047	TT	TC
049	TT	TC
050	TT	TC
051	TC	CC
052	TT	TT
053	CC	TC
054	TT	TC
056	CC	TC
058	TT	TC
059	TT	TC

060	TT	TC
061	TT	TC
062	TT	TT
063	TT	TT
064	TT	TT
065	TT	TC
066	TT	TT
067	TT	TC
068	TT	TT
069	TT	TT
070	TT	TC
071	TC	TC
072	TT	TT
073	TT	TT
075	TT	TT
076	TC	TC
077	TC	CC
078	TT	TC
079	TT	CC
080	TT	TT
081	TT	CC
082	TT	CC
084	TT	CC
085	TC	TC
086	TC	TT
087	TT	TC
088	TT	TC
090	TT	TT
091	TC	CC
093	TT	TC
094	TT	TT
095	CC	TT
096	TT	TC
098	TT	TT
099	TT	TC
101	TT	TT
103	TT	CC

Appendix E

Table D: Demographic data for 80 study subjects.

Sample ID	Gender	Age	Ethnicity
001	Female	23	Iban
002	Male	19	Chinese
003	Female	21	Chinese
004	Female	21	Chinese
005	Female	21	Chinese
006	Female	22	Chinese
007	Female	22	Chinese
009	Female	33	Chinese
010	Female	39	Chinese
012	Male	48	Chinese
014	Female	24	Chinese
017	Male	62	Chinese
018	Female	46	Chinese
020	Male	47	Chinese
022	Female	20	Chinese
024	Female	32	Chinese
026	Female	59	Chinese
027	Female	66	Chinese
028	Female	48	Chinese
029	Female	23	Chinese
030	Male	50	Chinese
031	Female	57	Chinese
033	Male	50	Chinese
034	Female	65	Chinese
036	Female	26	Chinese
037	Female	57	Chinese
039	Male	28	Chinese
040	Female	22	Chinese
041	Female	23	Chinese
043	Female	22	Indian
044	Male	22	Chinese
045	Female	22	Chinese
046	Female	22	Chinese
047	Female	22	Chinese
049	Female	22	Chinese
050	Female	22	Chinese
051	Female	22	Chinese
052	Female	34	Malay
053	Female	38	Malay
054	Female	36	Malay
056	Male	22	Chinese
058	Male	38	Kenyah
059	Female	22	Chinese

060	Female	22	Chinese
061	Female	25	Chinese
062	Female	22	Chinese
063	Male	22	Chinese
064	Male	22	Chinese
065	Male	22	Chinese
066	Female	22	Chinese
067	Female	23	Chinese
068	Male	21	Chinese
069	Female	54	Chinese
070	Male	63	Chinese
071	Male	80	Chinese
072	Female	78	Chinese
073	Male	56	Chinese
075	Female	27	Chinese
076	Female	81	Chinese
077	Male	51	Chinese
078	Male	47	Chinese
079	Female	36	Chinese
080	Female	59	Chinese
081	Female	48	Chinese
082	Female	37	Chinese
084	Male	30	Chinese
085	Female	31	Malay
086	Female	43	Chinese
087	Female	45	Indian
088	Female	39	Chinese
090	Female	45	Chinese
091	Female	36	Indian
093	Male	82	Chinese
094	Female	67	Chinese
095	Female	49	Chinese
096	Male	19	Chinese
098	Female	46	Chinese
099	Male	66	Chinese
101	Female	58	Chinese
103	Male	23	Chinese

Appendix F

Table E: TCM body constitutions for 80 study subjects based on the calculated adjusted score.

