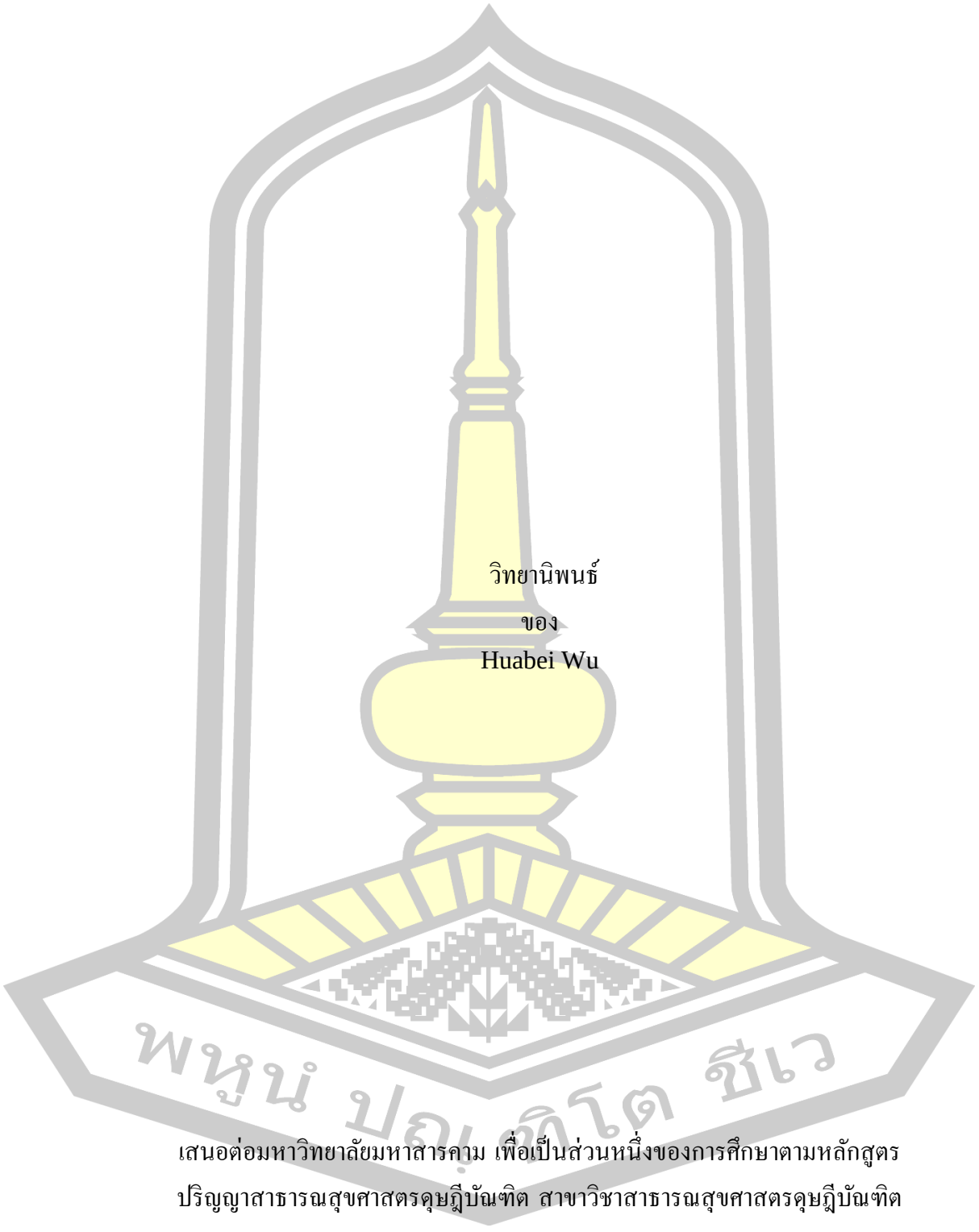
The prediction model of genetic and risk factors to hemorrhoids

Huabei Wu

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Doctor of Public Health in Doctor of Public Health
April 2024

The prediction model of genetic and risk factors to hemorrhoids



วิทยานิพนธ์

ของ

Huabei Wu

เสนอต่อมหาวิทยาลัยมหาสารคาม เพื่อเป็นส่วนหนึ่งของการศึกษาตามหลักสูตร

ปริญญาสาธารณสุขศาสตรดุษฎีบัณฑิต สาขาวิชาสาธารณสุขศาสตรดุษฎีบัณฑิต

เมษายน 2567

The prediction model of genetic and risk factors to hemorrhoids

Huabei Wu

A Thesis Submitted in Partial Fulfillment of Requirements

for Doctor of Public Health (Doctor of Public Health)

April 2024

The examining committee has unanimously approved this Thesis, submitted by Ms. Huabei Wu , as a partial fulfillment of the requirements for the Doctor of Public Health Doctor of Public Health at Mahasarakham University

Examining Committee

..... Chairman
(Assoc. Prof. Chulaporn Sota , Ph.D.)

..... Advisor
(Assoc. Prof. Sumattana Glangkarn , Ph.D.)

..... Co-advisor
(Asst. Prof. Nachalida Yukalang , Ph.D.)

..... Committee
(Assoc. Prof. Suneerat Yaugyuen , Ph.D.)

..... Committee
(Assoc. Prof. Thidarat Somdee , Ph.D.)

..... Committee
(Asst. Prof. Jaruwan Viroj , Ph.D.)

Mahasarakham University has granted approval to accept this Thesis as a partial fulfillment of the requirements for the Doctor of Public Health Doctor of Public Health

....
(Assoc. Prof. Sumattana Glangkarn , Ph.D.) (Assoc. Prof. Krit Chaimoon , Ph.D.)
Dean of The Faculty of Public Health Dean of Graduate School

TITLE The prediction model of genetic and risk factors to hemorrhoids

AUTHOR Huabei Wu

ADVISORS Associate Professor Sumattana Glangkarn , Ph.D.

Assistant Professor Nachalida Yukalang , Ph.D.

DEGREE Doctor of Public Health **MAJOR** Doctor of Public Health

UNIVERSITY Mahasarakham University **YEAR** 2024

ABSTRACT

Hemorrhoids is the most common anorectal disease. At present, the research on hemorrhoids has focused on the external environment and behavioral factors, while the research on genetic factors is rare. This study used mixed methods to establish a preliminary hemorrhoid risk prediction model with genetic and risk factors, providing clues and theoretical basis for further research on hemorrhoid prevention. The research objectives of this study include the following four aspects: (1)To explore the risk factors of hemorrhoids in Guangxi population. (2)To explore the causal association of body mass index and hemorrhoids. (3) To explore the association of FOXC2 polymorphism and its interaction with other risk factors with the susceptibility to hemorrhoids in Guangxi population. (4)To establish a prediction model for hemorrhoids.

This study can be divided into two phases. The first phase includes two parts. In the first part, a case-control study was used to analyze the relationship between the indicators in the questionnaire survey and the rs34221221 polymorphism of FOXC2 gene and hemorrhoids. The second part is to analyze the causal relationship between BMI and hemorrhoids using Mendelian randomization method.

In the case-control study, 200 patients with hemorrhoids and 200 patients without hemorrhoids were included according to the inclusion and exclusion criteria. A self-made questionnaire was used to collect the general information, environmental and behavioral factors that may be related to hemorrhoids, and the venous blood of patients was collected for single nucleotide polymorphism detection. T-test was used to analyze the quantitative data of the two groups, Chi-square test was used to analyze the classification data, and logistic regression was used to analyze the correlation between variables and hemorrhoids. The results showed that age, high education level, constipation and chronic gastritis will increase the risk of hemorrhoids (Adjusted OR=1.03, 95% CI: 1.01-1.05; Adjusted OR=2.02, 95% CI: 1.16-3.52; Adjusted OR=2.70, 95% CI: 1. 50-4. 87; Adjusted OR=1.96, 95% CI: 1.14-3.36), while bland dietary taste and CT genotype could reduce the risk of hemorrhoids (Adjusted OR=0.50, 95% CI: 0.28-0.89, Adjusted OR=0.642, 95% CI: 0.424-0.972).

In the second part, using BMI as the exposure factor and hemorrhoids as the outcome, a two sample Mendelian randomized analysis was performed. A total of

68 SNPs of BMI in east asian population were selected as genetic instrumental variables, and total of 67 SNPs in south asian population were included. The causal relationship between BMI and hemorrhoids was analyzed using IVW, MR- Egger and weighted median models. No causal effect was found between BMI and hemorrhoids. In East Asian population, in IVW model, B=1.790, 95% CI: 0.628-5.107, P=0.276; In MR-Eegger model, B=0.316, 95% CI :0.013-7.908, P=0.486; In the WME model, B=4.018, 95% CI: 0.816-9.794, P=0.087. In the South Asian population, in the IVW model, B=0.753, 95% CI 0.417-1.362, P=0.349; In MR-Egger model, B=0.642, 95% CI : 0.144-2.862, P=0.563; In WME model, B=0.369, 95% CI: 0.146-0.993, P=0.035. Cochran's Q test results showed that there was no heterogeneity in instrumental variables of MR analysis (P>0.05), and there was no pleiotropy in MR-Egger based on intercept (P>0.05). MR-PRESSO results showed that there was no outlier (Gglobal>0.05).

The second phase is to use the variables related to hemorrhoids discovered in the first phase as independent variables and hemorrhoids as the dependent variable for logistic regression analysis. The regression coefficients of the obtained independent variables are used as weights to establish a hemorrhoid risk prediction model($Logit(P)=-1.4+0.03X_1+0.79X_2-0.71X_3+1.02X_4+0.65X_5-0.45X_6$, where X_1 represents age, X_2 represents education level, X_3 represents dietary taste, X_4 represents constipation, X_5 represents chronic gastritis, and X_6 represents the genotype of the rs34221221 locus of the FOXC2 gene). The sensitivity of this model is 0.561, the specificity is 0.645, the cut-off value is 0.6, the accuracy of predicting the incidence of hemorrhoids is 76%, the accuracy of predicting the incidence of non hemorrhoids is 90%, and the accuracy of predicting the incidence of hemorrhoids in the entire group is 87.8%.

In conclusion, This study found that age, education level, constipation, and chronic gastritis can increase the risk of hemorrhoids, while a bland diet can reduce the risk of hemorrhoids; the rs34221221 polymorphism of the FOXC2 gene is associated with susceptibility to hemorrhoids, people with CT genotype are less susceptible to hemorrhoids than those with CC genotype. The final established model is $Logit(P)=-1.4+0.03X_1+0.79X_2-0.71X_3+1.02X_4+0.65X_5-0.45X_6$, when the predicted value is greater than 0.6, the predicted result indicates the possibility of developing hemorrhoids, and if it is less than 0.6, it indicates the possibility of not developing hemorrhoids.

Keyword : Hemorrhoids, FOXC2 gene, Risk factors, Prediction model

ACKNOWLEDGEMENTS

Time flies, and in a twinkling of an eye, the three-year doctoral study will soon be over. I clearly remember the excited temperament when I got the admission notice from the school of public health of Mahasarakham University, the nervous look when I was afraid of not understanding and speaking good English in my first class, and the fear when I went abroad to Thailand for the first time.

However, all this has passed, and I will soon graduate from Mahasarakham University. I would like to thank the faculty of public health of Mahasarakham University for providing me with the opportunity to learn. Here I not only learned new knowledge, but also learned a lot about Thai culture, customs and habits, which broadened the width and depth of my knowledge. I want to thank all the teachers who have taught me the knowledge, and thank you for your hard work and help in my study. Thank you to all the staff of the faculty of public health, especially associate professor Vorapoj Promasatayaprot, associate professor Suneerat Yaugyuen and teacher Nan. You are very kind and friendly, which makes me feel the warmth of home here.

I would like to thank my advisor associate professor Sumattana Glangkarn in particular. She not only teaches me scientific research skills, helps me complete research and thesis writing, but also cares about me , understands me, like a big sister, both my good teacher and friend. And my co-advisor assistant professor Nachalida Yukalang, my research advisor Dr. Rujira Nonsa-ard , thank you for your help in my research and thesis.

I want to thank my classmates for your company and help in learning and life. Having you makes me feel more at ease and happy in a foreign country.

In addition, I would also like to thank my family for their support for my study. Without their support, I cannot finish my studies.

I am happy every day when I study in the school of public health of Mahasarakham University. I sincerely wish Mahasarakham University better and better.

Huabei Wu

TABLE OF CONTENTS

Page

ABSTRACT ..... D

ACKNOWLEDGEMENTS ..... F

TABLE OF CONTENTS ..... G

LIST OF TABLES I

LIST OF FIGURES ..... J

Chapter I Introduction 1

1.1 Background 1

1.2 Research questions 3

1.3 Objectives of the Study 3

1.4. Scope of the study 4

1.5 Definition of Terms 4

1.6 Expected benefits of research 7

Chapter II Literature Review 8

2.1 Introduction to Hemorrhoids 8

2.2 Epidemiology of hemorrhoids 9

2.3 Symptoms of hemorrhoids 10

2.4 Risk factors related to hemorrhoids 12

2.5 Pathogenesis of hemorrhoids 18

2.6 Diagnosis of hemorrhoids 27

2.7 Treatment of Hemorrhoids 29

2.8 Prevention of Hemorrhoids 38

2.9 Introduction to Mendelian Randomization method (MR) 41

2.10 Conceptual framework 45

CHAPTER III MATERIALS AND METHODS 48

3.1 Study design 48

3.2 Technical Flowchart 50
3.3 Study population and samples 51
3.4 Sampling 52
3.5 Research instrument and Methods. 52
3.6 Statistical analysis 62
3.7 Research Progress Schedule 63
3.8 Regarding data missing and data organization 63
3.9 Ethical approval 64
CHAPTER IV RESULTS 65
4.1 Questionnaire data analysis 65
4.2 Analysis of the causal relationship between BMI and hemorrhoids(Mendelian randomization analysis) 70
4.3 Comparison of blood lipids between the two groups 75
4.4 Results of association analysis between rs34221221 polymorphism of FOXC2 gene and hemorrhoids. 75
4.5 Establishment of hemorrhoids risk prediction model and evaluation 82
CHAPTER V DISCUSSIONS 84
5.1 Summary of research results 84
5.2 Discussion 85
5.3 Summary, Strengths, limitations and suggestions of the study 97
5.4 Conclusion 98
REFERENCES 99
Reference 100
Appendix 1 Ethics Review Certificate 115
Appendix 2 Questionnaire 117
Appendix 3 East Asian population instrumental variables 122
Appendix 4 South Asian population instrumental variables 124
Appendix 5 Concentration and purity of sample DNA 126
BIOGRAPHY 150

LIST OF TABLES

Page

Table 1 Main experimental instruments 53
Table 2 Primer information of PCR amplification 56
Table 3 PCR amplification system 56
Table 4 PCR amplification program 57
Table 5 MIX Liquid Preparation Method 57
Table 6 Research progress schedule 63
Table 7 Analysis results of demographic data 66
Table 8 Correlation analysis results of various factors and hemorrhoids 67
Table 9 Independent variables and their assignments in logistic regression analysis 68
Table 10 Odds ratio and 95% confidence interval of binary logistic regression for each factor 69
Table 11 Two sample MR analysis results of the causal relationship between BMI and hemorrhoids 70
Table 12 Sensitivity and heterogeneity test results of two sample MR analysis of BMI and hemorrhoids 72
Table 13 comparison results of blood lipids between the two groups(Unit: mmol/l) 75
Table 14 Test results of Hardy-Weinberg genetic equilibrium law 77
Table 15 Distribution of allele frequency and genotype frequency in case group and control group and their relationship with hemorrhoids susceptibility 78
Table 16 Stratified analysis of the relationship between rs34221221 polymorphism and hemorrhoids susceptibility 79
Table 17 The interaction between the rs4221221 polymorphism of FOXC2 gene and various external factors on hemorrhoids 81
Table 18 correlation analysis between independent risk factors and hemorrhoids 82

LIST OF FIGURES

Page

Figure 1 Three core assumptions of instrumental variables in Mendelian randomization method 43

Figure 2 Conceptual Framework 47

Figure 3 Technical flowchart of case-control study 50

Figure 4 Research design 60

Figure 5 Scatter plot of MR analysis on the relationship between BMI and hemorrhoids. 71

Figure 6 Funnel plot for MR analysis of the relationship between BMI and hemorrhoids. 72

Figure 7 Sensitivity analysis of the "leave-one-out "method in MR analysis of BMI and hemorrhoids in East Asian populations. After removing each SNP, the overall error line did not change much (all error lines were to the right of 0), indicating that the results were robust. 73

Figure 8 Sensitivity analysis of the "leave-one-out "method in MR analysis of BMI and hemorrhoids in South Asian populations. After removing each SNP, the overall error line did not change much (all error lines were to the left of 0), indicating that the results were robust. 74

Figure 9 The whole genome DNA agarose gel electrophoresis map shows that the first lane from left to right is mark, and the second lane to the sixth lane is the whole genome DNA. 76

Figure 10 Map of Sanger sequencing results for rs34221221 polymorphic loci 77

Figure 11 ROC curve of hemorrhoid risk prediction model 83

Chapter I
Introduction

1.1 Background

Hemorrhoids are one of the most common anorectal diseases, whose prevalence, multiplicity are in consensus, and there are ten claims of nine hemorrhoids in Chinese folk. Data showed that the prevalence of anorectal disease was 50.10% in urban and rural residents of China, among whom the prevalence of hemorrhoids was up to 49.14%, with adults predominating [1].

Hemorrhoids can be divided into internal hemorrhoids, external hemorrhoids and mixed hemorrhoids according to different locations. At present, it is believed that internal hemorrhoids are pathological changes or displacements of the supporting structure, vascular plexus and arteriovenous anastomotic branches of the anal pad (anal vascular pad). External hemorrhoid is the pathological expansion or thrombosis of the subcutaneous vascular plexus at the far side of the dentate line. Mixed hemorrhoid is a mixture of internal hemorrhoids and external hemorrhoids [2]. The most widely accepted classification is Go ligers' classification, which divides hemorrhoids from light to heavy into first degree, second degree, third degree and fourth degree hemorrhoids [3].

This disease can cause many troubles and pains to patients, and common symptoms are bleeding during defecation, perianal swelling, itching, and pain. Hemorrhoids have many risk factors for morbidity, and constipation and prolonged mental tension are commonly recognized as causes of hemorrhoids [4]. However diarrhea has also been shown to be a risk factor for the development of hemorrhoids [5], and pregnancy also results in congestion of the anal pads to produce hemorrhoids, which resolve spontaneously shortly after production [6]. Certain occupational factors are also responsible for producing hemorrhoids. In addition, many dietary factors, including a low fiber diet, spicy food, and alcohol intake are also associated with hemorrhoids, but the reported data are inconsistent [7]. It has also been reported that obesity and high body mass index can be considered independent risk factors for hemorrhoids [8-9].

The pathogenesis of hemorrhoids has not been uniformly recognized, and

currently the accepted theories of hemorrhoid genesis in the international community are: the theory of varicose veins, the theory of moving down the anal pads, the theory of vascular proliferation, the theory of bacterial infection, the theory of pelvic floor motility, etc., of which the theory of moving down the anal pads, the theory of vascular proliferation, and the theory of varicose veins are the most recognized [10]. In China, numerous scholars have endorsed the theory of varices, which holds that hemorrhoids are a chronic disease of one or more soft venous masses formed by varicose veins at the base of the anorectum and along the venous plexus of the anal mucosa. Some recent studies have shown a close relationship between the occurrence and genetics of hemorrhoids. According to an epidemic survey, the prevalence of hemorrhoids among residents with a family history of anorectal disease was 81.00%, which was much higher than that among patients without a family history (45.14%), and the occurrence of hemorrhoids and genetic genes have a close relationship [11].

If the genetic factors associated with the development of hemorrhoids can be identified, and the interaction between these genetic factors and external environmental factors has an impact on the development of hemorrhoids, then corresponding preventive measures can be taken in the population, leading to a reduction in the prevalence of hemorrhoids and a reduction in patient pain and a corresponding economic burden.

Forkhead box C2 (FOXC2) or mesenchyme forkhead box 1 (MHF1) is a transcription factor belonging to the human forkhead or winged helix family. It has been confirmed that FOXC2 is closely related to the pathogenesis of varicose veins [12,13]. Polymorphic sites highly related to varicose veins and hemorrhoids have been found in the proximal upstream region of FOXC2 gene [12]. Therefore, FOXC2 gene may also be associated with hemorrhoids, which is a genetic factor in the occurrence and development of hemorrhoids. However, there is no further study on the association between FOXC2 gene and hemorrhoids in East Asian population.

Therefore, this study intends to explore the relationship between the FOCX2 gene polymorphism and its cross effects with major risk factors and hemorrhoids, and initially establish a hemorrhoids risk prediction model with genetic factors, which provides clues and theoretical basis for further research on hemorrhoids prevention and treatment. Since race and nationality are the confounding factors in genetic factor

research, genetic research should be conducted in the same ethnic group with the same genetic background. Therefore, this study intends to select Zhuang people living in Guangxi, China, as the research object to eliminate the interference of race and ethnic factors on the research results.

The Zhuang nationality is distributed in 31 provinces, autonomous regions and municipalities directly under the central government in China, mainly in the south. Guangxi Zhuang Autonomous Region is the main distribution area of Zhuang nationality, with a total of 15.722 million people (2023). Most of the Zhuang people live in mountainous areas, and most of them live in concentrated communities. they have the same genetic background, so the national population is more suitable for the study of hemorrhoids genetic factors.

In addition, Zhuang people eat rice as their staple food, like to eat sour and pickled food, and often eat vegetables and meat after pickling. Zhuang people like to drink and entertain guests with wine. Zhuang people often carry heavy objects on their shoulders or shoulders when working.

The occurrence of anorectal diseases is closely related to gender, work, life, diet and so on. Research shows that work posture, bad life behavior, excessive drinking, often eating spicy food and pickled food are closely related to the occurrence of hemorrhoids[14]. The Zhuang people just have these characteristics, so it is speculated that the prevalence of hemorrhoids in this nation should not be low. However, there is no research on hemorrhoids in the Zhuang population. Based on the above reasons, this study selected the Zhuang population as the research object.

1.2 Research questions

How to establish an effective hemorrhoid risk prediction model containing genetic and risk factors?

1.3 Objectives of the Study

1.3.1 To explore the risk factors of hemorrhoids in Guangxi Zhuang population.

1.3.2 To explore the association of FOXC2 polymorphism and its interaction with other risk factors with the susceptibility to hemorrhoids in Guangxi Zhuang population.

1.3.3 To explore the causal association of body mass index and hemorrhoids.

1.3.4 To establish a prediction model for hemorrhoids.

1.4. Scope of the study

1.4.1 The research contents include the risk factors of hemorrhoids, the causal relationship between body mass index and hemorrhoids，the association between FOXC2 gene polymorphism and hemorrhoids, and the risk prediction model of hemorrhoids.

1.4.2 The study population is Zhuang people in Guangxi, China. The number of samples in the case group and the control group was 200.