Sample ID	Adjusted Score (AS)									TCM body constitutions
	Neutral	Yang-deficiency	Yin-deficiency	Phlegm-dampness	Damp-heat	Blood-stasis	Specific diathesis	Qi-deficiency	Qi-depression	
001	34	0	53	28	29	25	21	61	50	Qi-depression, Qi-deficiency, Yin-deficiency
002	75	11	6	6	29	11	18	21	21	Neutral
003	72	29	31	19	33	18	18	21	21	Neutral
004	63	68	9	13	33	32	46	46	25	Yan-deficiency, Qi-deficiency
005	66	14	50	19	13	21	25	29	7	Yin-deficiency
006	75	39	22	9	13	39	4	14	0	Neutral
007	66	25	19	25	17	36	29	54	36	Qi-deficiency
009	75	39	25	22	4	7	18	32	11	Neutral
010	88	18	22	13	25	18	18	11	14	Neutral
012	81	0	0	0	0	0	0	0	0	Neutral
014	53	18	44	28	33	50	46	25	50	Blood-stasis, Ying-deficiency, Qi-depression
017	100	0	19	47	50	18	0	29	11	Phlegm-dampness, Damp-heat

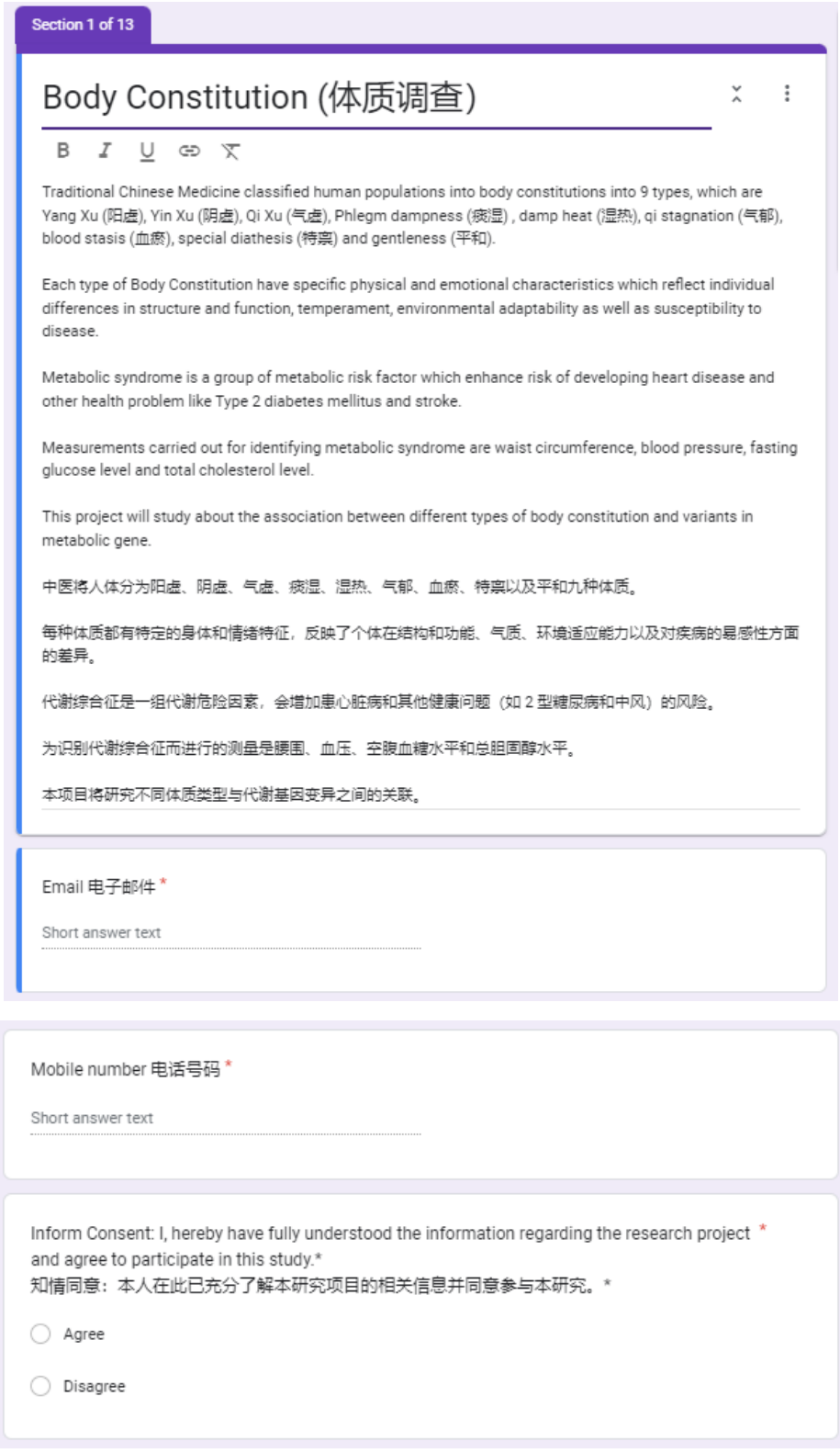
018	75	0	50	38	33	68	7	29	21	Blood-stasis, Yin-deficiency
020	100	0	0	28	8	0	0	21	4	Neutral
022	63	14	50	34	21	11	0	43	39	Yin-deficiency
024	53	0	25	6	21	14	14	32	50	Qi-depression
026	69	0	38	6	13	25	29	11	4	Neutral
027	50	0	22	25	8	32	29	32	4	Blood-stasis
028	75	11	25	19	38	43	4	32	25	Blood-stasis
029	59	18	16	9	29	29	11	29	11	Neutral
030	91	0	9	0	0	0	4	32	0	Neutral
031	69	14	31	16	0	36	18	14	0	Neutral
033	100	0	13	0	17	14	11	11	0	Neutral
034	81	18	0	0	0	21	11	14	4	Neutral
036	97	0	28	13	25	21	0	7	14	Neutral
037	78	0	3	0	0	21	11	11	7	Neutral
039	78	4	0	6	4	4	11	11	4	Neutral
040	69	11	13	16	25	18	11	36	11	Neutral
041	47	14	16	19	29	29	18	43	54	Qi-depression
043	97	18	9	22	17	21	18	29	11	Neutral
044	66	68	25	16	25	4	39	14	14	Yang-deficiency
045	72	11	16	6	38	11	7	46	25	Qi-deficiency
046	38	25	38	9	25	25	39	25	57	Qi-depression
047	53	18	44	28	33	50	46	25	50	Qi-depression, Qi-deficiency, Yang -deficiency, Special diathesis