1.4.3 The research setting is a provincial general hospital in Guangxi, China. All participants were selected from the hospital

1.4.4 The research period is from November 2022 to December 2023.

1.5 Definition of Terms

1.5.1 Hemorrhoids, it refers to the soft venous mass formed by the expansion and flexion of the venous plexus under the mucosa and anal skin at the end of the human rectum.

1.5.2 Gene polymorphism, it refers to the simultaneous and frequent presence of two or more discontinuous variants or genotypes or alleles in a biological population (This study focuses on the polymorphism of the rs34221221 locus of the FOXC2 gene).

1.5.3 Susceptibility, it refers to the state of being very likely to be influenced, harmed or affected by something. In this study, it refers to the likelihood of individuals developing hemorrhoids.

1.5.4 Prediction model, it is a statistical model that estimates the probability of a certain disease or a certain outcome according to a series of individual characteristics.

1.5.5 Mendelian randomization(MR), it is a data analysis technique used in epidemiological research to evaluate the causal relationship between exposure factors and outcomes, using genetic variations that are strongly correlated with exposure factors as instrumental variables.

1.5.6 Body mass index(BMI), this indicator is calculated by dividing weight (kilograms) by the square of height (meters), and the ratio obtained from this formula

can to some extent reflect human density. In China, the adult body mass index falls within the normal range of 18.5 to 23.9kg/m² .

1.5.7 Hardy-Weinberg equilibrium means that in an infinite random mating population without mutation, migration and selection, the gene frequency and genotype frequency will remain unchanged generation by generation. It is the most important principle in population genetics.

1.5.8 Sensitivity, it can reflect the ability of the evaluation method to accurately determine the actual disease case as having a certain disease. In this study, it was used to reflect the ability of the hemorrhoid prediction model to accurately predict the predicted person as having hemorrhoids.

1.5.9 Specificity, it can reflect the ability of the method to be evaluated to correctly judge the research object who is not actually sick as not suffering from a disease. In this study, it is used to reflect the ability of hemorrhoids prediction model to correctly predict the predicted person as not suffering from hemorrhoids.

1.5.10. Youden index, also known as the Correct index, indicates that the method to be evaluated can correctly judge the total ability of patients and non patients. It is equal to the sum of sensitivity and specificity minus 1. When the sensitivity and specificity are the largest, the Youden index is the largest. It means, at this value, the judgment ability of the method to be evaluated is the strongest. Therefore, this study uses the maximum value of the Youden index to determine the cut-off value of the prediction model. It means when the maximum value of the Youden index is taken, the corresponding model predictive value is the value to judge whether the predicted person will suffer from hemorrhoids. When the predictive value is greater than this value, it means that the predicted person has the risk of suffering from hemorrhoids. If it is less than this value, there is no risk of suffering from hemorrhoids.

1.5.11 Positive predictive value, which represents the likelihood of a subject developing a disease when their predicted result is positive. In this study, it represents the likelihood of hemorrhoids if the predicted value of the subject is greater than the cut-off value.

1.5.12 Negative predictive value, which represents the likelihood of excluding a subject's disease if their prediction result is negative. In this study, it represents the likelihood of the predicted person not developing hemorrhoids when their predicted

value is less than the cut-off value.

1.5.13 The cut-off value, it is the judgment standard, which is the boundary value for judging the positive and negative of the test. It determines the normal value of a certain index to distinguish between normal and abnormal. A reasonable cut-off value is to make the authenticity of the test the best, to make the sensitivity and specificity as high as possible, or to maximize the correct index (Youden index).

1.5.14 ROC curve, namely the receiver operating characteristic curve, is a curve made with sensitivity as the ordinate and 1-specificity as the abscissa, indicating the relationship between sensitivity and specificity. It is the most commonly used method to determine the cut-off value.

1.5.15 Definition of variables in the questionnaire:

1) Higher education, in this study, it refers to the education of junior college or above.

2) Family income, it refers to the per capita monthly income of a household. According to statistics from the Nanning Municipal Bureau of Statistics in Guangxi, the per capita disposable income of urban residents in Nanning in 2022 was 42636yuan, which is an average of about 4000 yuan per month (http://tj.nanning.gov.cn/tjsj/tjgb/t5575662.html).

3) Drinking, it refers to drinking more than 50 milliliters each time, more than once a month, is considered to be drinking. Guangxi Zhuang people generally drink rice wine, which has an alcohol content of about 20%. According to Chinese literature reports, consuming more than 10 milligrams of alcohol each time can be harmful to the body. Therefore, it is calculated that drinking more than 50 milliliters each time can be harmful to the body[15]. Therefore, in this study, drinking more than 50 milliliters per time is referred to as alcohol consumption.

4) Regular eating was defined as eating more than three times a week, while occasional eating was defined as eating less than two times a month[16].

5) Eating more meat than vegetables in each meal is defined as mainly meat based, on the contrary, it is defined as vegetarian oriented, and eating as much meat as vegetables was defined as uniformity of meat and vegetables[16].

6) Working in a sitting position for more than 4 hours a day is defined as working in a sitting position. This definition is based on the literature on the impact of

prolonged sitting on mortality[17-19].

7) Activities with more rapid breathing than normal, at least three times a week, at least 10 minutes each time, are defined as high strength physical activity; Moderate strength physical activity refers to the activity that takes more effort to breathe than normal, at least 3 times a week, at least 10 minutes each time; Low strength physical activity mainly refers to walking, including walking at work, at home, from somewhere to somewhere, entertainment, games or leisure. These definition were developed based on the International Physical Activity Questionnaire(IPAQ)[20].

8) Constipation refers to poor defecation at least in the past 3 months, which is characterized by less frequent defecation, difficult defecation, or both[21].

9) Chronic gastritis was determined according to the history of the subjects.

1.6 Expected benefits of research

The hemorrhoid risk prediction model can be used to predict hemorrhoids in high-risk groups, and corresponding preventive measures can be taken according to the prediction results, so as to reduce the prevalence of hemorrhoids and reduce the economic burden of diseases caused by hemorrhoids.

Chapter Ⅱ

Literature Review

The research is entitled: *Association between FOXC2 gene polymorphism and susceptibility to hemorrhoids*. The aim of this study is to explore the genetic factors of hemorrhoids and their interaction with external environmental factors, and to establish a hemorrhoid risk prediction model. The researchers reviewed the concept, pathogenesis and mechanism, prevalence, related risk factors, treatment and prevention of hemorrhoids, and the introduction to Mendelian Randomization method as follows:

2.1 Introduction to Hemorrhoids

2.2 Epidemiology of hemorrhoids

2.3 Symptoms of hemorrhoids

2.4 Risk factors related to hemorrhoids

2.5 Pathogenesis of hemorrhoids

2.6 Diagnosis of Hemorrhoids

2.7 Treatment of Hemorrhoids

2.8 Prevention of Hemorrhoids

2.9 Introduction to Mendelian Randomization method

2.10 Conceptual framework of the study

2.1 Introduction to Hemorrhoids

Hemorrhoids are one of the most common anal and rectal diseases in clinical practice. Hemorrhoids have been extensively documented in traditional Chinese medicine literature. Thomson proposed the modern concept of hemorrhoids in 1975 [22]: Hemorrhoids are pathological hypertrophy of the anal pad at the lower end of the rectum. Hemorrhoids can be divided into internal hemorrhoids, external hemorrhoids, and mixed hemorrhoids according to their location of occurrence. Internal hemorrhoids are soft venous masses formed by the enlargement, varicose veins, and congestion of the hemorrhoid venous plexus above the dentate line of the anus and under the mucosa at the end of the rectum. External hemorrhoids are diseases that occur below the dentate line, caused by the dilation or rupture of the external venous plexus, recurrent

inflammation, blood stasis, thrombosis, or tissue proliferation. According to the pathological characteristics of the organization, external hemorrhoids can be divided into four categories: connective tissue external hemorrhoids, thrombotic external hemorrhoids, varicose external hemorrhoids, and inflammatory external hemorrhoids. Mixed hemorrhoids are the fusion of internal hemorrhoids and corresponding external hemorrhoid vascular plexus across the dentate line into a whole [23-24]. Hemorrhoids can also be classified based on the location and severity of prolapse. The most widely accepted classification is the Goligers classification system, which divides hemorrhoids from mild to severe into first degree, second degree, third degree, and fourth degree hemorrhoids [25].

2.2 Epidemiology of hemorrhoids

The universality and multiplicity of hemorrhoids are recognized. There is a saying that ten Chinese people have nine hemorrhoids. The general survey of diseases organized by the Chinese Association of Anorectal Medicine from 1975 to 1997 showed that the total incidence of anorectal diseases in China was 59.1% (33,837/57,297), of which the incidence rate of hemorrhoids was the highest (51.56%), accounting for 87.25% of all anorectal diseases, of which the incidence rate of internal hemorrhoids was the highest (52.23%), followed by mixed hemorrhoids (21.05%) and external hemorrhoids (14.04%). An epidemiological survey of common anorectal diseases among urban residents in 31 provinces (autonomous regions, municipalities directly under the Central Government) in mainland China carried out from 2013 to 2014 showed that 51.14% (21,885/42,792) of the total survey population reported anorectal diseases among adults, with the highest incidence rate of hemorrhoids (50.28%) [26]. A recent epidemiological survey on residents aged 18-80 in five rural communities in Fengxian District, Shanghai showed that the total incidence rate of hemorrhoids among the surveyed population was 40.27% (2416/6000), with mixed hemorrhoids and external hemorrhoids having significantly higher incidence rates than internal hemorrhoids. The majority of hemorrhoids (80.63%) were diagnosed as damp heat descending syndrome and spleen deficiency qi sinking syndrome in traditional Chinese medicine. After analyzing the incidence of hemorrhoids at different age stages, it was found that the incidence of hemorrhoids increases with age, with the highest incidence occurring in

the age group of 35-59 years old [27-28]. The epidemiological survey results in the United States show that the incidence of hemorrhoids ranges from 4% to 55%, with nearly 4 million visits per year. The risk of hemorrhoids is highest among people aged 45 to 65 [29-31], while the incidence rate of hemorrhoids in major states is uneven [32]. According to research reports in South Korea, the overall prevalence of hemorrhoids is 14.4%, with females (15.7%) having a higher prevalence than males (13.0%) [33]. According to the research report of Austria, the incidence rate of hemorrhoids is 38.9%, of which Grade I hemorrhoids have the largest number, accounting for 72.89% of the total number of patients; The age group with the highest incidence rate is 45 to 49 years old, with a prevalence rate of 60.87% [34]. Hemorrhoids are also the most common rectal disease in Italy, with an estimated prevalence of 4.4%, and the peak age of onset is between 45 and 65 years old. In addition, 50% of the population aged 50 and above have experienced issues related to hemorrhoids [35]. In a survey conducted in Ethiopia, it was found that the overall prevalence of hemorrhoids in the area was 13.1%, with 64.1% of patients suffering from grade I hemorrhoids. The prevalence of hemorrhoids in females was 18.8%, higher than 7.7% in males, and 68.7% of female patients had a history of childbirth [36]. The incidence rate of hemorrhoids in Britain is 13.3% every year, but this figure is only the statistics of patients coming to see a doctor. The number of asymptomatic or asymptomatic hemorrhoid patients without statistical data is about three times higher [37]. The incidence of hemorrhoids among Russians has also reached 13.4% [38].

Brief summary

In general, the prevalence of hemorrhoids has significant regional differences, which varies from country to country, while the prevalence in China is relatively high. The regional differences in the prevalence of hemorrhoids may be related to climate, water and soil, food, eating habits, living habits, and even race and nationality. It may also be related to the diagnostic criteria, preventive health care, and people's awareness of hemorrhoids in various countries and regions.

2.3 Symptoms of hemorrhoids

The symptoms of hemorrhoids may overlap with those of other anorectal diseases, such as skin growths, abscesses, fissures, polyps, inflammatory bowel disease, and

anorectal tumors. The most common manifestation of hemorrhoids is painless rectal bleeding that occurs during or immediately after defecation. Usually, patients observe mild to moderate bright red bleeding on feces or toilet paper [39-42]. Repeated bleeding may lead to secondary iron deficiency anemia. Sometimes, hemorrhoids may cause massive bleeding and require emergency hospitalization and blood transfusions [43,44]. Other symptoms include swelling, prolapse, infection, perianal skin irritation, itching, and discomfort. In addition, prolapse of large hemorrhoids may cause a feeling of rectal fullness and, in rare cases, can lead to difficulty in defecation. For uncomplicated hemorrhoids, pain is rare. However, the presence of pain symptoms may indicate the presence of other conditions, such as fissures, abscesses, perineal neuropathy, transient rectal pain, and anorectal tumors. Regardless of the bowel movement, acute edema and thrombosis of external hemorrhoids can lead to acute anal pain.

Below is a summary of the main clinical manifestations of hemorrhoids according to their classification:

The main clinical manifestations of internal hemorrhoids are bleeding, prolapse, perianal dampness, itching, and may be accompanied by thrombosis, entrapment, strangulation, and difficulty in defecation.

The surface of external hemorrhoids is covered by skin and is not prone to bleeding. The main clinical manifestations are soft tissue masses in the anal area, which may cause discomfort, dampness, itching, or foreign body sensation. If blood clots or inflammation occur, there may be pain.

The main clinical manifestation of mixed hemorrhoids is the coexistence of symptoms of internal and external hemorrhoids, and in severe cases, it manifests as prolapse of circular hemorrhoids.

Brief summary

Although hemorrhoids are not an immediate life-threatening emergency, their symptoms can also cause discomfort to patients, thereby affecting the quality of work and life. Due to its high incidence rate and the improvement of people's health awareness, the medical expenses of medical examination, diagnosis, treatment, and hospitalization caused by hemorrhoids are also constantly increasing, and the disease burden cannot be underestimated.

2.4 Risk factors related to hemorrhoids

Regarding the triggering factors of hemorrhoids, the *Neijing* in traditional Chinese medicine was the first to propose the concept of "*therefore satiety*". Later, the *Zhu bing yuan Hou Lun* comprehensively proposed that the causes of hemorrhoids include feeling wind evil, improper diet, emotional factors, careless sexual activity, and fatigue. Modern medicine believes that hemorrhoids are closely related to anatomy, age, diet, occupation, bowel movements, genetics, living environment, education level, lifestyle habits, sleep status, physical exercise, and pregnancy history. Foreign literature studies have also found that factors that increase the risk of developing hemorrhoids mainly include dietary fiber intake, constipation, diarrhea, pregnancy, prolonged sitting, and excessive stress load [45]. Chen Ping[46] et al. found through investigation that irregular lifestyle factors such as long-term lack of sleep, excessive fatigue, frequent staying up late, irregular dietary habits such as hunger and satiety, overeating, and dietary preference are related to the occurrence of hemorrhoids. Tan Naizhi [47]et al. found through investigation that lack of exercise, poor mental health, and prolonged bowel movements are related factors for the occurrence of hemorrhoids. Tian Yu[48] et al. found that a large proportion of patients with hemorrhoids have a history of hemorrhoids in their immediate family members. Amina [49] et al.'s research has shown that frequent consumption of spicy and stimulating foods, high-fat foods, and minimal intake of vegetables and fruits can easily lead to hemorrhoids. Below is a summary of the factors that affect hemorrhoids that have been extensively studied.

2.4.1 External factor

2.4.1.1 Poor bowel habits and abnormal bowel movements

Many individuals have varying degrees of bad bowel habits or abnormal bowel movements[50]. The so-called bad bowel habits include: prolonged bowel movements can easily cause congestion in the anus and cecum, leading to diseases; Smoking while using the toilet can buffer the brain's bowel reflex, which can easily cause constipation; Excessive force during defecation may increase unnecessary burden and local congestion on the muscles of the cecum, anus, and pelvic floor, leading to the occurrence and spread of diseases, and so on. Abnormal bowel movements, including diarrhea and constipation, are important causes of hemorrhoids. Constipation is the biggest culprit. Long term retention of toxic substances in the cecum can not only cause

colon cancer, but also lead to fecal accumulation, affecting blood circulation. Forcefully removing dry fecal masses will inevitably cause significant pressure on the anus, leading to a series of pathological changes such as blood stasis, swelling, and fissures. Research has shown that patients with constipation are more likely to develop hemorrhoids. This is also related to the degeneration of the supporting tissue in the anal canal, as well as the tearing of the elastic supporting tissue caused by prolonged tension during defecation and defecation. This can lead to distal displacement of the anal pad and the development of hemorrhoids [51,52]. In addition, studies have also shown that hard stool and increased intra-abdominal pressure can hinder venous return, leading to congestion of the hemorrhoid plexus and arteriovenous anastomosis at the anorectal junction, thereby leading to the development of hemorrhoids [53].

But there are also inconsistent research results. A study have shown that hemorrhoids and constipation have different epidemiological characteristics, including age, gender, race, and socio-economic status, which raises doubts about constipation as a risk factor[54]. A study by the Milwaukee Veterans Medical Center found that diarrhea rather than constipation is associated with hemorrhoids[30]. In a study using national VA data, the comorbidities associated with hemorrhoids were all diarrhea related diseases (colitis, malabsorption, intestinal bypass surgery, chronic pancreatitis) rather than constipation[55].

2.4.1.2 Long term mental stress

Long term mental stress is usually considered to be the cause of hemorrhoids. In modern society, due to the acceleration of people's pace of life, people have been in a state of high mental tension for a long time, resulting in a long-term high intestinal load. In this state, constipation will occur, resulting in adverse stimulation of the anus and increasing its pressure. Over time, it is often easy to cause anorectal tail vein abnormal congestion, and then cause hemorrhoids [4].

2.4.1.3 Pregnancy

Pregnancy can lead to anal pad congestion, resulting in hemorrhoids, which will subside soon after birth [6]. During pregnancy, progesterone hormone can relax the smooth muscle of the whole body. This is very important for maintaining pregnancy and preventing premature uterine contraction. This can also lead to other sequelae of the gastrointestinal tract, including the decline of the whole intestinal motility (resulting

in delayed gastric emptying and contraction), as well as the reduction of the tonic contraction of sphincter (including the lower esophageal sphincter that causes esophageal reflux and heartburn), and possibly the anal sphincter [56-58].

2.4.1.4 Occupational factors

In modern medical anatomy, it has long been proved that human rectum and its branches do not have venous valves, and there is no "valve" for venous blood flow. Because human beings walk upright, when venous tissue fluid flows back to the heart from bottom to top through the rectum under the action of gravity, the tail of human anorectum is prone to blood stasis, which will lead to anal mucosal venous plexus lesions, varicose, hyperplasia and other phenomena, and finally form hemorrhoids. However, some occupations need to maintain posture for a long time, often standing or sitting, and anorectal venous reflux is more likely to be difficult. Blood is deposited at the bottom of the rectum, which is more likely to lead to anal mucosal congestion and hemorrhoids [59]. Among the people with different employment status, the prevalence rate of hemorrhoids in school students is the lowest, which is 4.88%. According to the research of Chen Ping et al, the prevalence rate of hemorrhoids in employees is 44.42%, among which the prevalence rate of drivers is the highest, which is 65.00%. The prevalence rate of people working in high temperature and humidity environment was 71.10%, which was higher than that of people working in comfortable environment and general environment; The prevalence of hemorrhoids is different in different weight-bearing work. The higher the degree of weight-bearing work, the higher the prevalence of hemorrhoids[46].

2.4.1.5 Dietary factors

Many dietary factors, including low fiber diet, spicy food and alcohol intake, are also related to hemorrhoids, but the reported data are inconsistent [7]. Whether the diet pattern or diet matching is reasonable is an important factor affecting the occurrence and aggravation of hemorrhoids. For example, the quality of food, the type and quantity of vegetables, the content of protein, fat, starch and cellulose, and the amount of water intake will directly affect the composition of feces, leading to anorectal and Cecal Diseases. For people who drink alcohol or eat spicy food for a long time, the incidence of anorectal disease is significantly increased, because wine and spicy food can stimulate gastrointestinal mucosa, cause vasodilation and colon dysfunction.

2.4.1.6 Obesity and high body mass index

It has also been reported that obesity and high body mass index can be regarded as independent risk factors for hemorrhoids [8-9]. This may be due to increased intra-abdominal pressure due to high body weight and visceral fat, which is thought to cause congestion in the veins of the distal rectum. Obesity can induce the release of inflammatory cytokines and acute phase proteins, and ultimately activate the innate immune system and affect metabolic homeostasis, leading to the formation of hemorrhoids. At present, there are relatively few studies on overweight, obesity and body mass index, and the relevant mechanisms have not been clarified.

2.4.1.7 Depression

This study found that depression and hemorrhoids have independent associations [33]. Eating disorders and physical activity in patients with depression may be a possible mechanism of the association between depression and hemorrhoids [60,61]. Conversely, it seems that the impact of symptomatic hemorrhoids on the quality of life and mood may also lead to depression. However, at present, people know little about the relationship between hemorrhoids and depression, and it seems necessary to carry out further research[33].

2.4.1.8 Long term intestinal diseases and infectious diseases.

A literature[62] has put forward the "infection theory" on the etiology of hemorrhoids, because infectious diseases are easy to compress anal mucosal venous vessels to produce hemorrhoids, such as bacillary dysentery, typhoid fever, chronic intestinal infection, etc. Chenchangxiang and other researchers found that the detection rate of intestinal pathogenic bacteria in patients with hemorrhoids was higher than that in patients without hemorrhoids, and the number of patients with frequent diarrhea in patients with hemorrhoids was significantly higher than that in patients without hemorrhoids, which also supported the infection theory of hemorrhoids [63].

2.4.1.9 Hemorrhoids and sexual life

Male reproductive and proctology doctors have found that men with poor sexual habits or abnormal sexual activity are more prone to hemorrhoids. This is because during sexual activity, the muscles of the whole body are in a highly tense state, especially the pelvic and gluteal muscles continue to contract, which increases the resistance of blood circulation around the anus and causes blood circulation disorders.

If sexual activity is too frequent, anal veins often experience blood circulation disorders, varicose veins, congestion, protruding from the mucosa or outside the anus, leading to irreversible hemorrhoids[64].

2.4.1.10 Exercise factors

Physical exercise has a great effect on the prevention and treatment of hemorrhoids. The statistical results show that the incidence of hemorrhoids in middle-aged workers who have been engaged in sitting posture for a long time is significantly higher than others. Proper physical exercise can reduce venous pressure, strengthen the function of cardiovascular system, eliminate constipation and enhance muscle strength, which play an important role in the prevention and treatment of hemorrhoids[64].

2.4.2 Internal Factors

2.4.2.1 Age

In the study of Chinese people, after analyzing the prevalence of hemorrhoids at different ages, it was found that the prevalence of hemorrhoids increased with the increase of age, and the prevalence of hemorrhoids at the age of 35-59 was the highest [5-6]. Riss et al. Showed that the prevalence of hemorrhoids by age was the highest (60.8%) in the population between 45 and 49 years old[65]. Johanson and Sonnenberg [55] reported that the age range with the highest prevalence of hemorrhoids was 45 to 65 years old. The study of Lee J H et al. Also found that the prevalence of self-reported hemorrhoids (18.3%) and doctors' diagnosed hemorrhoids (9.7%) were the highest among the people aged 40 to 49 [33]. Although the prevalence of hemorrhoids in different regions and races is slightly different, it can be seen from the summary that the main age of hemorrhoids is about 40-60 years old. This may be related to the gradual decline of the body's morphology and system function at this age, the relaxation and weakness of anorectal nerves, blood vessels, muscles, ligaments, etc., the relaxation of fibrous tissue reduces the ability of the tissue to support and suspend the anal canal "vascular pad" and is prone to prolapse, and the distortion and congestion of blood vessels due to the loss of proper support [66]. It may also be that with the increase of age, bad lifestyle and bad behavior factors are increasing, leading to the occurrence of hemorrhoids. Or age interacts with other hemorrhoids risk factors.

2.4.2.2 Gender

The prevalence of hemorrhoids was not consistent between different genders. Haas

et al.[67] and Johanson and Sonnenberg [30] reported that there was no difference in the prevalence of hemorrhoids between men and women, while Riss et al. reported that the prevalence rate of women (40.78%) is higher than that of men (37.09%)[34]. In the study of Lee J H et al., the prevalence of self-reported hemorrhoids in women was higher than that in men, while there was no difference in the prevalence of hemorrhoids diagnosed by doctors between women and men. The researchers believe that the reason for this finding is that women are more likely than men to avoid receiving anal examination to diagnose hemorrhoids. Therefore, they believe that the prevalence of hemorrhoids in men seems unlikely to be higher than that in women [33] . In short, the relationship between gender and the incidence of hemorrhoids is still inconclusive, and the results reported in different studies are different, which needs to be confirmed by larger sample size data.

2.4.2.3 Family history

The investigation found that hemorrhoids also have a genetic tendency. The mechanism may be related to genetic defects, multiple colon polyps, P-J syndrome and other genetic anorectal diseases. In addition, it may also be related to abnormal embryonic development. The statistical results showed that 77.81% of the patients with genetic factors were hospitalized, indicating that the patients with hemorrhoids caused by genetic factors are generally more serious and need hospitalization to alleviate or recover. According to the epidemiological survey, the prevalence of hemorrhoids in residents with a family history of anorectal disease was 81.00%, which was much higher than that in patients without a family history (45.14%), and the occurrence of hemorrhoids was closely related to genetic genes[11]. Chen changxiang and other researchers also said that the incidence of hemorrhoids in the children of parents with hemorrhoids increased. The mechanism may be that the venous wall is congenital weak, poor resistance, and can not tolerate vascular pressure, which can cause hemorrhoids over time[63].

Brief summary

In short, hemorrhoids is a chronic disease with a long formation period. Hemorrhoids have many complicated causes in daily life. Long term bad habits and physical problems may lead to hemorrhoids. At present, the research on the etiology of hemorrhoids is mostly limited to observational research, which can find out the related

risk factors of hemorrhoids, but it has not yet been able to verify that these factors are the related etiology of hemorrhoids, let alone clarify the pathogenesis of hemorrhoids caused by these risk factors. Therefore, more efforts and further research are needed on the etiology and pathogenesis of hemorrhoids.

2.5 Pathogenesis of hemorrhoids

2.5.1 Hemorrhoids pathogenesis theory

There is no unified understanding of the pathogenesis of hemorrhoids. At present, internationally recognized hemorrhoids theories include varicose vein theory, anal pad downward movement theory, vascular proliferation theory, bacterial infection theory, pelvic floor dynamics theory, etc. Among them, the theory of anal pad downward movement theory, the theory of vascular proliferation and the theory of varicose veins have the highest recognition [68] .

2.5.1.1 pad downward movement theory

In 1975, Thomson[69] based on the work of Gass et al. [70], proposed that the rupture and degeneration of anal submucosal supporting tissue led to the formation of hemorrhoids, thus basically forming the theory of anal pad downward. The anal pad is composed of blood vessels, smooth muscle, connective tissue and elastic fibers, which can assist the internal and external sphincter to maintain anal canal closure under physiological conditions. The theory of anal pad downward movement holds that when the fixed supporting structure of anal pad is damaged, the anal pad will move downward and come out, thus forming hemorrhoids [71].

The theory of anal pad downward movement can well explain the typical clinical manifestations of hemorrhoids, but it is difficult to explain the phenomenon that some hemorrhoids only bleed without prolapse, nor can it explain the good therapeutic effect of various microcirculation regulating drugs on early hemorrhoids

2.5.1.2 Neovascularization theory

Vascular proliferation theory: In the 19th century, many scholars discovered that hemorrhoids are a very thick vascular tumor like sponge like tissue, believing that this structure plays a certain role in the self-control mechanism of the anus and helps to close it. In the 1960s, it was confirmed that there was arteriovenous communication in the tissue, indicating that the tissue may have erectile function, which can enhance the

self-control ability of the anus. Some scholars speculate that hemorrhoids originate from the proliferation of this sponge like tissue, but this speculation also lacks evidence because there are no signs of vascular proliferation in any specimen. Although the theory of vascular proliferation lacks evidence, the discovery of arteriovenous communication can explain why hemorrhoids bleed bright red instead of dark red, indicating that hemorrhoids are not simply venous diseases [71].

In the new angiogenesis theory, a large number of new blood vessels can accumulate to form hemorrhoids, which can sag and prolapse with the further increase of hemorrhoids. The new angiogenesis theory can explain the clinical symptoms such as mass and prolapse, but it cannot fully explain the clinical manifestations of hemorrhoids.

2.5.1.3 Varicose vein theory

The theory of varicose veins can be traced back to the period of Hippocrates and Galen, which stems from the observation of scattered expansion of veins in hemorrhoids. In the 18th century, Hunter, an English doctor, found varicose veins in the removed hemorrhoids. He clearly believed that this was the cause of hemorrhoids, so venous dilation was regarded as the basic pathological change of hemorrhoids. There are two main reasons for varicose veins: one is the increase of local venous pressure, and the other is the weakness of the local venous wall. The increase of venous pressure is related to the lack of venous valve in the portal vein system and the lack of portal systemic circulation in the anal canal. In 1749, Morgagni, an Italian pathologist, proposed that anal varices were related to standing posture, and agreed that increased intra-abdominal pressure could be transmitted to anal veins[71].

Varicose vein theory can well explain the clinical manifestations of hemorrhoids, such as hematochezia and mass. Tortuous and dilated veins can gather to form vascular mass. At the same time, varicose veins are also more prone to rupture, which can rupture and bleed in the case of defecation and other conditional stimuli. However, the varicose vein theory has some limitations in explaining the symptoms of "prolapse".

In China, many scholars have accepted the varicose vein theory and believe that hemorrhoids is a chronic disease caused by varicose veins at the bottom of anus and rectum and anal mucosa. According to the varicose vein theory, the pressure of anal vein is too high, and the venous return of hemorrhoids is blocked, which leads to the

bending and expansion of hemorrhoids vein, and then forms hemorrhoids vein mass [72]. Especially in patients with portal hypertension, the venous blood flow is blocked due to the obstruction of the superior vena cava reflux, and the blood flows into the superior and inferior veins of hemorrhoids through the inferior vena cava. In the hemorrhoid nucleus of external hemorrhoids, vascular proliferation and dilation, and edema of vascular endothelial cells are often seen. This is due to discontinuous venous dilation of hemorrhoids, congestion of hemorrhoids venous plexus in anorectal mucosa, or mucosal damage during defecation, obstruction of arteriovenous anastomosis, resulting in capillary spasm, venous plexus dilation, hemorrhoids nuclear congestion and swelling [73].

There are many theories about the pathogenesis of hemorrhoids, but there is no theory that can fully explain the formation of hemorrhoids, which needs further study. Many scholars have tried to clarify the occurrence and development of hemorrhoids from the perspective of molecular biology, and many scholars believe that the occurrence and development of hemorrhoids are also closely related to genetics.

2.5.2 Molecular biological pathogenesis of hemorrhoids

At present, the related researches on the molecular biological pathogenesis of hemorrhoids include: increased expression of matrix metalloproteinase-9 (MMP-9), abnormal expression of smooth muscle actin (SMA) in vascular wall, increased expression of nitric oxide synthase (NOS) and transient receptor potential vanilloid subtype 1 (TRPV1) in hemorrhoids, new angiogenesis in hemorrhoids, down-regulation of microRNAs (miRNA, miRNA or MIR), and overexpression of vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF2), etc.

2.5.2.1 Overexpression of MMP-9 destroys the supporting structure of anal pad

MMP is a zinc dependent matrix metalloproteinase, and MMP-9 is one of its most complex MMP [74]. Qin Lei et al. [75] studied 110 cases of hemorrhoids and the corresponding control group sections and found that MMP-9 showed a high expression level in hemorrhoids, and its expression level in stage II hemorrhoids was higher than that in stage I hemorrhoids, and its expression level was positively correlated with the severity of hemorrhoids, the development of disease course and the degree of interstitial edema. This result suggested that MMP-9 was probably related to the occurrence of hemorrhoids. Daihao[76] found that MMP-9 could hydrolyze a variety of protein

components such as elastic fibers and destroy the supporting structure of anal pad. The above results suggest that the overexpression of MMP-9 destroys the supporting structure of the anal pad, which causes the anal pad to move down and prolapse, leading to the occurrence of hemorrhoids.

Further analysis showed that the expression and activity of MMP-9 were regulated by a variety of molecules. Among them, the regulatory effects of human neutrophil gelatinase associated apolipoprotein (NGAL) and tissue inhibitor of metalloproteinase (TIMPs) on MMP-9 have been studied. Serra et al.[77] found that NGAL expression increased in stage IV internal hemorrhoids. NGAL can combine with MMP-9 to form MMP-9/NGAL complex, inhibit the degradation of MMP-9, thereby improving the biological activity of MMP-9 [78], and indirectly participate in the pathogenesis of hemorrhoids. TIMPs are the main endogenous inhibitors of MMPs in tissues, which can be produced by endothelial cells and fibroblasts [79]. TIMPs can inhibit the degradation of MMP-9 by combining its N-terminal with the N-terminal of MMP-9 [80]. However, there is no research on the expression level or activity of TIMPs in hemorrhoids.

2.5.2.2 Abnormal expression of SMA induces angiogenesis in myofibrous dysplasia, resulting in destruction of anal pad supporting tissue

Myofibrous dysplasia blood vessels are a kind of blood vessels with sclerosis and dysplasia of smooth muscle, which appear in the mucosa and submucosa of hemorrhoids. Myofibrous dysplasia blood vessels invade the mucosal muscle layer, making the mucosal muscle layer loose, fibrotic or broken, and the anal pad support function is weakened, which can lead to hemorrhoids [81]. Fengdayong et al. [82] found through immunohistochemical staining that the expression of SMA in the vascular wall of myofibrous dysplasia in hemorrhoids was uneven, and no positive expression of SMA was observed in some venous walls, while the expression of SMA in the venous wall of normal rectal mucosa was uniform. This result suggested that the abnormal expression of SMA may induce the formation of blood vessels in myofibrous dysplasia, thereby damaging the mucosal muscle layer and weakening the supporting effect of anal pad, eventually lead to the occurrence of hemorrhoids.

2.5.2.3 Overexpression of NOS increases vasodilator NO, leading to varicose veins

In mammalian cells, NO can be endogenously generated by L-arginine through

NOS [83]. NOS can be divided into three types: neuronal type NOS, inducible type NOS, and endothelial cell type NOS. Neuronal type NOS and endothelial cell type NOS belong to structural type and catalyze the production of NO in a relatively constant amount; Induced NOS belongs to the inducible type and can catalyze the production of a large amount of NO when stimulated by inflammatory factors. NO has the function of dilating blood vessels, inhibiting platelet adhesion and aggregation, and is also a highly active and cytotoxic free radical. Excessive NO can cause abnormal vasodilation, distortion, and support of connective tissue deformation [84]. Han Wei et al. [85] found that the expression level of inducible NOS was significantly increased in hemorrhoid tissue. Further research by Lohsiriwat et al. [86] found that the expression levels of neuronal type NOS, inducible type NOS, and endothelial cell type NOS in hemorrhoid tissue were significantly higher than those in normal rectal tissue. In addition, in the study by Gokce et al. [87], the expression level of asymmetric dimethylarginine, the main endogenous inhibitor of NOS in the rectal mucosa of hemorrhoid patients, was significantly reduced. The above research results suggest that the expression level of NOS in hemorrhoid tissue increases, catalyzing the production of a large amount of NO, causing varicose veins in hemorrhoid tissue, and thus participating in the pathogenesis of hemorrhoids.

2.5.2.4 Overexpression of TRPV1 increases vasodilator NO, leading to varicose veins

TRPV1 is a non selective cation channel that can be expressed in neurons, endothelial cells, and other areas. Capsaicin can activate TRPV1, increasing its permeability to Na+and Ca2+. The level of TRPV1 in hemorrhoid tissue is significantly higher than that in normal rectal tissue, and it is mainly distributed in the stratified flat epithelium at the junction of the anus and rectum [88]. In addition, TRPV1 can promote the production of important vasodilator factor NO. Varela L ó pez et al[89] . treated Wistar rats with capsaicin, and TRPV1 activation increased NO production by 29% compared to the control group. The above research results suggest that the increase in TRPV1 expression level in hemorrhoid tissue is likely to be caused by promoting NO production, leading to varicose veins and the occurrence of hemorrhoids. However, there is currently no direct research indicating the relationship between the increase in TRPV1 expression level and the increase in NO generation in hemorrhoid tissue. The

mechanism by which the increase in TRPV1 expression level leads to the onset of hemorrhoids still needs further research.

2.5.2.5 Downregulation of miR-412-5p and miR-4729 expression leads to abnormal proliferation of endothelial cells and promotes neovascularization

MiRNAs are endogenous single stranded non coding RNA molecules that can regulate post transcriptional gene expression by inhibiting mRNA translation or promoting mRNA degradation [90]. The study by Song et al. [91] suggests that the occurrence of hemorrhoids may be closely related to miRNA transcription abnormalities. Upregulation or downregulation of miRNA expression can lead to abnormal expression of target genes and imbalance of certain signal transduction pathways. A study [92] found that miR-412-5p located in the delta-like homolog - iodothyroxine deiodinase 3 imprinting cluster was significantly downregulated in hemorrhoid tissue, causing high expression of target protein Xpo1, resulting in a large amount of p53 being removed from the nucleus, and the p53 p66SHC-p16 pathway being inhibited, leading to imbalanced regulation of endothelial cell cycle, proliferation of endothelial cells, and neovascularization of hemorrhoid tissue. Liu et al. [93] found that overexpression of exogenous miR-4729 in vascular endothelial cells can inhibit the expression of methyltransferase like protein 14, interfere with the methylation of tyrosine kinase 1 (Tyrosine kinase with immunoglobulin like and epidermal growth factor homology 1, Tie1) mRNA, reduce the stability of Tie1 mRNA, and inhibit the expression of the Tie1/VEGFA signaling molecule loop, Causing inhibition of endothelial cell proliferation. The expression of miR-4729 in hemorrhoid tissue is significantly downregulated, indicating a weakened ability of miR-4729 to inhibit vascular proliferation and cause neovascularization. MiRNAs play an important role in regulating normal physiological functions and the progression of various diseases in the human body, but their regulatory mechanisms in the development of hemorrhoids have not been fully studied. The existing literature suggests that the significant downregulation of miR-412-5p and miR-4729 expression in hemorrhoid tissue may be the main cause of hemorrhoid vascular proliferation. miR-4729 is closely related to VEGFA, which explains the pathogenesis of hemorrhoids from an epigenetic perspective. Inhibiting the decrease in miR-412-5p and miR-4729 content is expected to inhibit new blood vessel formation and correct pathological structural changes in

hemorrhoids.

2.5.2.6 Overexpression of VEGF and FGF2 leads to abnormal microvascular proliferation

VEGF is a mitogen of endothelial cells and plays an important role in neovascularization. VEGF can promote neovascularization by inducing endothelial cell proliferation and migration, while also increasing vascular permeability [94], allowing nutrients in plasma to seep out and promoting the formation of capillary networks. FGF2 is distributed in most tissues of the body and has a strong promoting effect on cell proliferation, which can induce endothelial cell proliferation [95]. Studies [96] have shown that there is an interaction between VEGF and FGF2, and FGF2 can promote the expression of VEGF. VEGF is also the basis for FGF2 to function. Liang Wenlong et al. [97] found that the expression level of VEGF receptors in hemorrhoid tissue was significantly higher than that in normal anal cushion tissue. High expression of VEGF receptors induced the production of more VEGF, leading to excessive proliferation of microvessels. Wang Qi et al. [98] found that the expression levels of VEGF and FGF2 in hemorrhoid tissue were significantly higher than those in normal rectal mucosal tissue, and the expression of VEGF and FGF2 increased with the increase of hemorrhoid stage. The above studies suggest that VEGF and FGF2 play important roles in the neovascularization of hemorrhoids, and reducing the expression of VEGF and FGF2 is expected to improve the clinical symptoms of hemorrhoids. Porwal et al. [99] found that the traditional Chinese herbal preparation Anoac-H can exert good therapeutic effects in the treatment of hemorrhagic hemorrhoids by reducing the expression of VEGF and other mechanisms, suggesting that VEGF has important value as a therapeutic target for hemorrhoids.

Brief summary

The molecular biology research of hemorrhoids not only further explains and confirms the classic clinical theory of hemorrhoid pathogenesis at the micro level, but also may provide new potential targets for the clinical treatment of hemorrhoids, which will be beneficial for exploring effective prevention and treatment methods, and has important social value and practical significance.

2.5.3 Genetic pathogenesis of hemorrhoids

2.5.3.1 Results of Hemorrhoid Genetics Research

Recent studies have shown that the occurrence of hemorrhoids is closely related to genetics. According to the epidemiological survey, the prevalence of hemorrhoids among the residents with a family history of anorectal diseases was 81.00%, which was much higher than that of the patients without a family history (45.14%). The occurrence of hemorrhoids is closely related to genetic genes [11]. In addition, some genes and gene polymorphic sites related to hemorrhoids were also found in the association study of some genes and hemorrhoids. Salnikova Le found that the SNP site rs735854 of MYH9 gene was highly correlated with hemorrhoids by searching the PheWAS(Perform a Phenome-wide association study)directory [100]. The study of Chiang CP found that the expression of acetaldehyde dehydrogenase was significantly increased in hemorrhoids patients compared with normal controls, suggesting that the direct metabolite of acetaldehyde may play an etiological role in the pathogenesis of hemorrhoids [101]. Other studies showed that the expression of mir-412-5p was significantly decreased in hemorrhoid tissues of hemorrhoid patients. This study also showed that the down-regulation of endogenous mir-412-5p expression in vascular endothelial cells of internal hemorrhoids led to the high expression of target gene xpo1 and the nuclear translocation of p53 protein, making it unable to activate p66Shc and p16, which ultimately weakened the regulation of vascular endothelial cell cycle, thereby accelerating the division of hemorrhoid vascular endothelial cells and leading to angiogenesis [102]. The study of Song C also suggested that the results of high-throughput RNA-Seq screening showed that hemorrhoids may be closely related to abnormal miRNA transcription, leading to abnormal target gene expression and imbalance of some signal transduction pathways. Abnormal expression of microRNAs is a potential key factor in hemorrhoids [103]. The study of Al-batayneh KM and Al-battah RM also found the genes related to hemorrhoids and their polymorphic sites. In the recruited hemorrhoid patients, they found that the SNP (-91 C G) in the proximal upstream region of the FOXC2 gene was highly correlated with both varicose veins and hemorrhoids, showing that varicose veins and hemorrhoids are closely related vascular abnormalities, which means that they may have a common genetic etiology [104].

2.5.2.2 FOXC2 gene polymorphism and hemorrhoids

Forkhead box C2 (FOXC2), or mesenchymal forkhead box 1 (mhf1), is a transcription factor belonging to the human forkhead or winged helix family. The

common feature of this family is that it has an independent conserved DNA binding domain with a length of about 110 amino acids, called fox (forkhead box) domain, which is called forkhead (or winglike helix) DNA binding domain. This binding domain is a large loop structure folded into three helices (helix 1, 2, 3) and two wings (Winged). According to this feature of the DNA binding domain, These transcription factors are also called forkhead / winged helix transcription factors. This family is characterized by a conserved homologous DNA binding domain consisting of 100 amino acids. This family plays an important role in embryogenesis and cell differentiation of many organisms. Among the human genes, forkhead transcription factors are currently divided into foxa-q, with 17 subfamilies and 43 members. Foxx2 is one of the members and is encoded by the human chromosome 16q24.1 region gene. The full length of FOXC2 gene is 3288bp, of which the full length of cDNA is 1506bp. It has no intron and only contains a single coding exon. The protein encoded by FOXC2 has a total of 494 amino acid residues.

At present, the research on FOXC2 gene function mainly focuses on the expression of mouse embryos and the relationship between FOXC2 gene mutation and human diseases. FOXC2 gene has been found to be associated with a variety of human diseases. It is an indispensable transcription factor in the genesis and development of cardiovascular and axial bones.

Studies have shown that FOXC2, together with TIE2, VEGFR-3, rasa1, KRIT1, MGC4607, PDCD10, Nemo, SOX18, EEG, ACVRLKL, MADH4, NDP, TIMP3, NOTCH3, COL3A1, PTEN and other important regulators of angiogenesis, plays an important role in the process of angiogenesis [105]. Before the occurrence of the circulatory system, the genetic program first determines the characteristics of the arteries and veins. A signal cascade amplification system including SHH, VEGF, VEGFR2, FOXC1, FOXC2, COUP-TFII and other factors determines the differentiation of the arteries and veins [106]. FOXC2 is highly expressed in mouse embryonic venous valves, and mutation of FOXC2 gene can cause loss of function and venous reflux of lower limb venous valves. It has been confirmed that FOXC2 is closely related to the pathogenesis of varicose veins [104, 107]. Mellor RH, Lim CS, etc. have all found mutations in FOXC2 gene in varicose vein patients [108,109]. The linkage study of twins by Ng et al confirmed the heritability of FOXC2 mutation in the occurrence and

development of primary varicose veins; Further studies also showed that FOXC2 mutants had evidence of venous reflux on ultrasound [110]. The varicosity theory of hemorrhoids also believes that there is venous congestion and varicosity in the hemorrhoid venous plexus. Therefore, FOXC2 gene may also be associated with hemorrhoids, which is a genetic factor for the occurrence and development of hemorrhoids, as found by Al batayneh km and Al battah RM in hemorrhoid patients of Arab nationality [104]. However, there is no further study on the association between FOXC2 gene and hemorrhoids in the East Asian population.