049	13	43	28	41	38	43	57	50	64	Qi-depression, Yang-deficiency, Special diathesis
050	88	7	41	16	54	7	18	14	4	Damp-heat
051	75	18	34	3	8	18	21	32	4	Neutral
052	72	43	13	6	8	14	4	18	4	Yang-deficiency
053	69	21	0	0	8	7	0	0	7	Neutral
054	47	57	44	47	8	29	50	32	21	Yang-deficiency, Special diathesis, Phlegm-dampness
056	59	64	28	44	42	29	54	43	57	Yang-deficiency, Qi depression, Special diathesis
058	72	0	3	9	4	29	7	18	7	Neutral
059	13	36	47	44	46	64	39	82	64	Qi-depression, Blood-stasis, Qi-deficiency
060	69	21	0	25	4	11	0	18	29	Neutral
061	75	29	19	19	50	14	0	32	29	Damp-heat
062	75	11	13	6	13	14	7	25	21	Neutral
063	84	4	34	9	17	4	7	39	11	Neutral
064	78	25		13	17	18	32	21	4	Neutral
065	38	36	44	16	21	25	11	46	36	Yin-deficiency, Qi-deficiency
066	66	11	13	6	13	14	7	25	25	Neutral
067	59	11	19	28	29	36	7	50	11	Qi-deficiency
068	59	50	13	19	83	32	25	18	50	Qi-depression, Yang-deficiency