In addition, FOXC2 gene is a newly discovered transcription factor related to fat metabolism. Studies have shown that FOXC2 can activate the main transcription factor of adipocyte differentiation and is considered as the main effector gene of adipocyte differentiation [111]. In addition, it also has the function of regulating blood lipid and body fat. In the study of Dutch and Finnish populations, it was shown that the genetic gene of familial mixed hyperlipidemia with reduced HDL was located at 16q24.1, and FOXC2 was located near this site. Sequencing of FOXC2 gene found that its allelic variation was related to triglyceride level [112]. In patients with type 2 diabetes, the C / C genotype of foxc2-512c > t is associated with increased body mass index. In the multiple regression model including age, sex and emotional status, foxc2-512c > T polymorphism is a significant independent predictor of body mass index [113]. There is evidence that obesity and high body mass index can be regarded as independent risk factors for hemorrhoids [8-9]. Therefore, FOXC2 gene may also affect the occurrence and development of hemorrhoids by affecting obesity and body mass index.

Brief summary

At present, there are not many studies on hemorrhoids related genes, and the relevant genes are also limited, but it can also be seen that the occurrence of hemorrhoids has a certain correlation with gene polymorphism. This provides clues for the pathogenesis of hemorrhoids and the prevention of hemorrhoids in the future.

2.6 Diagnosis of hemorrhoids

The diagnosis of hemorrhoids can be completed according to the medical history, clinical symptoms and signs, physical examination and other auxiliary examinations[114-117].

Medical history

A comprehensive understanding of the characteristics of the medical history is an important measure to make a clear diagnosis, formulate a correct treatment plan, grasp the opportunity of operation and eliminate the contraindications of operation. Before the physical examination, the following information should be asked:(1) Condition: main symptoms such as prolapse, bloody stool or pain and other inducing factors and onset characteristics; (2) Diet and living habits: including intake of water and cellulose, health problems, frequency of defecation and stool properties, whether there are bad living habits such as sitting and squatting for a long time; (3) Past medical history: including the patient's personal medical history and family history of intestinal tumor. For patients with rectal bleeding, it should focus on the investigation of colorectal conditions; (4) Medication history: focus on understanding the current medication situation of patients, especially anticoagulants, antihypertensive drugs and hypoglycemic drugs; (5) If the patient is female, the pregnancy and childbirth history and menstruation should be inquired.

Clinical signs

In order to accurately diagnose the morphology and distribution characteristics of hemorrhoids and exclude other anal lesions, visual examination of the entire anal canal and rectum (such as anorectal endoscopy) should be performed when conditions permit.

Visual examination mainly observes whether there is redness, swelling, fistula, eczema, on the external skin of the anus in a resting state, whether there are external hemorrhoids protrusions or internal hemorrhoids eversion, and abnormal anal canal morphology.

All patients should receive digital rectal examination routinely, except those with anal stenosis or severe pain. The patient with prolapse as the main complaint should take the squat position and simulate defecation. The doctor should observe the shape and tissue characteristics of the prolapse and record it with pictures. Before anorectal digital diagnosis, necessary communication and prompt should be carried out with the patient, supplemented by oily substances to fully lubricate the gloves, move gently, press gently with the pulp of the fingers and then slowly enter the fingers to determine whether the anal canal is narrow, anal sphincter tension, and whether the anal canal surface is smooth, and then check whether the mucosal surface of the middle and lower segments of the rectum is smooth and whether it touches tumors or feces along the anatomical route, and judge the change of anal angle and the coordination of anal sphincter through rest, force discharge, and lifting the anus. The finger retraction should also be slow. At the same time, observe whether the fingertips are stained with mucus, pus, blood and other secretions.

Before anoscope, ask the patient to open his mouth and breathe to cooperate with the examination. Under the microscope, observe the morphology and tissue characteristics of hemorrhoids above and below the dentate line. At the same time,

judge whether there are ulcers, cracks, anal papilla hypertrophy, bleeding points and abnormal secretions accumulated in the intestinal cavity.

Auxiliary inspection

The purpose of auxiliary examination is to make clear the diagnosis of hemorrhoids, exclude whether it is complicated with other serious gastrointestinal diseases, such as inflammatory bowel disease and colorectal tumor, and understand the basic situation of the whole body to exclude the contraindications of surgery.

Fecal occult blood test: as the simplest and cheapest screening method, it is recommended to be used routinely. With informed consent, fecal gene testing can be recommended. This method is a new cancer detection technology without intestinal preparation, which has the advantages of noninvasive, convenient and accurate, and has been included in the international guidelines for colorectal cancer screening.

Indications for colonoscopy: Colonoscopy is required for any one or more of

the following conditions.

1. Age>50 years old (has not undergone colon examination in the past decade);

2. Symptoms of digestive tract, such as bloody stools, mucous stools, and abdominal pain;

3. Unexplained anemia or weight loss;

4. Have a history of colorectal cancer or precancerous diseases such as colorectal adenoma, ulcerative colitis, Crohn's disease, schistosomiasis, etc;

5. Direct relatives with colorectal cancer or colorectal polyps;

6. Have a history of pelvic radiation therapy;

7. The fecal occult blood test result is positive.

2.7 Treatment of Hemorrhoids

At a symposium in 1980, Professor Marino Martin outlined the treatment of hemorrhoids [118], that is, the treatment principle we followed later, that is, only symptomatic hemorrhoids need treatment. There are many clinical treatments for internal hemorrhoids, but each treatment has its corresponding indications, advantages and disadvantages.

2.7.1 Conservative treatment

2.7.1.1 General treatment

The general treatment includes increasing the amount of dietary fiber, drinking

more water, exercising frequently, reducing the intake of spicy food, defecating, avoiding going to the toilet for too long, avoiding sitting for a long time, sitting in warm water, etc. Labidi Asma et al. [119] found in a 100 case-control study that people with low fiber diet and low water intake are more likely to suffer from hemorrhoids. Similarly, a meta-analysis of 378 patients with hemorrhoids showed that fiber supplements had certain benefits in alleviating symptoms and reducing the risk of bleeding[120]. Although the therapeutic effect of changing diet and lifestyle on hemorrhoids is limited, it can be considered as part of conservative treatment and preventive measures.

2.7.1.2 Drug therapy

This includes topical drugs (such as ointments, suppositories, lotions, etc.), intravenous active drugs (such as plant extracts flavonoids, saponins, ginkgo biloba leaves, etc.), anti-inflammatory and analgesic drugs, etc. Zagriadskiĭevgeny A et al. [121] found in a multicenter observational study involving 1952 patients that flavonoids were beneficial for alleviating hemorrhoid symptoms in most patients and were most effective for stage I and II internal hemorrhoids. An Zhiying et al. [122] used Bai Zhi San Huang Tang for fumigation and washing, and found that the effective rates for treating stage I, II, and III internal hemorrhoids were 94.4%, 73.3%, and 23.1%, respectively. They also believed that Bai Zhi San Huang Tang had a good therapeutic effect on stage I and II internal hemorrhoids. Na Yunlang et al. [123] used Huaihua powder to treat stage I internal hemorrhoids and found an effective rate of 74.1%. The research report by Qijano Ce et al. [124] shows that the use of medication in pregnant women with hemorrhoids poses a risk of fetal malformation or even death. When using medication for pregnant women, it is important to carefully weigh the pros and cons. Drug therapy is mainly used to treat acute symptoms of hemorrhoids, and as part of adjuvant therapy, it has the advantages of improving symptoms and enhancing efficacy. However, caution should be exercised when using it for pregnant women and patients.

2.7.2 Device therapy

2.7.2.1 Rubber ring binding

The mechanism of rubber ring ligation [125] is to use rubber rings to wrap around the upper pole area of internal hemorrhoids, thereby blocking blood supply, inducing aseptic inflammatory reactions, leading to fibrosis and scar formation, and achieving

the therapeutic goal. Aram [126] collected data from 890 patients with internal hemorrhoids who underwent rubber ring ligation and conducted a retrospective study. The cure rate of rubber ring ligation was 76%, the recurrence rate within 2 years was 20%, and the incidence of complications was 4%. Rubber ring ligation had a better effect in treating stage II and III internal hemorrhoids. Yang Liang [127] found through comparing the therapeutic effects of endoscopic rubber ring ligation and Diosmin tablets, that rubber ring ligation.

The effective rate of treatment is 94%, drug treatment is 80%, and the cure rate of rubber ring ligation is higher. Abiodun Adekunle Adedapo et al. [128] found that compared with 50% glucose sclerotherapy, endoscopic rubber ring ligation treatment for stage II and III internal hemorrhoids has a higher cure rate and lower recurrence rate, but also a higher degree of pain. The research report by Xiao Zaoxue [129] shows that compared with microwave radiation surgery, automatic hemorrhoid ligation has a higher treatment effectiveness, lower incidence of complications, less surgical time and cost, and shorter postoperative recovery time. There are also relevant literature [130] indicating that rubber ring ligation has the advantages of mild postoperative pain and fewer complications, but its long-term efficacy is lower than that of hemorrhoidectomy. Peng Huabin[131] found in his research that the total effective rate of both the rubber ring ligation surgery and the PPH procedure for the treatment of mixed hemorrhoids is 100%. However, the rubber ring ligation group has the advantages of mild pain, fewer complications, shorter surgical time, and less intraoperative bleeding. From this, it can be seen that the rubber ring ligation surgery is mainly suitable for the treatment of stage I, II, and III internal hemorrhoids, as well as stage IV internal hemorrhoids and mixed hemorrhoids. It has the advantages of significant therapeutic effect, fewer postoperative complications, short hospital stay, low treatment cost, and low recurrence rate. However, its long-term efficacy is lower than that of hemorrhoidectomy, and the postoperative pain is also higher than that of sclerotherapy.

2.7.2.2 Hardening method

The commonly used hardening agents in the hardening method include: poly (cinnamyl alcohol), Xiao Zhi Ling, 5% sodium cod liver oil, 5% phenolic almond oil, 50% glucose, etc. The mechanism is to inject a hardening agent into the hemorrhoid nucleus, causing a sterile inflammatory reaction in the tissue and leading to fibrosis,

ultimately resulting in vascular occlusion and atrophy. Tomikl Yuichi et al. [132] selected 83 patients with internal hemorrhoids and treated them with aluminum potassium sulfate tannic acid sclerotherapy. The effective rate of sclerotherapy was as high as 97.6%, with 4 cases of complications and a recurrence rate of 9.6%. Huang Changxi et al. [133] selected 120 patients with internal hemorrhoids and treated them with different sclerotherapy agents. They found that the total effective rate of polyguanol sclerotherapy was 96.7%, and that of Xiaozhiling sclerotherapy was 93.3%, with similar therapeutic effects. However, after six months of postoperative follow-up, the incidence of sclerotic complications with Xiaozhiling was 18.3%, and the incidence of sclerotic complications with polyguanol was 6.7%. Liang Qishou et al.[134] also obtained the same results in a research report in 2015. However, in a prospective study report by Huang Junjie [135] in 2017, it was shown that compared with the traditional sclerosant Xiaozhiling, the efficacy of polyguanol injection treatment is higher, the recurrence rate is lower, the pain is mild, and the complications are fewer. The long-term recurrence rate of polyguanol injection treatment is lower than that of Xiaozhiling injection. Kodak[136] selected 116 patients with stage II and III internal hemorrhoids and treated them with closed hemorrhoidectomy and sclerosing injection of paclitaxel. The results showed that the effective rates of the two were similar, but the sclerosing group with paclitaxel had more advantages in terms of surgical time, symptom disappearance time, intraoperative bleeding volume, and incidence of complications. The same results also appeared in the research report of Liu Xians et al.[137]. Fu Xinyao et al.[138] conducted a study on the treatment of stage III internal hemorrhoids using the injection method of polyoxymethylene alcohol and the Procedure for prolapsing hemorrhoids (PPH) surgery. The results showed that the cure rates and incidence of most complications were similar in the two groups, but the incidence of postoperative pain and anal edema was high in PPH surgery. Polycinnamyl alcohol hardener has better therapeutic effects than traditional hardeners and is the most commonly used one. Sclerosing agent injection therapy is suitable for stage I, II, and III internal hemorrhoids, with advantages such as high effectiveness, low recurrence rate, mild pain, and fewer complications. Compared with traditional hemorrhoidectomy and PPH surgery, sclerosing agent injection therapy has advantages in treating mild to moderate internal hemorrhoids.

2.7.2.3 Binding method and hardening method

Pu Wanyun et al.[139] used an automatic hemorrhoid ligation stapler combined with 50% glucose injection to treat internal hemorrhoids. The results showed that compared with the group treated with sclerotherapy alone, the combination of ligation and sclerotherapy had a higher overall effective rate, fewer complications, shorter wound recovery time, and lower pain scores. Zhu Yong [140] collected 72 patients with internal hemorrhoids and studied the efficacy of automatic ligation combined with lidocaine sclerotherapy injection. It was found that the combined group had a higher effective rate than the single ligation group, and the postoperative improvement was significant, with a lower recurrence rate and incidence of complications. It can be used as a part of conservative treatment and preventive measures. Zeng Lusun et al. [141] treated 68 elderly patients with internal hemorrhoids using a combination of rubber ring ligation and polyoxymethylene injection. The total effective rate of the combination group (91.18%) was higher than that of the single ligation group (73.53%), and the incidence of complications was low. Zhu Zhanqiu [142] used the injection of Xiaozhiling sclerosing agent combined with automatic hemorrhoid ligation to treat 126 cases of internal hemorrhoids. It was found that the combined group had better postoperative bleeding, pain, hospital stay, and difficulty urinating compared to the single ligation group. Li Kangping et al. [143] treated a total of 200 cases of stage I, II, and III internal hemorrhoids with negative pressure rubber ring ligation and injection of Xiaozhiling sclerosing agent. They found that the combined group had a higher effective rate (96.7%) than the single ligation group (88.6%) and the single sclerosing group (80.0%). Rubber ring ligation combined with injection of sclerosing agent had the advantages of high effective rate and low complications. The combination of ligation and sclerotherapy combines the advantages of both, and compared with the treatment of ligation or sclerotherapy alone, it has advantages such as higher effective rate, lower incidence of complications, lower degree of pain, and shorter hospital stay. It is suitable for the treatment of mild to moderate internal hemorrhoids.

2.7.2.4 Other therapies include infrared coagulation therapy, laser therapy

Cryotherapy, acupoint selection therapy, electrocoagulation therapy, etc. Most mechanisms involve the degeneration and necrosis of tissues, causing hemorrhoids to shrink, and then achieving treatment through self-repair. An early study [144] showed

that rubber ring ligation was more advantageous than infrared coagulation therapy, but a study published in 2006 [145] tended to have similarities between the two. Lu Can et al. [146] treated 80 cases of stage I and II internal hemorrhoids with pricking the Jiaojiao acupoint, and found that the pricking group was significantly better than the Taining suppository drug treatment in improving the bleeding symptoms of patients. They believe that pricking the Jiaojiao acupoint has a high therapeutic effect on stage I and II internal hemorrhoids. Wei-Liang Loh et al.[147] used single-stage electrocoagulation to treat 100 cases of stage II and III internal hemorrhoids, with an effective rate of up to 94%. After 36 months of follow-up, the recurrence rate was 6%, and it was pointed out that it was more advantageous compared to a study on the treatment of internal hemorrhoids using a large rubber ring ligation technique [148]. Paulo Boarini et al. [149] selected 50 patients with stage I, II, and III hemorrhoids, all of whom were treated with semiconductor fiber laser electrocautery. The total satisfaction rate was 89%, the symptom relief rate was 84%, and the hemorrhoid grading was reduced by at least one level by 80%. Although these treatment methods have certain therapeutic effects, there are relatively few literature reports on these treatment methods, and their efficacy still needs to be proven through long-term clinical practice.

2.7.3 Surgical treatment

2.7.3.1 Hemorrhoidectomy

Hemorrhoidectomy includes Milligan Morgan hemorrhoidectomy (MMH), semi open hemorrhoidectomy, Ferguson hemorrhoidectomy (FH), combined treatment with sclerotherapy, and transanal hemorrhoidectomy. The mechanism is to achieve the treatment goal by removing hemorrhoids, mainly suitable for stage III and IV internal hemorrhoids, mixed hemorrhoids, partial thrombotic external hemorrhoids, acute incarcerated hemorrhoids, severe bleeding, and non surgical treatment ineffective. MMH surgery was first proposed by Miles and later improved by Milligan and Morgan. This surgery is based on the theory of varicose veins, which involves ligating internal hemorrhoids and removing external hemorrhoids without suturing. The FH procedure invented by Fergusone sutures the surgical incision for external hemorrhoids, but it is believed that the sutured incision is prone to infection. In a meta-analysis [150], it was pointed out that in addition to the advantages of light pain, fast wound healing, and low risk of bleeding, the external stripping and internal ligation closed surgery is similar to

the open surgery in terms of postoperative recurrence, complications, and infections. MMH surgery is considered the "gold standard" for the surgical treatment of hemorrhoids [151], but its disadvantage is high postoperative pain, which can easily lead to complications such as anal edema and stenosis. Adrian Medina-Gallardo et al. [152] analyzed the analgesic needs of 117 patients with internal hemorrhoids who underwent MMH surgery and found that 22.2% of patients needed opioid drugs. With the continuous development of technology, people have invented some new surgical tools to alleviate the pain of patients and speed up the recovery of surgical wounds, such as bipolar electric knife, ultrasonic knife, etc. Bilbin Yusuf et al.[153] used ultrasonic scalpel hemorrhoidectomy (HSH) and stapler hemorrhoid fixation (SH) to treat 99 cases of stage III and IV internal hemorrhoids. The study found that the pain level and incidence of complications were similar between the two, but the HSH group required significantly shorter surgical time and had a lower recurrence rate. In the study conducted by Nienhuijs et al.[154] using bipolar electric knife and traditional hemorrhoidectomy to treat internal hemorrhoids, it was found that the required surgical time and pain rate were significantly reduced using electric knife treatment. A meta-analysis conducted in 2013 [155] showed that there was no significant difference in VAS pain score, recurrence rate, and postoperative bleeding between LigaSure hemorrhoidectomy (LH) and stapler hemorrhoid fixation, and stapler hemorrhoid fixation had a higher recurrence rate. However, some studies[156] have shown that compared with traditional surgery, there is no significant difference in terms of efficacy, complications, recurrence rate, etc., but it actually increases the cost of surgery. Due to the severe pain caused by traditional hemorrhoidectomy, people have improved their surgical methods, such as transanal hemorrhoidectomy. A recent high-quality meta-analysis [157] showed that transanal hemorrhoidectomy and stapler hemorrhoid fixation had similar rates of complications, readmission, length of hospital stay, resumption of work, and patient satisfaction, but significantly higher long-term recurrence rates than stapler hemorrhoid fixation.

2.7.3.2 Procedure for prolapse and hemorrhoids with stapler (PPH)

The indications for PPH surgery mainly include stage III and IV internal hemorrhoids, as well as stage II internal hemorrhoids with recurrent bleeding. This surgical method was invented by Professor LONGA, and its mechanism is to remove

and anastomose the prolapsed anal cushion mucosal tissue above the dentate line, and the prolapsed anal cushion is reduced. Liu Qingsheng et al. [158] used a retrospective study method to analyze 4580 patients with stage II-IV internal hemorrhoids treated with PPH surgery, and concluded that PPH surgery had a high cure rate (98.03%) and a low recurrence rate (0.09% in 2 years and 0.18% in 5 years). Liang Mingchao et al. [159] used PPH and MMH surgery to treat 100 cases of stage III and IV internal hemorrhoids. Compared with the effective rate (82%) of the MMH group, the total effective rate (96%) of the PPH group was not only high, but also had advantages such as fewer complications, shorter surgical time, and shorter hospital stay. A research report [160] indicates that there is no significant difference in recurrence rate between PPH surgery and traditional hemorrhoidectomy. A high-quality research report [161] indicates that the recurrence rate of PPH is higher after long-term follow-up. Based on this drawback, people have made improvements on the advantages of PPH surgery, such as transanal stapler partial rectal resection (STARR), selective hemorrhoid mucosal resection and stapling (TST), modified transanal stapler rectal resection (TST STARRPPlus), PPH surgery combined with external dissection and internal ligation, PPH surgery combined with Ligaure hemorrhoidectomy, TST combined with polyoxymethylene alcohol sclerotherapy, and so on. Li Guobin et al. [162] used STARR surgery to treat 47 patients with mixed hemorrhoids, resulting in 44 cases of symptom disappearance and 3 cases of symptom improvement, all of which had no serious complications. However, a study by Stuto A et al. [163] in 2011 found that 2171 patients underwent STARR surgery with significant improvement in symptoms, but there were more postoperative complications (4.5% urgency, 3.6% bleeding, 3.4% perineal sepsis, and 0.05% rectovaginal leakage). There are also studies [164] showing that TST and PPH have similar efficacy and recurrence rates. The indications, efficacy, complications, and recurrence rates of each improved stapler vary, and their long-term effects still need to be continuously proven in clinical practice.

2.7.3.3 Hemorrhoid artery ligation surgery

The mechanism of hemorrhoid artery ligation surgery mainly includes the following two aspects: firstly, by ligating the hemorrhoid artery, the blood supply to the internal hemorrhoids is reduced, and the hemorrhoids will shrink; The second is to fix the mucosal tissue on the muscle layer after ligation, which serves as a suspension for

the anal cushion. This surgery was first reported by Morinaga and has since been clinically practiced in multiple countries, achieving certain therapeutic effects. LIU H et al. [165] used hemorrhoid artery ligation to treat patients with internal hemorrhoids, and showed that hemorrhoid artery ligation had satisfactory therapeutic effects in the treatment of stage II and III internal hemorrhoids, but had poor therapeutic effects on stage IV internal hemorrhoids. A study by Brown SR et al. [166] included 370 patients with stage II and III internal hemorrhoids and observed their recurrence rate one year later. It was found that there was no significant difference in the recurrence rate between the two groups compared to the use of rubber ring ligation, but ligation was more prone to pain, higher cost, and more postoperative complications. Hemorrhoid artery ligation has a significant therapeutic effect on internal hemorrhoids, mainly suitable for stage II and III internal hemorrhoids. It has the advantages of minimally invasive, safe, and simple operation, but the improvement effect on prolapsed internal hemorrhoid symptoms is not good, and the recurrence rate is also high. People have improved their surgical methods, such as hemorrhoid artery ligation and internal hemorrhoid circumcision. Liu You et al. [167] selected 40 patients with mixed hemorrhoids for treatment. Hemorrhoid artery ligation combined with internal hemorrhoid ligation had a similar cure rate to traditional external and internal hemorrhoid ligation, but the combined treatment group had a shorter course of treatment and fewer complications.

2.7.3.4 Hemorrhoid artery embolization

Endovascular embolization can accurately identify the branches of the hemorrhoid artery, making it possible for the hemorrhoid artery to completely occlude, and avoiding surgical trauma to the anus and rectum. In 2014, Vidal et al. [168] reported three cases of hemorrhoid patients treated with hemorrhoid artery embolization. Both patients had a history of surgery, which resulted in rectal bleeding after embolization. The other patient without a history of surgery stopped bleeding after one embolization. Zakharchenko A et al.[169] used metal coils and synthetic polyvinyl alcohol particles to embolization the hemorrhoid artery in 40 cases. The satisfaction rates of patients with stage III, I-II hemorrhoids were 83% and 94%, respectively. After embolization, the hemorrhoid nucleus shrank by 43%, and there were no recent complications. Arterial embolization is technically feasible for the treatment of hemorrhoids, with the advantages of safety and good tolerability. More research is needed to evaluate the

efficacy of this new technology in the treatment of hemorrhoids.

Brief summary

According to the treatment principle of hemorrhoids, asymptomatic hemorrhoids do not require treatment. In clinical practice, conservative treatment and instrument therapy are generally used to improve the symptoms of patients. When non-surgical treatment is ineffective, surgical treatment is considered. But each treatment method has its corresponding indications and efficacy, and clinical workers should choose appropriate treatment methods based on the characteristics of the disease, the patient's wishes, and local medical level. With the advancement of modern technology and the continuous innovation of surgical instruments, the treatment methods for hemorrhoids are becoming increasingly diverse. Seeking a treatment method that can not only eliminate hemorrhoid symptoms but also minimize postoperative complications while protecting the anal and anal structures is the overall goal and trend, and it is also the direction of unremitting efforts and exploration by clinical workers.

2.8 Prevention of Hemorrhoids

Although the etiology of hemorrhoids is not yet clear and the influencing factors are diverse, some preventive measures have been proposed based on past experience and clinical practice results.

2.8.1 Prevention of constipation: (1) Diet: Diet is an important factor in preventing hemorrhoids and reducing their recurrence. In daily diet, it is recommended to choose foods such as vegetables, fruits, and legumes that contain more vitamins and crude fiber. Eat more foods such as black sesame, walnuts, and honey to increase gastrointestinal peristalsis, lubricate the intestines, and facilitate defecation. Eat less spicy and stimulating foods, such as alcohol, chili, pepper, ginger, mustard, etc. Diet should not be too much or too full to avoid worsening the occurrence of hemorrhoids due to dry stools and difficulty in elimination Developing a habit of regular bowel movements: It is best to develop a habit of regular bowel movements every morning. Drinking a glass of cold water in the morning can stimulate gastrointestinal movement and prevent constipation. When feeling the urge to defecate, do not hold back from defecating, as prolonged tolerance can cause habitual constipation correcting bad bowel habits: squatting in the toilet for too long or exerting excessive force during defecation

can easily cause poor local blood circulation and lead to hemorrhoids over time choosing the correct method for treating constipation: For general constipation patients, a reasonable diet can be adjusted to develop a habit of regular bowel movements. You can also massage the lower abdomen in a clockwise direction for 15-20 minutes before going to bed every day to increase gastrointestinal motility. For stubborn constipation or constipation caused by a certain disease, early diagnosis and treatment should be given, and long-term use of laxatives or enemas should not be allowed[170].

2.8.2 Strengthening exercise, it is beneficial for promoting blood circulation, harmonizing the body's qi and blood, promoting gastrointestinal peristalsis, improving pelvic congestion, effectively avoiding venous blood stasis in the anus and intestines, preventing varicose blood vessels and constipation, and avoiding the occurrence of hemorrhoids[171].

2.8.3 Pay attention to pregnancy health: During pregnancy, due to the significant increase in progesterone and relaxin in the female body, pelvic and rectal blood vessels can dilate, leading to hemorrhoids. Pregnant women experience reduced activity, slowed gastrointestinal motility, prolonged retention of feces in the intestinal cavity, absorption of water, resulting in dry feces and difficulty in defecation. Therefore, during pregnancy, activities should be increased appropriately. Avoid prolonged standing and sitting, and pay attention to maintaining unobstructed bowel movements. After each bowel movement, use warm water to fumigate and wash the local area of the anus to improve local blood circulation, which is very beneficial for preventing hemorrhoids. At the same time, during pregnancy, the fetus gradually increases, causing an increase in abdominal pressure and aggravation of pressure on the inferior vena cava. Especially when the fetal position is not correct, the pressure is more pronounced, causing obstruction of venous return in the lower rectum and anal canal, leading to the dilation of the hemorrhoid venous plexus, which can also exacerbate the onset of hemorrhoids. Hemorrhoids should be mainly treated with high fiber foods, warm water sitz baths, and ointment suppositories[172].

2.8.4 Avoid prolonged standing and sitting in a fixed position, and engage in appropriate physical exercise: People who stand, sit, or squat for a long time are prone to hemorrhoids, as prolonged exposure to these positions can affect pelvic blood circulation, causing perianal venous congestion and dilation, leading to the formation

of hemorrhoids. Proper physical exercise, such as walking, jogging, and anal lifting exercises, are beneficial for promoting blood circulation, harmonizing the body's qi and blood, promoting gastrointestinal peristalsis, improving pelvic congestion, effectively avoiding venous blood stasis in the anus and intestines, preventing blood vessel varicose and constipation, and thus avoiding the occurrence of hemorrhoids[173].

2.8.5 Pay attention to keeping the lower body warm, keeping blood unobstructed, drinking plenty of hot water, avoiding dehydration, and avoiding dry stools caused by dehydration[174].

2.8.6 Diseases that can cause an increase in intra-abdominal pressure should be treated promptly, such as dysentery, diarrhea, cirrhosis, etc, Timely treatment of systemic diseases such as heart, lung, and liver to prevent increased abdominal pressure and hemorrhoid venous hypertension[174].

2.8.7 Often do anal lifting exercises, the specific method is to relax the whole body, either sitting, standing, or lying down. Abandoning all distractions, intentionally contracting the anus, slowly lifting it up, intending to lift the sinking qi to the dantian and then relax. Repeat this process several to dozens of times, Generally, doing this exercise thirty times a time, twice a day, can be done anytime, anywhere. It works well when working, traveling, watching TV, or walking[174].

2.8.8 Self massage: Hemorrhoids are the result of local blood stasis. Massage is one of the traditional methods for fitness and disease control in China. The Changqiang acupoint (located in front of the coccyx tip) on the meridians of traditional Chinese medicine is the preferred acupoint for treating hemorrhoids. Taking the Changqiang acupoint for massage can significantly improve local blood circulation. It is very effective in both prevention and treatment[174].

2.8.9 Take medication in a timely manner. Once there are signs of hemorrhoid attacks, such as mild discomfort, pain, itching, and bloody stools, medication should be taken in a timely manner, and it is twice as effective as usual[175].

Summary

There are many influencing factors for hemorrhoids, but its prevention focuses on nine aspects, including preventing constipation, strengthening exercise, avoiding sitting or standing for a long time, paying attention to the lower body warmth, often doing anal lifting exercise, self massaging Changqiang acupoint, timely treatment of diseases that

can cause increased intra-abdominal pressure, and early treatment.

2.9 Introduction to Mendelian Randomization method (MR)

2.9.1 The Origin, Principle, and Application of MR

With the successful completion of the human genome project, HapMap and the thousand human genome project, researchers have obtained a detailed map of common variation sites in the human genome. On this basis, genome wide association studies (GWAS) have developed rapidly, and genome data related to complex diseases or traits have surged [176], which has led to the emergence and application of MR.

The core of MR design is the use of instrumental variables. The concept of instrumental variables originated from econometrics and was later widely adopted in medical research [177]. Instrumental variables refer to measurable variables that are related to the target risk factor but not related to other factors or confounding factors. In MR research design, genetic variation following Mendelian inheritance law is used as an instrumental variable to replace unmeasurable risk factors to be studied. By analyzing the association between genetic variation and risk factors, as well as genetic variation and disease risk, we can infer the causal relationship between risk factors and disease risk [178]. Katan[179] first proposed the concept of MR in 1986. Due to the research being based on Mendelian's second law of inheritance, which randomly assigns parental alleles to offspring during human gamete formation, it is called Mendelian randomization research.

The analysis model of MR is "genetic variation risk factors disease risk". It is known that genetic variation is strongly correlated with risk factors. If there is a causal relationship between this risk factor and disease risk, carrying these genetic variations can also alter disease risk. MR uses the opposite approach to infer: it is known that genetic variation is strongly correlated with risk factors. By analyzing whether genetic variation is related to disease risk, the causal relationship between risk factors and disease risk can be derived.

Traditional medical statistics and epidemiological research have certain limitations. Traditional correlation analysis includes two statistical methods: correlation analysis and regression analysis. The correlation results obtained from statistical analysis based on established parameter models can only indicate the presence of

covariant trends among variables, and cannot directly infer causal relationships between variables[180]. In addition, due to the frequent influence of potential confounding factors and reverse causal relationships, the results of traditional observational studies cannot directly reflect causal relationships[181]. Randomized controlled trials (RCTs) are the gold standard for testing the causal relationship between medical related exposure and outcomes. However, the implementation of RCTs is difficult and often limited by medical ethics [182].

MR can compensate for the shortcomings of traditional methods mentioned above. MR relies on the natural and random allocation of genetic variations during meiosis, resulting in

Genetic variation is randomly distributed in a population[183]. Individuals naturally and randomly carry genetic variations that affect risk factors at birth, such as genetic variations that lead to elevated levels of low-density lipoprotein cholesterol (LDL-C) or genetic variations that are not inherited. In a given population, groups the population based on whether they carry the genetic variation, and then compare the occurrence of outcomes between the two groups. This grouping method based on innate genetic variation of individuals is completely random and not affected by confounding factors such as other characteristics of the population, environment, socio-economic status, etc., and the causal time series is reasonable, similar to the random grouping process in randomized controlled trials. The difference in disease risk between two subgroups carrying different alleles will indicate the causal effect of this risk factor (such as LDL-C) on the disease.

In clinical applications, MR not only helps to understand the etiology of diseases, but also provides new strategies for disease treatment. For example, several large-scale observational studies have shown that a decrease in plasma LDL-C levels is associated with a decrease in the incidence of coronary heart disease, but these studies are difficult to avoid confounding effects of LDL-C related factors. Research has shown that mutations in the pre protein converting enzyme Bacillus subtilis protease Kexin 9 type (PCSK9) gene are associated with a decrease in LDL-C levels. PCSK9 is a glycoprotein mainly synthesized in the liver and can bind to LDL receptors on the liver surface, weakening the liver's ability to metabolize plasma LDL-C and leading to an increase in LDL-C levels. MR uses PCSK9 gene variation analysis to investigate the relationship

between LDL-C and coronary heart disease. Evidence suggests that genetic variation is associated with LDL-C levels, as well as the risk of coronary heart disease. This study provides supporting evidence for the causal association between LDL-C and coronary heart disease, and suggests that PCSK9 may be a new target for reducing LDL-C[183]. At present, it has been confirmed that PCSK9 monoclonal antibody, as a novel lipid-lowering drug, can reduce LDL-C to unprecedented levels and has a protective effect on cardiovascular diseases .

2.9.2 Research Procedures

The research step of MR design mainly includes three steps: (1) Determine the risk factors (exposure) and disease risk (outcome) to be studied, and the correlation between the two can be detected in the observational study design. (2) Select appropriate genetic variations [single nucleotide polymorphisms (SNPs) significantly associated with risk factors obtained from GWAS] as instrumental variables. (3) MR statistical analysis is used to detect whether genetic variations associated with risk factors are also associated with disease risk, in order to evaluate the causal effect of risk factors on disease risk.

The selection of the aforementioned instrumental variables (genetic variation) is a crucial step in the research. The instrumental variable (genetic variation) in MR research must meet three core assumptions: ① the instrumental variable (genetic variation) must be closely related to risk factors; ② The instrumental variable (genetic variation) should not be associated with confounding factors that affect the "risk factor disease risk" relationship; ③ The instrumental variable (genetic variation) can only be associated with disease risk through risk factors, and cannot affect disease risk through other pathways [184].(Figure 1)

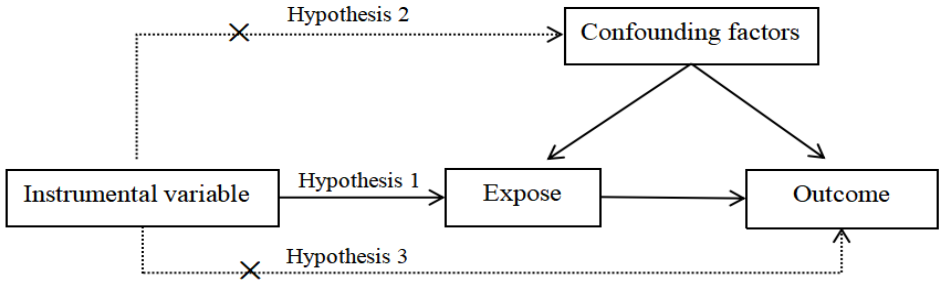


Figure 1 Three core assumptions of instrumental variables in Mendelian randomization method

2.9.4 The research types of Mendelian randomization

The commonly used types of MR research currently include single sample, two sample, bidirectional, and two-stage MR[185.186]. The classic research design is a single sample MR, which refers to the study population coming from the same research sample. Individual level data can be used to measure risk factors (exposure), disease risk (outcome), and genetic variation (instrumental variables) within the same sample. Two sample MR refers to the association data between genetic variation and risk factors, as well as between genetic variation and disease risk, from two independent samples of the same population. Usually, summary data from previous GWAS studies is used, and individual level data can also be used. Two sample MR requires two samples to have similar age, gender, and racial distributions. This method can make causal inference without evaluating the correlation information between risk factors and disease risk[185]. Bidirectional MR is mainly used when the causal direction of risk factors and disease risks is uncertain. MR analysis is conducted from two directions to determine the direction of causal association between the two. Two stage MR can detect the degree to which the causal relationship between risk factors and disease risk is mediated by intermediate variables of interest. The development of two-stage MR methods originated from epigenetic epidemiology, used to study the degree of causal association between methylation mediated exposure and medical related outcomes [187]. Currently, other variables such as biomarkers are also commonly used as intermediate variables for evaluation.

2.9.3 Limitations of MR

Although MR is superior to traditional observational epidemiological studies, it also has certain limitations: (1) it is difficult to obtain suitable genetic variations. Although a large number of genetic loci related to complex traits have been identified, there are still some interesting risk factors that are difficult to obtain related genetic variations due to the lack of GWAS research or public data resources. Even if genetic variation is obtained, it may not be possible to effectively infer causal relationships due to not meeting the three core assumptions. (2) Sufficient statistical ability. Insufficient statistical ability will reduce the accuracy of the results. The determining factors of statistical ability in MR research include the frequency of genetic variation, the effect

of genetic variation on risk factors, and the sample size of the study. Integrating multiple variants into a multi gene risk score or increasing sample size can help improve statistical ability. (3) The result is not easy to interpret. The biological mechanisms underlying most genetic variations are still unclear, making it difficult to explain the underlying mechanisms of causal associations. Combining biological knowledge, bioinformatics analysis, and subsequent basic experiments will help to interpret research results. (4) Beavis effect: Complex diseases or traits are often associated with a large number of gene loci, while GWAS studies only report the most significant small portion of genetic variation, which may overestimate the association between genetic variation and risk factors, thereby affecting causal inference in MR studies.

Summary

In summary, hemorrhoids are likely a hereditary, multifactorial chronic disease that occurs at the bottom of the rectum and anal mucosal venous plexus. Currently, there are multiple treatment methods for hemorrhoids, but their risk factors and pathogenesis are still unclear. Especially the genetic mechanism has not been reported in the East Asian population so far, but existing results indicate that the genetic mechanism is likely related to the FOXC2 gene. Therefore, this study aims to explore the risk factors and distribution patterns of hemorrhoids in the Zhuang population of Guangxi, and analyze the relationship between FOXC2 gene polymorphism and its interaction with major risk factors and the occurrence and development of hemorrhoids. A preliminary prediction model for the risk of hemorrhoids with added genetic factors is established, providing clues and theoretical basis for further research on the mechanism and prevention of hemorrhoids.

2.10 Conceptual framework

The four problems to be solved in this research are:

2.10.1 Determine the external environmental risk factors for hemorrhoids based on a questionnaire survey.

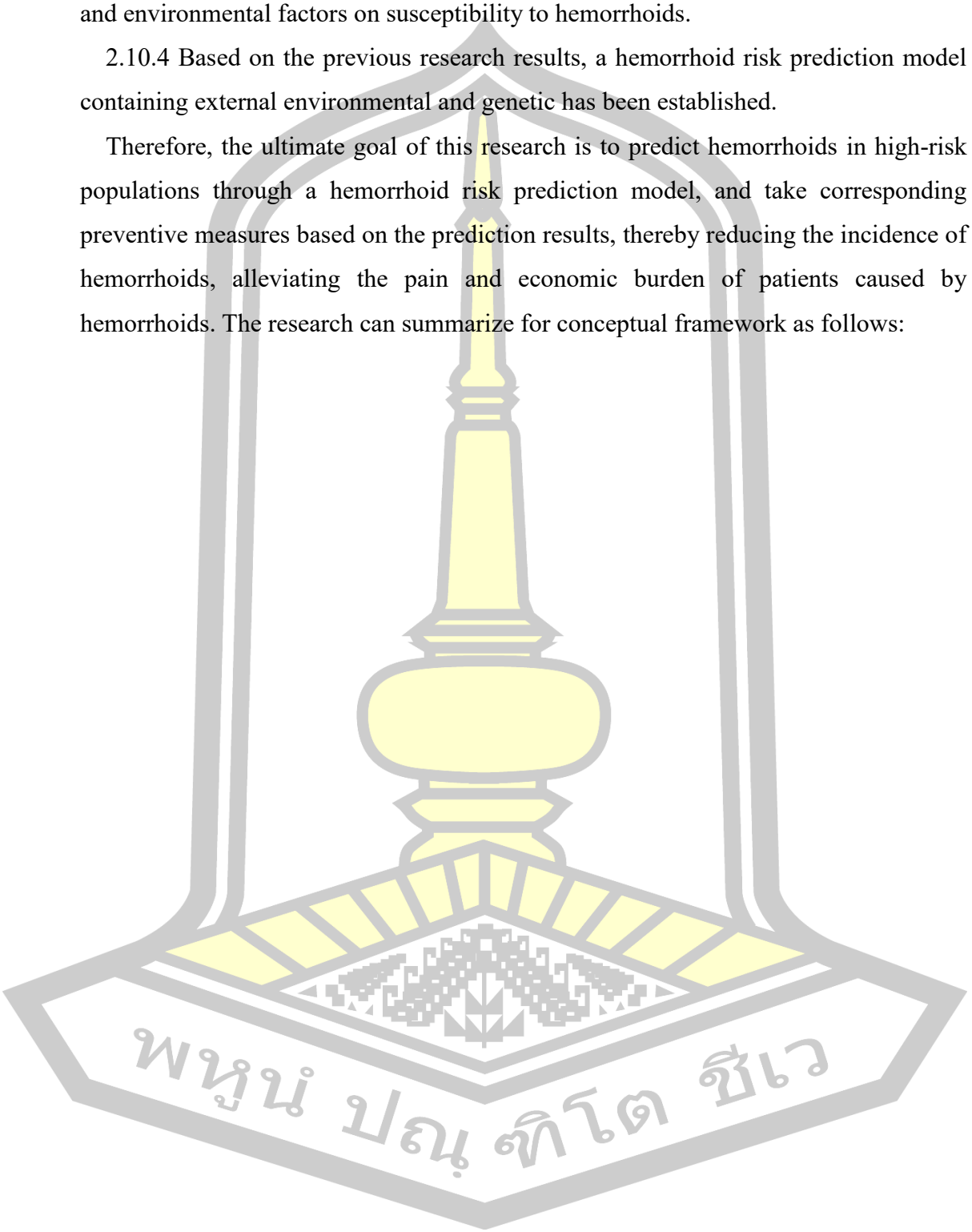
2.10.2 Determine the causal relationship between BMI and hemorrhoids based on MR analysis results.

2.10.3 Determine the relationship between the rs34221221 polymorphism of the FOXC2 gene and susceptibility to hemorrhoids based on the genotyping results of two

groups of research subjects, and determine the impact of the interaction between genes and environmental factors on susceptibility to hemorrhoids.

2.10.4 Based on the previous research results, a hemorrhoid risk prediction model containing external environmental and genetic has been established.

Therefore, the ultimate goal of this research is to predict hemorrhoids in high-risk populations through a hemorrhoid risk prediction model, and take corresponding preventive measures based on the prediction results, thereby reducing the incidence of hemorrhoids, alleviating the pain and economic burden of patients caused by hemorrhoids. The research can summarize for conceptual framework as follows:



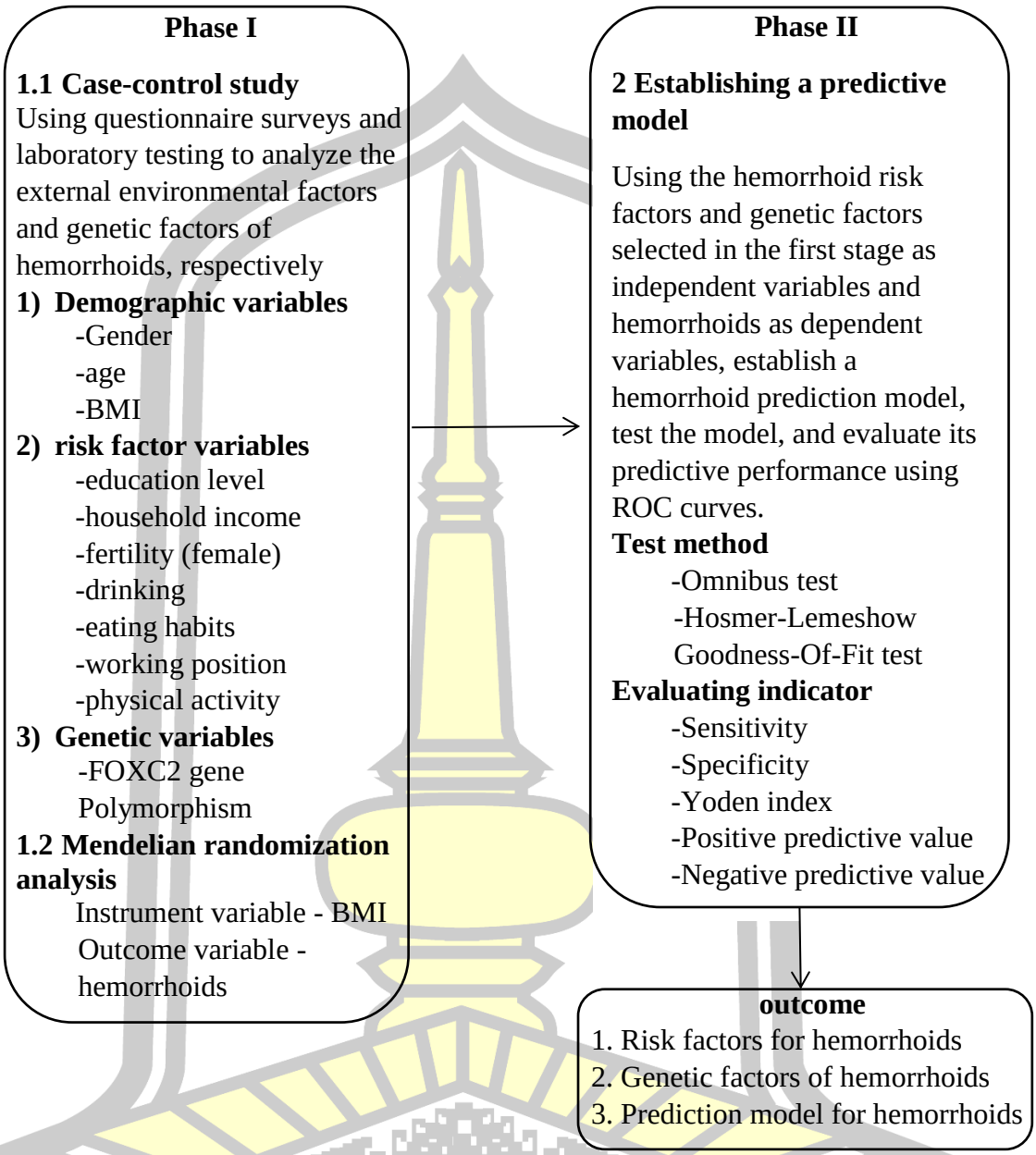


Figure 2 Conceptual Framework

CHAPTER III
MATERIALS AND METHODS

This study has four objectives, the first of which is to explore the risk factors for hemorrhoids in the Zhuang population in Guangxi. The second is to explore the relationship between FOXC2 gene polymorphism and its interaction with other risk factors and susceptibility to hemorrhoids. The third is to explore the causal relationship between body mass index and hemorrhoids. Finally, a hemorrhoid prediction model is established based on the first three objectives. In this study, a mixed method was used. The research is divided into two phases. At the first phase, the first is a case-control study, the basic information, demographic information and possible hemorrhoids related factors of the subjects were collected through a questionnaire survey, and the main risk factors of hemorrhoids were analyzed. Secondly, the FOXC2 gene polymorphism of the subjects was detected to analyze its relationship with hemorrhoids. In addition, the causal relationship between body mass index and hemorrhoids was analyzed by two sample Mendelian randomized analysis. In the second phase, the binary logistic regression method was used to construct the hemorrhoids risk prediction model and ROC curve was used to evaluate the prediction performance of the model.