069	84	4	13	38	33	14	6	25	54	Qi-depression
070	75	0	4	9	4	18	4	11	4	Neutral
071	78	11	28	13	4	7	14	4	4	Neutral
072	97	0	19	13	0	18	0	7	0	Neutral
073	63	4	13	9	0	18	4	39	36	Neutral
075	72	18	19	9	13	39	4	21	25	Neutral
076	78	0	6	3	13	18	0	14	7	Neutral
077	84	0	3	9	4	4	14	25	0	Neutral
078	84	18	13	25	25	21	11	21	32	Neutral
079	47	25	38	44	29	46	25	25	32	Phlegm-dampness, Blood-stasis
080	84	0	56	3	17	50	0	7	0	Yin-deficiency, Blood-stasis
081	91	4	6	9	0	36	0	29	25	Neutral
082	84	4	9	22	17	18	4	18	4	Neutral
084	38	7	41	13	29	25	43	29	39	Yin-deficiency
085	47	39	9	9	8	25	21	0	4	Neutral
086	31	36	50	50	46	46	36	75	54	Qi-deficiency, Yin-deficiency, Qi-depression, Phlegm-dampness
087	41	25	47	34	17	21	0	32	14	Yin-Deficiency
088	63	64	47	53	21	43	57	75	36	Qi-Deficiency, Yang-deficiency, Special diathesis, Phlegm-dampness
090	44	43	13	0	8	14	11	4	0	Yang-deficiency

091	53	0	19	16	17	21	0	18	29	Neutral
093	31	14	38	31	25	32	54	32	21	Neutral
094	56	32	34	25	13	29	11	25	71	Qi-depression
095	81	7	31	38	46	29	25	25	0	Damp-heat, Ying-deficiency
096	22	4	13	13	21	11	14	14	14	Neutral
098	66	43	3	9	0	21	7	43	25	Yin-deficiency
099	50	0	22	13	17	25	14	11	18	Neutral
101	34	0	22	38	25	32	32	50	7	Qi-deficiency
103	50	29	28	9	21	21	29	29	14	Qi-deficiency

Appendix F



Section 1 of 13

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。

Email 电子邮件 *

Short answer text

Mobile number 电话号码 *

Short answer text

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

Section 2 of 13

Demographic 个人资料

Description (optional)

Name 姓名 *

Short answer text

Gender 性别 *

- Male
- Female

Age 年龄 *

Short answer text

Ethnicity 种族 *

- Malay 马来人
- Chinese 华人
- Indian 印度人
- Other...

Section 3 of 13

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

(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

Section 4 of 13

Yang-deficiency (阳虚)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 5 of 13

Yin-deficiency (阴虚)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 6 of 13

Qi-deficiency (气虚)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

3. Did you get palpitations? *
你有心悸吗?

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 7 of 13

Phlegm-dampness (痰湿)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 8 of 13

Damp-heat (湿热)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 9 of 13

Blood-stasis (血瘀)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：>每周 6 次

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 10 of 13

Special diathesis (特禀)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 11 of 13

Qi-depression (气郁)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不: 每周0次
(2) 很少: 每周1-2次
(3) 有时: 每周3-4次
(4) 经常: 每周5-6次
(5) 总是: > 每周 6 次

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

Section 12 of 13

Gentleness (平和)

Please recall frequency of specific conditions stated below occurred in NEAR A MONTH.
(1) Never: 0 time per week
(2) Seldom: 1-2 times per week
(3) Sometimes: 3-4 times per week
(4) Often: 5-6 times per week
(5) Always: >6 times 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) 从不：每周0次
(2) 很少：每周1-2次
(3) 有时：每周3-4次
(4) 经常：每周5-6次
(5) 总是：＞每周 6 次

1. Were you energetic? *
你是个有活力的人吗?

	1	2	3	4	5	
Never	○	○	○	○	○	Always

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	
Never	○	○	○	○	○	Always

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

	1	2	3	4	5	
Never	○	○	○	○	○	Always