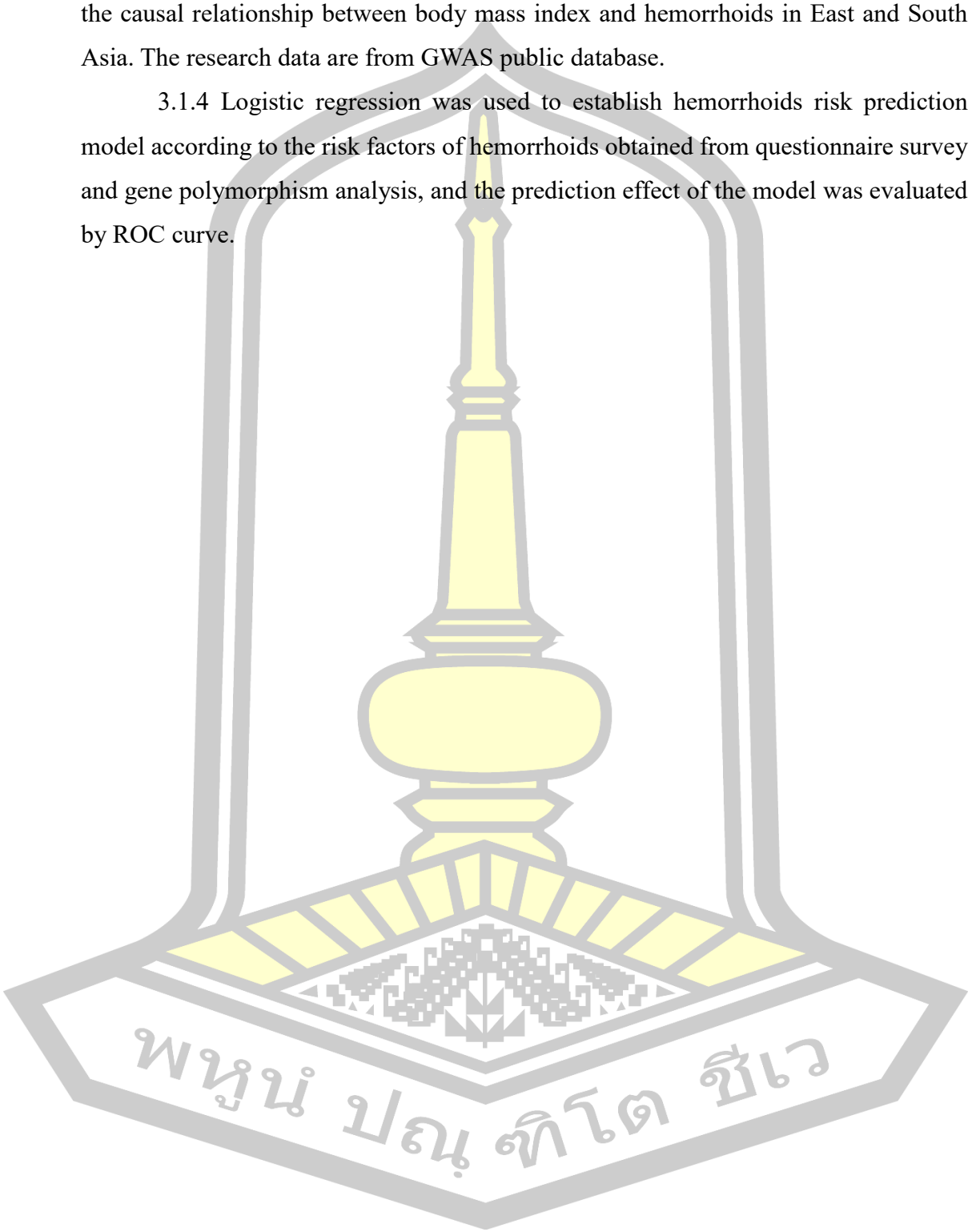
3.1 Study design

3.1.1 Collect basic information, demographic information, and possible factors related to hemorrhoids from participants through a questionnaire survey, and analyze the main risk factors for hemorrhoids through case-control studies. The questionnaire content includes the following variables: gender, age, heigh, weight, ethnicity, occupation, education level, family income, alcohol consumption, dietary habits, dietary taste, spicy food consumption, pickled food consumption, work position, physical activity, and disease situation .

3.1.2 The blood samples of the subjects were collected and genomic DNA was extracted. The FOXC2 gene polymorphism was detected in the laboratory, and the correlation between FOXC2 gene polymorphism and hemorrhoids was studied by case-control study.

3.1.3 Two samples of Mendelian randomization method were used to explore the causal relationship between body mass index and hemorrhoids in East and South Asia. The research data are from GWAS public database.

3.1.4 Logistic regression was used to establish hemorrhoids risk prediction model according to the risk factors of hemorrhoids obtained from questionnaire survey and gene polymorphism analysis, and the prediction effect of the model was evaluated by ROC curve.



3.2 Technical Flowchart

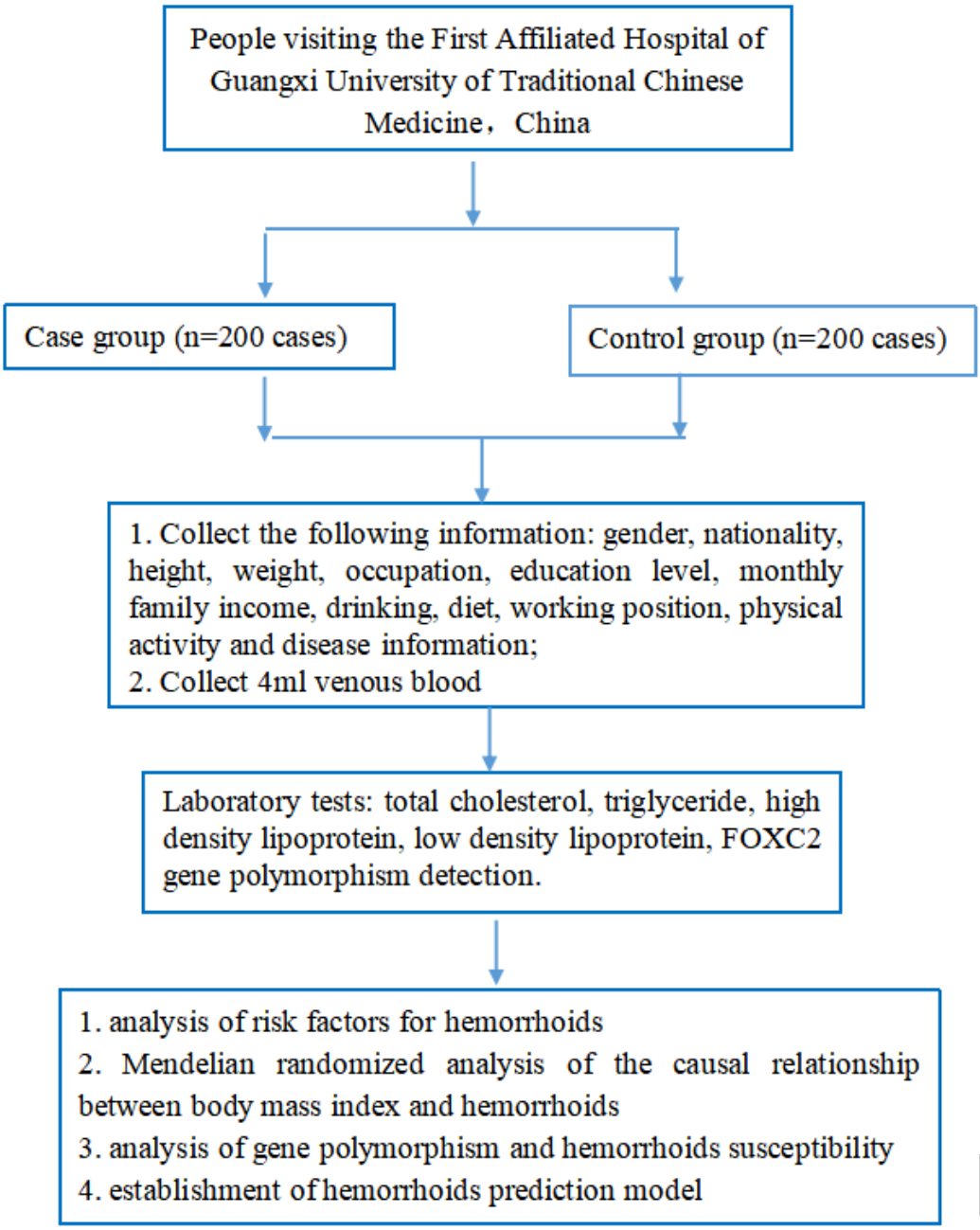


Figure 3 Technical flowchart of case-control study

3.3 Study population and samples

3.3.1 Study population

The study population was Guangxi Zhuang people in China. All participants (Hemorrhoids patients or non hemorrhoids patients) were local residents of the Zhuang ethnic group in Guangxi, who underwent colonoscopy at the Spleen and Stomach Department of the First Affiliated Hospital of Traditional Chinese Medicine in Guangxi.

Inclusion and exclusion criteria for case and control subjects

Case group

Inclusion criteria: According to the Chinese guidelines for the diagnosis and treatment of hemorrhoids (2020)[117], local Zhuang hemorrhoids patients living in Guangxi were selected, including patients with internal hemorrhoids, external hemorrhoids and mixed hemorrhoids, aged 18-65 years.

Exclusion criteria: Exclude the following personnel: patients with birth defects, colorectal cancer and other malignant tumors. Patients with serious illness who are not suitable for the study. Minors under the age of 18 and the elderly over the age of 65. Pregnant women and lying-in women.

Control group

Inclusion criteria: the subjects selected in the control group must be local Zhuang people living in Guangxi without hemorrhoids and no blood relationship with any individual in the case group, and the age requirement is 18-65 years old.

Exclusion criteria: Exclude the following personnel: Patients with varicose veins (including primary varicose veins of lower extremity, varicocele of spermatic cord, portal varicose veins, etc., all control subjects must be diagnosed) and their family history. Patients with a family history of hemorrhoids. Patients with birth defects, colorectal cancer and other malignant tumors. Patients with serious illness who are not suitable for the study. Minors under the age of 18 and the elderly over the age of 65. Pregnant women and lying-in women.

3.3.2 Calculation of sample size

The highest test efficiency is obtained when the sample content is equal between cases and controls. Therefore, the sample size estimation method in this study when the number of cases and the number of controls are equal in non-matched design was used to estimate the sample size needed for the study, which was calculated using EpiCalc

2000 software(https://en.cnki.com.cn/Article_en/CJFDTOTAL-BJXB201202027.htm). Based on the relevant literature, the OR was set at 2.12, the proportion of exposed in the control group was 22.6%, the significance was 0.05 (two-sided test), and the power was 90%, and finally, we calculated 179 in the control and case groups, respectively, with a sample size of 358 cases. Because hemorrhoids may be a multifactorial and complex disease, considering confounding factors, the actual sample content should increase by about 10% based on ideal estimation, so about 200 cases and controls were included in this study.

3.4 Sampling

All research subjects were selected from patients who underwent colonoscopy at the Department of Spleen and Stomach, First Affiliated Hospital of Guangxi University of Chinese Medicine, based on inclusion and exclusion criteria.

3.5 Research instrument and Methods.

3.5.1 Research instruments

Questionnaire: the questionnaire is one of the tools used in the study, which is used to collect the baseline data of the research object and the influencing factors of hemorrhoids. The questionnaire includes the following six parts.

Part 1: Basic personal information of the research subject, including current address, gender, age, Height, weight, ethnicity, parental ethnicity, occupation, education level, and family income.

Part 2: The alcohol consumption of the research subjects, including whether they have consumed alcohol, the length of time they have consumed alcohol, the age at which they first consumed alcohol, the types of alcohol consumed, and the frequency and quantity of alcohol consumed per week.

Part 3: The dietary situation of the research subjects, including their usual habits of meat and vegetables, staple food tendencies, habits of eating spicy food, habits of eating pickled products, and dietary tastes.

Part 4: The working positions of the research subjects, including sitting positions, standing and walking positions, and a combination of multiple positions.

Part 5: The physical activity of the research subjects, mainly including three types

of physical activity intensities: high, medium, and low.

Part 6: Diseases of the subjects and their parents and siblings, including hypertension, diabetes, cancer, chronic diarrhea, chronic gastritis, constipation, hemorrhoids, varicose veins and other diseases.

R software: Use the TwoSampleMR package in R 4.0.2 software package to conduct Mendelian randomization analysis on the causal relationship between body mass index and hemorrhoids in East and South Asian populations.

The main instruments used for DNA extraction and FOXC2 gene rs34221221 polymorphism detection :

Table 1 Main experimental instruments

Instrument	Production company
high speed centrifuge PICO 17	Thermo
NANO-DROP	Thermo
BIO RAD POWER PAC 3000	BIO RAD
BIO RAD DNA SUB CELL	BIO RAD
Gel Imaging System GIS-1600	Shanghai Tianneng Technology limited company
ABI 9700 PCR System	Applied Biosystems
ABI 3730 Water bath	Applied Biosystems TAISITE

3.5.2 Methods

3.5.2.1 Case-control study

A case-control study was conducted to analyze the association between various indicators in the questionnaire survey and the rs34221221 polymorphism of the FOXC2 gene with hemorrhoids, in order to preliminarily examine the possible risk factors for hemorrhoids.

Questionnaire investigation

The investigator first informs the patient or the physical examinee of the purpose and content of the survey. After obtaining the consent of the other party, the investigator asks questions. After the respondent truthfully answers, the investigator truthfully fills in the information in the corresponding place of the questionnaire. After the investigation, the investigator will enter the questionnaire information into the database of SPSS software after verifying that the questionnaire information is correct.

Blood sample collection

After the subjects signed the informed consent, 4ml of peripheral venous blood was collected and centrifuged in EDTA-K2 anticoagulant tube at 5000 rpm/min for 10 minutes. The upper plasma and lower blood cells were extracted into a new centrifuge tube, marked and frozen at -80 ℃. After the sample collection was completed, it was mailed to Hefei Linmei Biotechnology limited company in Fujian Province by cold chain for blood lipid detection, DNA extraction and sequencing.

The DNA extraction and sequencing of this study were completed by Hefei Linmei Biotechnology limited company.

Gene and SNP screening

Using the method of literature data mining, we collected the literature related to the genetic research of varicose diseases, analyzed the genes and their polymorphisms in the literature, and selected the genes and polymorphic loci with the strongest association with varicose diseases as the research genes and polymorphic loci in this study.

Laboratory Testing

DNA extraction

The kit used was:

Hipure tissue DNA Mini Kit (company: Magen, Article No.: d3121-02)

Experimental steps:

1. Add 25 μl proteinase K into 1.5-2.0ml centrifuge tube.
2. Transfer 10~250 μl of anticoagulant blood sample into a centrifuge tube containing protease. Shake and mix for 5 seconds. If the sample<250 μL, adjust the total volume to 250 μl with buffer PBS or buffer AE.
3. Add 250 μl buffer al to the sample. Reverse it for 3-5 times and mix it with high-speed vortex for 15 seconds. 70℃ water bath for 10 minutes.
4. Add 250 μl absolute ethanol into the sample and vortex at high speed for 15 seconds.
5. Collect the droplets on the pipe wall by brief centrifugation.
6. Install the gDNA column into a new collection tube. Transfer the mixture to the column. Centrifuge 10000 xg for 1 minute. Discard the collection pipe and effluent.
7. Install the gDNA column into a new collection tube. Add 500 μl buffer dw1 to

the column. Reverse mixing for 2 times. Centrifuge 10000xg for 30-60 seconds.

8. Dump the effluent and put the column into the recovery header. Add 650 µl buffer dw2 to the column, 10000×g centrifuge for 30-60 seconds.
9. Dump the effluent and put the column into the recovery header. 10000×g centrifuge for 2 minutes.
10. Transfer the column to a new 1.5ml centrifuge tube (self-made), add 30~150 µl buffer AE preheated to 70℃to the center of the membrane of the column. Leave for 3 minutes. 10000 ×g centrifuge for 1 minute.
11. Discard the DNA binding column and keep the DNA at -20℃for long-term storage at 2-8 ℃.

Genomic DNA concentration and purity determination

The maximum UV absorption wavelength of genomic DNA is 260nm, protein is 280nm, and salt ions are 230nm. Based on this principle, add DNA solution 1 to Nanodrop 2000 µ L. Measure the absorbance values of the DNA solution at 260 nm, 280 nm, and 230 nm, respectively, to calculate the DNA concentration and purity. For standard samples, when the concentration is 1 µ g/ml, the OD260 of DNA sodium salt is 0.02. When OD260=1, the concentration of dsDNA is approximately 50 µ g/ml.

The evaluation criteria for DNA concentration are as follows:

1.A260/A280>1.8 indicates residual RNA in the sample, which can be digested using RNA enzymes;

2.A260/A280<1.6 indicates protein impurities in the sample, and the extraction step can be repeated to improve DNA purity;

3.A260/A230<2.0 indicates that the salt ions in the sample are too high and the purification steps can be repeated.

Operation steps

1. UV-240 spectrophotometer was turned on and preheated for 10 minutes
2. Washed the colorimetric dish with distilled water, dryed it with absorbent paper, added TE buffer, placed it on the S cell rack in the sample room, and closed the cover.
3. Zero calibration after setting the slit.

4. Diluted the standard sample and the tested sample appropriately (DNA 5 μl dilute with TE buffer to 1000 μl) , Afterwards, recorded the number and dilution.
5. Placed the colorimetric dish containing the standard sample or the sample to be tested on the S rack in the sample room, and closed the cover plate.
6. Setted the wavelength of ultraviolet light and measured the OD values at 230nm, 260nm, and 280nm wavelengths.
7. Calculated the concentration of the sample to be tested.

The concentration of DNA samples(μg/μl)= OD260×Dilution factor×50/1000

PCR amplification method:

1. Primer design: software used: primer 5.0. See excel primer table for specific primer information.

Table 2 Primer information of PCR amplification

Primer name	Primer sequence	Primer length(bp)	Product length(bp)
rs34221221-F1	CCGGGTGATTGGCTCAAAGT	20	
rs34221221-R1	GGCCCTGAGAGCGAGAGAG	19	523
rs34221221-R2	CCGATTCGCTGGGGGCTT	18	190

2. Primer synthesis method: Hefei Linmei Biotechnology limited company.
3. Reagent used for PCR: 2xTaqDNA polymerase, universal Biology (jkr-dem023).
4. Operation steps of PCR

PCR amplification system:

Table 3 PCR amplification system

Add items	**Volume**
2X Taq DNA polymerase	20μL
Forward primer (10 Mm)	1μL
Reverse primer (10 Mm)	1μL
Template DNA	2μL
ddH2O	16μL
Total	40μL

PCR amplification procedure:

Table 4 PCR amplification program

Temperature	Time	
94 ℃	5 min	
94 ℃	30 sec	×38 cycles
60 ℃	30 sec	
72 ℃	30 sec	
72 ℃	5 min	
4 ℃	save	

Purification of PCR products

① Preparation of digestive solution

Add items	Volume
TaKaRa Alkaline Phosphatase	0.5 μL
TaKaRa Exonuclease I	0.5 μL

Prepare digestive solution and mix shrimp alkaline enzyme and exonuclease in a 1:1 ratio. After centrifugation of PCR products, add more than 1μL of digestive solution to each well into the PCR reaction solution.

② Purification reaction: Perform a programmed reaction using a PCR instrument.

The program is SAP: 37℃ for 60 minutes, 80℃ for 15 minutes.

Sequencing reaction

① SEQ MIX Configuration

Reagent: ABI PRISM ® BigDye ® Terminator v3.1 Cycle Sequencing Kit

Prepare MIX according to the number of PCR reactions in the following table:

Table 5 MIX Liquid Preparation Method

Reagents Name	Dosage
BigDye	0.4 μL
Sequencing Buffer	0.8 μL
H2O	1.8 μL
Primer	1 μL
Template	1ul

Add 3ul or more of MIX to a 96 well plate according to the sequencing reaction table, with 1ul corresponding to primers and 1ul corresponding to PCR products (note that membrane sealing is required), and centrifuge.

The sequencing reaction only has one primer, and to avoid liquid consumption during the sampling process, the system needs to be overconfigured.

② SEQ program: Pre denaturation at 96℃ for 2 minutes, 96℃ for 10 seconds, 55 ℃ for 5 seconds, 60℃ for 90 seconds, 25 cycles.

Purification

This step is to remove as much impurities as possible from the sequencing reaction system except for the target single stranded nucleic acid fragment, in order to reduce the time required for ABI 3730 capillary electrophoresis

The impact of impurities on the quality of peak plots.

① Reagent preparation: 0.125 mol/L EDTA Na2 solution: Weigh 2.325g of EDTA Na2 2H2O into a 50ml centrifuge tube and add 40ml of deionized water,

Heat in a 65℃ water bath, oscillate intermittently several times until completely dissolved. Dilute to 50ml with deionized water and shake for 10 seconds to mix well.

Anhydrous ethanol (premium grade)

Deionized formamide HIDI

② Mix anhydrous ethanol with distilled water to prepare 85% ethanol and 70% ethanol, and use on the same day.

③ After centrifugation of the sequencing reaction plate, 2.5 μl of EDTA Na2 solution was added to each reaction well, and 40μl of 85% ethanol was fully shaken for 3 minutes before centrifugation

3000g, 4 ℃ , 30 min (EDTA as a metal ion chelating agent can bind with ions in the sequencing PCR reaction system to remove ions).

④ After centrifugation, invert the sequencing reaction plate on absorbent paper and stop immediately when the centrifugal force reaches 185g (or 900rpm).

⑤ Add 50 μ l of 70% ethanol to each well, shake thoroughly for min, centrifuge 3000g, 4 ° C, 15 min (DNA fragments dissolved in 70% ethanol)

Low solubility, can be precipitated by centrifugation.

⑥ Repeat step 4.

⑦ Dry in dark for 15-30 minutes, add 10 μ l of HIDI (denaturation treatment, carried out in a biosafety cabinet) to each well, centrifuge and react at 96 ℃ in a PCR instrument for 2 minutes, cool to 4 ℃ , and then remove.

On machine sequencing

After the denaturation is completed, the sequencing machine (ABI 3730) is used for sequencing.

1. Create sample board program

① Select GA Instruments>ga3730>Plate Manager;

② Click the New button and fill in the corresponding content in the pop-up New Plate dialog window (enter the name of the sample plate in the ID and Name blank fields; select the corresponding sequencing program in Application; select 96 or 384 well plates in Plate Type; define the sampling order in Scheduling; select Septa in Plate Sealing; and enter the operator in Owner Name and Operator Name).

③ Click the "OK" button; In the pop-up Sequencing Analysis Plate Editor window, fill in the relevant content (fill in the sample name in the Sample Name, fill in "1" in the A01 line for 96 well plates, and write 1, 2, 3, and 4 in the A01, B01, A02, and B02 lines for 384 well plates in sequence; select the data upload path in the Results Group 1 column; select the sequencing program in the Instrument Protocol 1 column; and select the data analysis program in the Analysis Protocol 1 column).

④ Then select all A01 rows, click Edit>Fill Down Special, select Fill Down Special (96 Cap), and 96 rows will be automatically generated; If it is a 384-hole plate, select all rows A01, A02, B01, and B02 in sequence, repeat the operation 4 times, and click "OK"

2. Load sample board

① Pull out the sample stack and open the injection stack door;

② Place the sample board (arranged from bottom to top for machine operation), paying attention to the direction of missing corners;

③ Close the sample loading stack door and push the sample loading stack back to its original position.

3. Call the computer program

① Select GA Instruments>GA 3730>Instrument Name>Run Schedule

② Click the Search button, enter the sample board name in the pop-up Add Plate to In Stack window, click the Search button, select the corresponding sample board name, click the Add button to bring it up, and click the Done button to close the window

4. Start the program

If the machine is in standby mode, click the green arrow button in the upper left corner of the DC software, and then click the OK button to start the machine; If the machine is constantly running, this step is not necessary.

5. Result analysis

After obtaining the peak map, rename and analyze it to determine the presence and category of mutations.

3.5.2.2 Mendelian randomization analysis:

Research Design

This research was a two sample MR study. Exploring the causal relationship between BMI and hemorrhoids in East and South Asian populations. Using genetic variation as instrumental variable, performed MR analysis with BMI as exposure and hemorrhoids as outcome.The research design is shown in Figure 4.

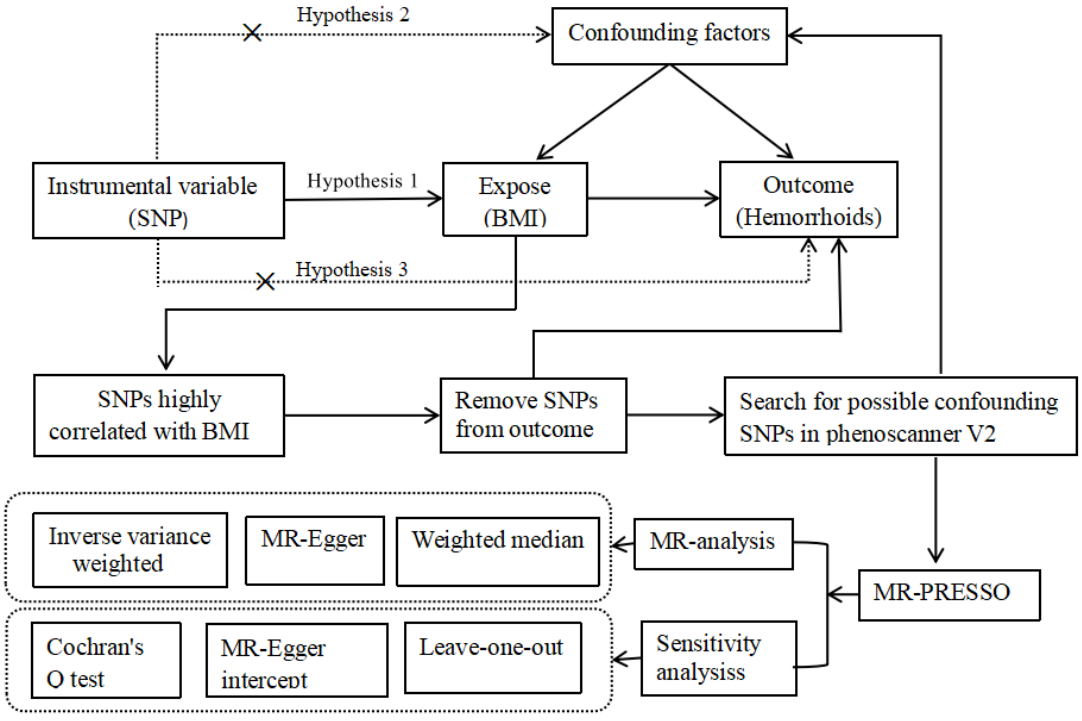


Figure 4 Research design

Data Source and Acquisition

BMI data was sourced from the GWAS summary statistical data of BMI in Japan's Biobank, which could be obtained for free on the website (https://pheweb.jp/downloads) , BMI was a continuous type of data, with a sample size of 163,835 cases.

The hemorrhoids data was sourced from two datasets of the South and East Asian

populations of Open GWAS (ukb-e-455_CSA, ukb-e-455-EAS). The sample size of the South Asian population was 8640, including 657 cases and 7983 controls(https://gwas.mrcieu.ac.uk/datasets/ukb-e-455_CSA/); The sample size for the East Asian population was 2658, with 228 cases and 2430 controls(https://gwas.mrcieu.ac.uk/datasets/ukb-e-455_EAS/).

Selection of genetic instrumental variables

Mendelian randomization analysis needed to meet the following three core hypothesis: (1) genetic IVs are strongly correlated with exposure; (2) There was no correlation between IVs and any confounding factors affecting exposure outcome association; (3) Genetic IVs could only affect the outcome through exposure factors, but not through other paths.

In order to meet the above three principles, the following procedures are strictly followed for the screening of instrumental variables: (1) SNPs strongly related to BMI are extracted from GWAS data of BMI ($P<5\times 10^{-8}$)； SNPs strongly associated with hemorrhoids were extracted from the GWAS database of hemorrhoids ($P<1\times10^{-5}$. Because the sample size of hemorrhoids outcome was relatively small, we could only relax the threshold of association, so we finally choose the more recognized 1×10^{-5} as the correlation threshold);(2) The linkage disequilibrium (LD) was clustered with $r^2<0.001$ and genetic distance=10000kb to exclude the effect of strong LD on subsequent analysis;(3) The intensity of correlation between loci and exposure factors is determined by the F value of each SNP, F value=$(\beta/SE)^2$. When F>10, it is generally considered that there was no bias of instrumental variables, so SNPs with statistical value of F less than 10 were screened out to exclude bias caused by weak instrumental variables; (4) IVs were extracted from the outcome dataset; (5) The Asian population (EAS) was used as a reference to coordinate the exposure and outcome loci to correct or exclude the palindromic sequence and chain ambiguous SNPs; (6) MR-PRESSO was used to exclude outliers to avoid potential level pleiotropy ($P<0.05$), and the phenoscanner database was searched to exclude instrumental variables related to gender, age, sedentary behavior, dietary fiber intake, constipation, diarrhea and other confounding factors ($P<1\times10^{-5}$).

MR analysis

The inverse variance weighted (IVW), Mr-Egger and weighted median (WME)

methods were used in the study. The IVW method assumes that all genetic variants were effective IVs, used the ratio method to calculate the causal effect value of a single instrumental variable, and summarizes each estimate for weighted linear regression to obtain the total effect value. The MR-Egger method used the inverse of the outcome variance as the weight to fit and evaluate the effect of exposure on the outcome . The main difference with IVW method was that the existence of intercept term was considered in regression, and its intercept can evaluate whether there was horizontal pleiotropy between instrumental variables. The WME method took advantage of the intermediate effects of all available genetic variants to obtain estimates by weighting the inverse variance of the correlation between each SNP and the result. Because IVW method has higher test efficiency than the other two MR methods, this study used IVW method as the preferred causal effect estimation method. The twosamplemr package of R 4.0.2 was used for bidirectional MR analysis.

Heterogeneity and sensitivity analysis

In order to ensure the validity of genetic instrumental variables, Cochran's Q was used to test IVW and MR-Egger models to assess the heterogeneity between instrumental variables . Secondly, MR-Egger test based on test intercept was used to identify potential horizontal multiple effects. In addition, MR-PRESSO was used to identify and eliminate outliers (10000 iterations), and the "leave-one-out" method is further used to evaluate potential outliers.

3.6 Statistical analysis

3.6.1 All questionnaire data and laboratory test data are entered into the database for statistical analysis. Quantitative data is expressed as mean ± standard deviation (SD), and classified data is expressed as absolute number (percentage). The statistical software was SPSS 26.0 (SPSS Corporation, Chicago, Illinois, USA). $P<0.05$ is statistically significant. The statistical methods used include:

Descriptive statistics including frequency, percentage, mean, standard deviation, used to explain the characteristics of the sample.

Independent sample t-test was used to test the hypothesis of height, weight and blood lipid content between the two groups.

Pearson chi-square test was used to evaluate the correlation between all influencing factors and hemorrhoids and Harvey balance test of gene frequency.

Logistic regression was used to analyze the correlation strength and 95% confidence interval of each risk factor and hemorrhoids after correction of confounding factors, the interaction between genotype and each risk factor, and to establish the risk prediction model of hemorrhoids.

ROC curve was used to evaluate the effectiveness of hemorrhoids risk prediction model.

3.6.2 The double sample Mendelian randomization package of R software was used to analyze the causal relationship between BMI and hemorrhoids.

3.7 Research Progress Schedule

Table 6 Research progress schedule

Time	Objectives and content
2022.7-2022.10	1. Determine research direction, purpose, and content, and complete literature review.
2022.11-2023.5	1. Complete the design of the questionnaire Training for on-site investigation personnel; Screening and signing of testing agreements for laboratory testing companies. 2. Mendelian randomization research method learning and data mining.
2023.6-2023.10	1.Complete the questionnaire survey and collect biological samples, and send the biological samples to the testing company for testing. 2. Complete the writing and submission of the first manuscript (MR analysis).

3.8 Regarding data missing and data organization

When collecting questionnaire data, due to various reasons, some variables were collected incompletely, so some variables were not analyzed during data analysis, such as occupation, menstrual and reproductive history, and some disease history. In addition, some variables have been classified or merged during data analysis, such as household income and work position.

3.9 Ethical approval

This research has been approved by the Ethics Committee of Guangxi Medical University (No.0168 of 2022) and the Ethics Committee of Mahasarakham University (208-075/2023). Participants will be informed of the research objectives, risks, and methods, and their participation will be voluntary. In order to maintain ethical principles, the information of all participants must be kept confidential.

CHAPTER IV
RESULTS

Introduction

All the results of the research are presented in this chapter, including five parts. Section 4.1 is the analysis results of questionnaire data, including the general data of all subjects, the correlation between various factors and hemorrhoids, the logistic regression results of various factors and hemorrhoids after correcting confounding factors, and the interaction between various factors.

Section 4.2 is the results of the Mendelian randomized study of the causal relationship between BMI and hemorrhoids. This part is the result of analyzing the causal relationship between BMI and hemorrhoids in East Asia and South Asia by using three models of the two sample Mendelian randomization method. In addition, it also shows the stability and sensitivity of the results.

Section 4.3 is the results of the comparative study of blood lipid items in patients with hemorrhoids and non-patients, including the results of the comparative study of total cholesterol, triglycerides, high-density and low-density lipoprotein in the two populations.

Section 4.4 is the analysis results of the association between the rs34221221 polymorphism of FOXC2 gene and hemorrhoids, including the analysis results of the interaction of genotype with blood lipids and external factors on hemorrhoids.

Section 4.5 is the result of hemorrhoids prediction model. This prediction model includes external factors and genetic factors. In addition, this section also includes an evaluation of the predictive model

4.1 Questionnaire data analysis

A total of 400 questionnaires were sent out (including 200 in the case group and 200 in the control group), and 400 questionnaires were recovered, with an effective rate of 100%. The 400 subjects were all Zhuang people, 148 males and 252 females; The oldest was 60 years old, the youngest was 18 years old, and the average age was 40.34 ± 10.92 years old.

4.1.1 Analysis of demographic data

The demographic data analysis of the two groups showed that there were no significant differences in age, gender, height, weight and BMI between the two groups. See Table 7 for details.

Table 7 Analysis results of demographic data

Group	n	Gender (M/F)	Age (years)	Height (cm)	Body weight (kg)	BMI （>24/≤24）
Case	200	75/125	41.28±10.36	162.08±7.24	60.92±11.58	70/132
Control	200	73/127	39.41±11.41	161.84±7.40	61.14±12.21	82/118
χ^2/t value		0.043	-1.716	-3.331	1.87	1.528
P value		0.836	0.087	0.741	0.852	0.216

Note: P<0.05 means the difference is statistically significant. All data were analyzed by using Chi square or t-test based on continuous data.

4.1.2 The distribution of various factors in the population

The questionnaire survey investigated the subjects' education level, household income, drinking, eating habits, working position, physical activity and other factors. After analysis, it was found that education, household income, working position, constipation and chronic gastritis were related to hemorrhoids, while other factors were not related to hemorrhoids (Table 8).

Table 8 Correlation analysis results of various factors and hemorrhoids

Variables, n (%)	Cases (n=200)	Controls (n=200)	χ^2 value	p-value
Education level			7.879	0.005
Higher education	121 (60.5)	93 (46.5)		
Non higher education	79 (39.5)	107 (53.5)		
Household income (CNY/month)			5.438	0.020
>4000	128 (64.0)	105 (52.5)		
≤4000	72 (36.0)	95 (47.5)		
Drinking			0.082	0.775
Yes	56 (28.5)	61 (30.5)		
No	143 (71.5)	139 (70.6)		
Dietary habit			2.661	0.264
Mainly meat based	73 (36.5)	59 (29.5)		
Vegetarian oriented	28 (14.0)	36 (18.0)		
Uniformity of meat and vegetables	99 (49.5)	105 (52.5)		
Spicy food			0.174	0.917
Eating regularly	59 (29.5)	56 (28.0)		
Occasionally eat	92 (46.0)	96 (46.0)		
Not eating	49 (24.5)	48 (24.0)		
Pickled food			0.058	0.971
Eating regularly	15 (7.5)	16 (8.0)		
Occasionally eat	144 (72.0)	142 (71.0)		
Not eating	41 (20.5)	42 (21.0)		
Dietary taste			5.883	0.053
Salty	88 (44.0)	87 (43.5)		
Bland	64 (32.0)	82 (41.0)		
Moderate	48 (24.0)	31 (15.5)		
Working position			6.895	0.009
Sitting position	99 (45.5)	73 (36.5)		
Non sitting posture	101 (50.5)	127 (63.5)		
Physical activity			2.396	0.302
High strength	24 (12.0)	25 (12.5)		
Medium strength	53 (26.5)	40 (20.0)		
Low strength	123 (61.5)	135 (67.5)		
constipation			10.714	0.001
Yes	44 (22.0)	20 (10.0)		
No	156 (78.0)	180 (90.0)		
chronic gastritis			6.498	0.011
Yes	48 (24.0)	28 (14.0)		
No	152 (76.0)	172 (86.0)		

Note: P<0.05 means the difference is statistically significant

4.1.3 Binary logistic regression analysis results of various factors

Perform binary logistic regression analysis with hemorrhoids as the dependent variable and each factor as the independent variable (Table 9), the crude ratio (unadjusted OR value) of each factor was calculated in the unadjusted binary logistic regression, the results showed education level, household income, work position, constipation, chronic gastritis and bland dietary taste were associated with hemorrhoids. And then education level, household income, work position, dietary taste, constipation and chronic gastritis were introduced into the model to calculate the adjusted OR value of each factor. After adjusting for confounding factors, household income and work position were not associated with hemorrhoids. However, after adjusting for confounding factors, it was found that age was statistically correlated with hemorrhoids and was an independent risk factor for hemorrhoids. In the variable of dietary taste, both in the uncorrected binary logistic regression model and the model corrected for confounding factors, the results showed that a bland diet can reduce the risk of hemorrhoids (Table 10).

Table 9 Independent variables and their assignments in logistic regression analysis

Variable code	Variable Label	Assignment
Y	Group	0=control group, 1=case group
X1	Gender	1=male, 2=female
X2	Age	Year
X3	BMI	1=>24,2=≤24
X4	Education level	1=Higher education, 2=Non higher education
X5	Household income (CNY/month)	1=>4000, 2=≤4000
X6	Working position	1=Sitting position, 2=Non sitting posture
X7	Drinking situation	1=Yes, 2=No
X8	Dietary habit	1=Mainly meat based, 2=Uniformity of meat and vegetables, 3=Vegetarian oriented
X9	Spicy food	1=Eating regularly, 2=Eating Occasionally, 3=Not eating
X10	Pickled food	1=Eating regularly, 2=Eating Occasionally, 3=Not eating
X11	Dietary taste	1=Salty, 2=Bland, 3=Moderate
X12	Physical activity	1=high strength, 2=Medium strength, 3=Low strength
X13	constipation	1=Yes, 2=No
X14	chronic gastritis	1=Yes, 2=No

Table 10 Odds ratio and 95% confidence interval of binary logistic regression for each factor

Variable	Unadjusted OR(95%CI)	p-value	Adjusted OR* (95%CI)	p*-value
Age	1.02 (1.00, 1.04)	0.820	1.03 (1.01, 1.05)	0.008
High education(ref:Low)	1.76 (1.19, 2.26)	0.005	2.02 (1.16, 3.52)	0.013
High household income (ref:Low)	1.54 (1.03, 2.29)	0.034	1.01 (0.60, 1.68)	0.983
Working position(ref:Non sitting)	1.64 (1.09, 2.44)	0.015	1.42 (0.91, 2.23)	0.124
Drinking situation(ref:No)	1.06 (0.70, 1.60)	0.796	1.35 (0.84, 2.17)	0.213
Dietary habit				
Vegetarian oriented(ref)				
Mainly meat based	1.60 (0.87, 2.90)	1.300	1.82 (0.99, 3.47)	0.068
Uniformity of meat and vegetables	1.21 (0.69, 2.13)	0.504	1.24 (0.67, 2.29)	0.489
Spicy food				
Not eating(ref)				
Eating regularly	1.03 (0.60, 1.77)	0.909	0.96 (0.53, 1.75)	0.901
Occasionally eat	0.94 (0.58, 1.53)	0.801	0.87 (0.51, 1.47)	0.602
Pickled food				
Not eating(ref)				
Eating regularly	0.96 (0.42, 2.19)	0.924	1.07 (0.44, 2.58)	0.886
Occasionally eat	1.04 (0.64, 1.69)	0.879	1.22 (1.72. 2.07)	0.465
Dietary taste				
moderate(ref)				
Salty	0.65 (0.38, 1.12)	0.122	0.62 (0.35, 1.09)	0.099
bland	0.50 (0.29, 0.80)	0.016	0.50 (0.28, 0.89)	0.019
Physical activity				
Low strength(ref)				
high strength	1.05 (0.57, 1.94)	0.867	0.95 (0.49, 1.83)	0.870
Medium strength	1.45 (0.90, 2.43)	0.124	1.33 (0.80, 2.21)	0.279
constipation(ref:No)	2.54 (1.43, 4.49)	0.001	2.70 (1.50, 4.87)	0.001
chronic gastritis(ref:No)	1.94 (1.16, 3.25)	0.012	1.96 (1.14, 3.36)	0.014

Note:*It refers to the corrected OR and P values for age, education level, family income, work position, constipation, and chronic gastritis. P<0.05 means the difference is statistically significan

4.2 Analysis of the causal relationship between BMI and hemorrhoids(Mendelian randomization analysis)

4.2.1 IV screening results

After strict screening of instrumental variables, a total of 8,191,759 SNPs of BMI in East Asian population were included in the study, and 68 SNPs were finally selected as genetic instrumental variables; A total of 9,812,032 SNPs were included in the study in the South Asian population, with 67 SNPs as the final instrumental variable. When the instrumental variables were screened, their F statistical values were greater than 10, indicating that the weak instrumental effect had been avoided(Appendix2-3) .

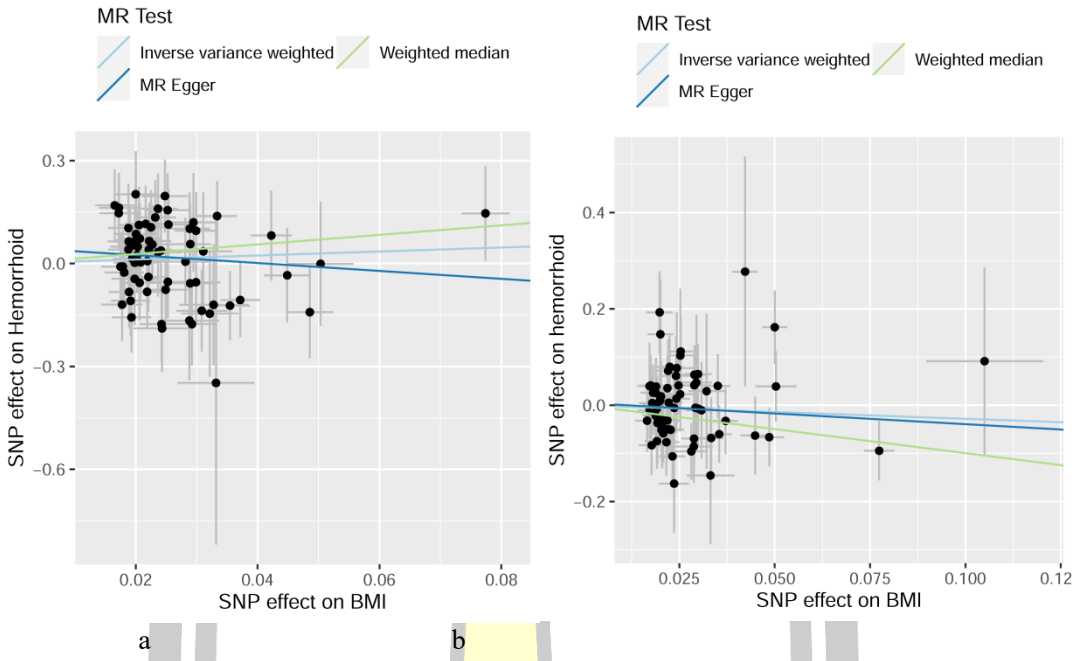
4.2.2 Causal effect of BMI and hemorrhoids

In East Asia and South Asia, the causal relationship between BMI and hemorrhoids was analyzed using IVW, MR- Egger and weighted median models, respectively. No causal effect was found between BMI and hemorrhoids. In East Asian population, in IVW model, B=1.790, 95% CI: (0.628-5.107), P=0.276; In MR-Eegger model, B=0.316, 95% CI (0.013-7.908), P=0.486; In the WME model, B=4.018, 95% CI (0.816-9.794, P=0.087). In the South Asian population, in the IVW model, B=0.753, 95% CI (0.417-1.362), P=0.349; In MR-Egger model, B=0.642, 95% CI (0.144-2.862), P=0.563; In WME model, B=0.369, 95% CI (0.146-0.993), P=0.035(See Table 11 for details). The regression slopes of MR-Egger, weighted median method, and weighted mode method are shown in Figure 5.

Table 11 Two sample MR analysis results of the causal relationship between BMI and hemorrhoids

Research object	Exposure/ outcome	Method	SNP(n)	*Beta*	95%CI	p-value
East Asian population	BMI/ hemorrhoids	IVW	68	1.79	0.628-5.107	0.276
		MR-Egger	68	0.316	0.013-7.908	0.486
		weighted median	68	4.018	0.816-19.794	0.087
South Asian population	BMI/ hemorrhoids	IVW	67	0.753	0.417-1.362	0.349
		MR-Egger	67	0.642	0.144-2.864	0.563
		weighted median	67	0.369	0.146-0.933	0.035

Note: P<0.05 means the difference is statistically significant



a b

Figure 5 Scatter plot of MR analysis on the relationship between BMI and hemorrhoids.

(1) a is the scatter plot for the East Asian population, and b is the scatter plot for the South Asian; (2)The three methods used in the current manuscript have been described. The bright blue line, green line and dark blue line represent IVW, weighted median and MR-Egger method respectively.

4.2.3 Heterogeneity and sensitivity analysis

The heterogeneity and sensitivity of genetic instrumental variables were analyzed. Cochran's Q test results showed that there was no heterogeneity in instrumental variables of MR analysis (P>0.05), and there was no pleiotropy in MR-Egger based on intercept (P>0.05). MR-PRESSO results showed that there was no outlier (Gglobal>0.05). (See Table 4-6 for details). The distribution of points in the funnel plot is roughly symmetrical, indicating the absence of imbalanced directional horizontal multi effect patterns(See Figure 6). By gradually deleting each instrumental variable from the MR analysis, the "leave-one-out" method was carried out to estimate the causal effect of the instrumental variable with outliers. The results of "leave-one-out" method were robust(see Figure 7 and Figure 8).

Table 12 Sensitivity and heterogeneity test results of two sample MR analysis of BMI and hemorrhoids

Population	Cochran's Q test (IVW)		Cochran's Q test (MR-Egger)		MR-Egger			Global test
	Q	*P*	Q	*P*	Intercept	se	*P*	*P*
East Asian	55.224	0.847	53.978	0.855	0.047	0.043	0.268	0.839
South Asian	55.520	0.886	52.468	0.869	0.005	0.021	0.820	0.876

Note: exposure is BMI, and the outcome is hemorrhoids. P<0.05 indicates statistical significance

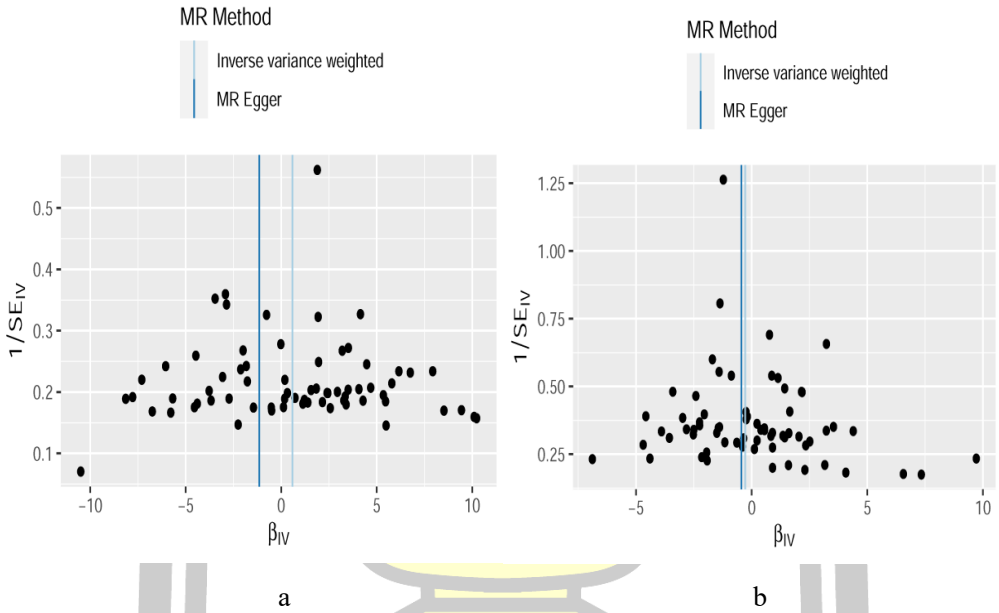


Figure 6 Funnel plot for MR analysis of the relationship between BMI and hemorrhoids.

(1) a is the Funnel plot for the East Asian population, and b is the Funnel plot for the South Asian; (2) The bright blue line and dark blue line represent IVW and MR-Egger method respectively

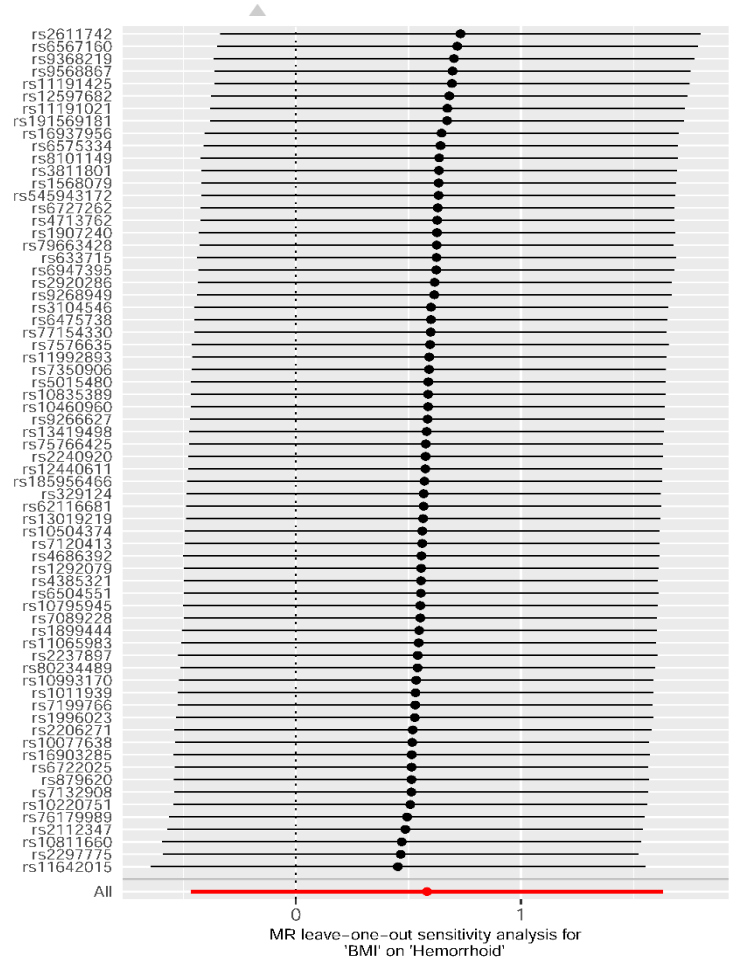


Figure 7 Sensitivity analysis of the "leave-one-out "method in MR analysis of BMI and hemorrhoids in East Asian populations. After removing each SNP, the overall error line did not change much (all error lines were to the right of 0), indicating that the results were robust.

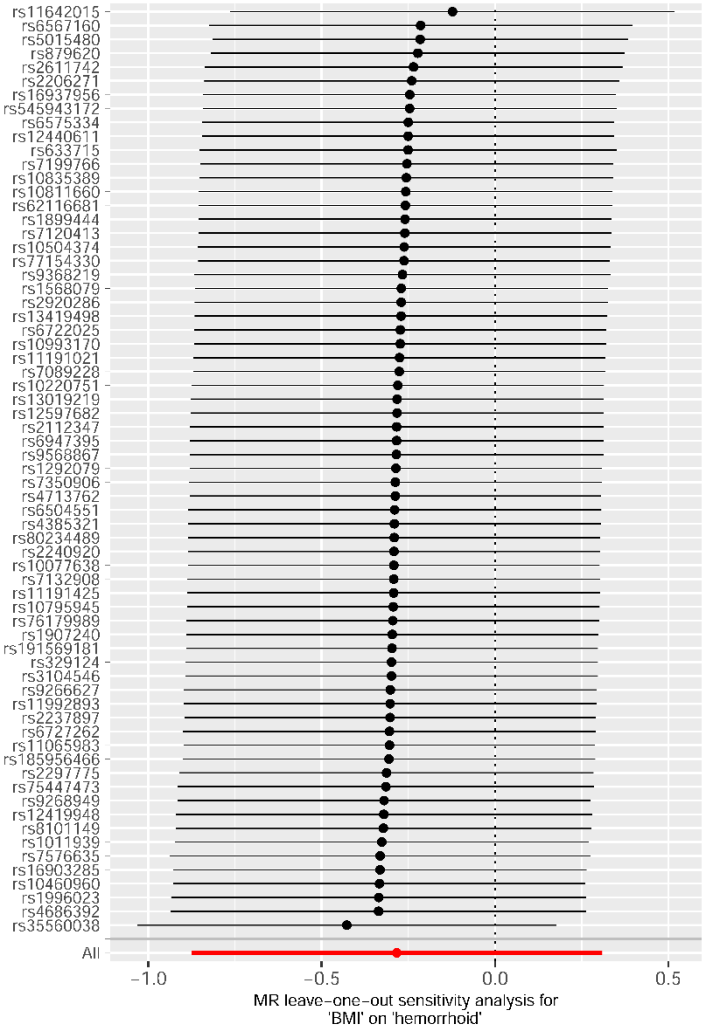


Figure 8 Sensitivity analysis of the "leave-one-out "method in MR analysis of BMI and hemorrhoids in South Asian populations. After removing each SNP, the overall error line did not change much (all error lines were to the left of 0), indicating that the results were robust.

4.3 Comparison of blood lipids between the two groups

The total cholesterol (TCHO), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) of the two groups were detected. After analysis, there was no significant difference in the content of four lipid items in the serum of the two groups.(see Table 13 for details)

Table 13 comparison results of blood lipids between the two groups(Unit: mmol/l)

Group	n	TCHO	TG	HDL-C	LDL-C
Case	200	3.32±1.24	3.07±3.88	0.89±0.32	1.87±0.81
Control	200	3.41±1.19	3.24±4.16	0.92±0.35	1.96±0.77
t value		-0.709	-0.401	-1.035	-1.097
P value		0.479	0.688	0.301	0.273

Note: P<0.05 means the difference is statistically significant

4.4 Results of association analysis between rs34221221 polymorphism of FOXC2 gene and hemorrhoids.

4.4.1 DNA quality inspection results

After the genomic DNA extracted from the whole blood is electrophoresis on the agarose gel, it can be clearly seen that the DNA bands are close to the spot sample, and it can be completely determined that the molecular weight of DNA is at least greater than 21Kb, indicating that the DNA extraction is successful, with good quality, and can fully meet the downstream experiments. As shown in Figure 9.

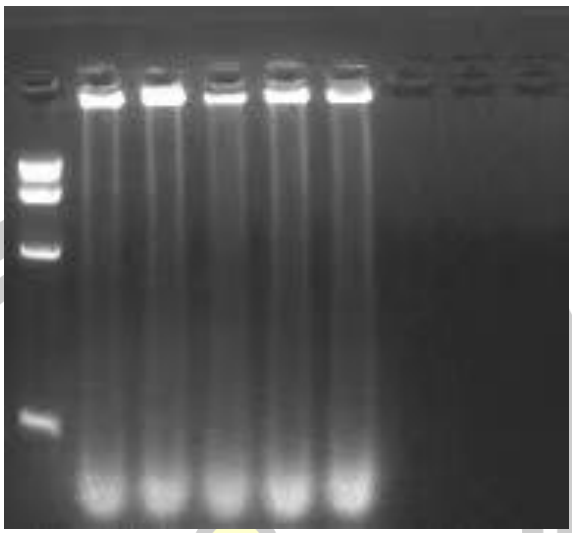


Figure 9 The whole genome DNA agarose gel electrophoresis map shows that the first lane from left to right is mark, and the second lane to the sixth lane is the whole genome DNA.

4.4.2 Genomic DNA concentration and purity determination results

The concentration of all DNA samples is>20ng/μL; The ratio of A260/A280 ranges from 1.6 to 1.8; The ratio of A260/A230 is between 2.00 and 2.20. The extracted DNA concentration and purity meet the experimental requirements. Please refer to Appendix 4 for details.

4.4.3 Detection results of rs34221221 polymorphism of FOXC2 gene

Sanger sequencing showed that 164 (41%) of 400 DNA samples carried CC genotype, and 234 (58.5%) carried CT genotype. The detection spectrum is shown in Figure 10 . The TT genotype was not detected in all samples. There were two cases of DNA sample test results that could not be judged, accounting for 0.5% of the total. The test results can be found in Appendix 5.

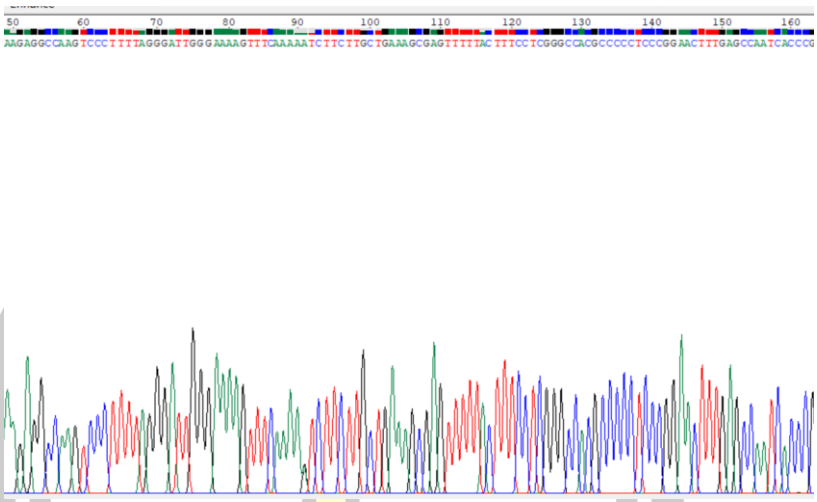


Figure 10 Map of Sanger sequencing results for rs34221221 polymorphic loci

4.4.4 Hardy-Weinberg genetic equilibrium law test results of genotype frequency distribution in the control group.

According to Harvey Weinberg's law of equilibrium, hardy Weinberg's genetic equilibrium coincidence test method was used to test the distribution of genotype frequency in the control group, which was consistent with Hardy Weinberg's law of equilibrium (P>0.05), as shown in table14.

Table 14 Test results of Hardy-Weinberg genetic equilibrium law

Gene locus	genotype	n	%	x ²value	p-value
rs34221221	CT	128	64	0.25	>0.05
	CC	72	36		

Note: P<0.05 means the difference is statistically significant

4.4.5 Genotype frequency and allele frequency distribution

The frequencies of allele C and T in the case group were 73.2% and 26.8%, respectively, and in the control group were 68% and 32%, respectively. There was no significant difference between the two groups (P=0.105). The number of cases with CC and CT genotypes in the case group was 92 and 106, respectively, and the frequency distribution was 46.5% and53.5% , respectively; The number of cases in the control group was 72 and 128 , and the frequency distribution was 36% and 64%, respectively. After statistical analysis, the distribution difference of the two genotypes between the two groups was statistically significant (p=0.034), see table15.

Table 15 Distribution of allele frequency and genotype frequency in case group and control group and their relationship with hemorrhoids susceptibility

Allele /Genotype	Case(n=198) n	Case(n=198) %	Control(n=200) n	Control(n=200) %	p_1-value[a]	Adjusted OR（95%CI）[b]	p_2-value[c]
C	290	73.2	272	68	0.105	1	
T	106	26.8	128	32		0.752(0.564-1.065)	0.115
CC	92	46.5	72	36	0.034	1	
CT	106	53.5	128	64		0.636（0.417-0.971）	0.036

Note: a is the chi-square p value of the distribution difference of allele frequency and genotype frequency between the case group and the control group, b and c are the logistic regression results after adjusting the education level, age, dietary taste, constipation and chronic gastritis, respectively. The difference was statistically significant when P<0.05.

4.4.6 Genetic polymorphisms and susceptibility to hemorrhoids

Multivariate unconditional logistic regression analysis showed that after adjusting for the confounding factors of education level, age, dietary taste, constipation and chronic gastritis , the minimum allele T and the risk of hemorrhoids had no statistical significance (adjusted OR=0.752, 95% CI: 0.564-1.065, P=0.115); Compared with CC genotype, carrying CT genotype was statistically correlated with the risk of hemorrhoids (adjusted OR=0.636, 95% CI: 0.417-0.971, P=0.036), shown in table15.

Further stratified analysis was conducted on the relationship between various genotypes at the rs34221221 locus of the FOXC2 gene and hemorrhoids, including gender, age, BMI, education level, family income, alcohol consumption, dietary habits, spicy and pickled food habits, dietary taste, work position, and activity intensity. The results showed that compared with the CC genotype, the CT genotype was statistically associated with the risk of hemorrhoids in the four population groups of males, non-alcoholic, non-spicy, and pickled foods (Adjusted OR=0.349, 95% CI: 0.168-0.722, P=0.005; Adjusted OR=0.576, 95% CI: 0.353-0.939, P=0.027; Adjusted OR=0.222, 95% CI: 0.088-0.560, P=0.001; Adjusted OR=0.596, 95% CI: 0.374-0.948, P=0.029); The stratified analysis of other factors did not find a statistical correlation between CT genotype and the risk of hemorrhoids, shown in Table 16.

Table 16 Stratified analysis of the relationship between rs34221221 polymorphism and hemorrhoids susceptibility

Stratification factors	Genotype	Case(n=198) n	Case(n=198) %	Control(n=200) n	Control(n=200) %	p-value[a]	Adjusted OR（95%CI）[b]
Gender							
Male	CC	37	50.0	21	28.8		1
	CT	37	50.0	52	71.2	0.005	0.349(0.168-0.722)
Female	CC	55	44.4	51	40.2		1
	CT	69	55.6	76	59.8	0.903	0.951(0.424-2.134)
Age（Year）							
<40	CC	42	45.7	37	37.0		1
	CT	50	54.3	63	63.0	0.182	0.662(0.361-1.214)
≥40	CC	50	47.2	35	35.0		1
	CT	56	52.8	65	65.0	0.096	0.613(0.344-1.091)
BMI							
<24	CC	55	42.3	40	33.9		1
	CT	75	57.7	78	66.1	0.149	0.676(0.398-1.150)
≥24	CC	37	54.4	32	39.0		1
	CT	31	45.6	50	61.0	0.066	0.533(0.272-1.042)
Education level							
higher education	CC	36	45.6	36	33.6		1
	CT	43	56.4	71	66.4	0.073	**0.572(0.311-1.053**）
Non higher education	CC	56	47.1	36	38.7		1
	CT	63	52.9	57	61.3	0.169	**0.675(0.386-1.182**）
Household income							
>4000**(CNY/month)**	CC	35	47.3	33	34.4		1
	CT	39	52.7	63	65.6	0.111	0.597(0.316-1.127)
≤4000**(CNY/month)**	CC	57	46.0	39	37.5		1
	CT	67	54.0	65	62.5	0.114	0.643(0.372-1.111)
Drinking situation							
Yes	CC	26	49.1	24	41.4		1
	CT	27	50.9	34	58.6	0.312	0.663(0.299-1.470)
No	CC	66	45.8	46	33.1		1
	CT	78	54.2	93	66.9	0.027	0.576(0.353-0.939)
Dietary habit							
Mainly meat based	CC	35	47.9	20	33.9		1
	CT	38	52.1	39	66.1	0.074	0.511(0.245-1.068)
Vegetarian oriented	CC	46	47.3	43	41.0		1
	CT	51	52.6	62	59.0	0.340	0.752(0.419-1.351)
Uniformity of meat and vegetables	CC	11	39.3	9	25.0		1
	CT	27	60.7	27	75.0	0.195	0.484(0.161-1.453)
Spicy food							
Eating regularly	CC	26	44.8	23	41.1		1
	CT	32	55.2	33	58.9	0.845	0.925(0.426-2.011)
Occasionally eat	CC	40	43.5	38	39.6		ref
	CT	52	56.5	58	60.4	0.456	0.794(0.432-1.457)
Not eating	CC	26	54.2	11	22.9		1
	CT	22	45.8	37	77.1	0.001	0.222(0.088-0.560)

Table 16 Stratified analysis of the relationship between rs34221221 polymorphism and hemorrhoids susceptibility(Continued Table)

Stratification factors	Genotype	Case(n=198)		Control(n=200)		p-value[a]	Adjusted OR（95%CI）[b]
		n	%	n	%		
Pickled food	CC	75	47.8	59	37.3		1
Yes	CT	82	52.2	99	62.7	0.029	0.596(0.374-0.948)
	CC	17	41.5	13	31.0		1
No	CT	24	58.5	29	69.0	0.468	0.708(0.279-1.197)
Dietary taste	CC	44	50.6	33	37.9		1
Salty	CT	43	49.4	54	62.1	0.098	0.594(0.321-1.102)
	CC	29	46.0	27	33.3		1
Bland	CT	34	54.0	54	66.7	0.126	0.580(0.289-1.164)
	CC	19	39.6	11	36.7		1
Moderate	CT	29	60.4	19	63.3	0.748	0.855(0.328-2.255)
Working position	CC	41	42.7	23	31.5		1
Sitting position	CT	55	57.3	50	68.5	0.159	0.626(0.326-1.202)
	CC	51	50.0	49	38.6		1
Non sitting posture	CT	51	50.0	78	61.4	0.098	0.637(0.373-1.087)
Physical activity	CC	17	73.0	12	48.0		1
High strength	CT	6	26.1	13	52.0	0.167	0.398(0.108-1.472)
	CC	26	49.1	13	34.2		1
Medium strength	CT	27	50.9	25	65.8	0.066	0.416(0.164-1.060)
	CC	49	40.2	46	34.1		1
Low strength	CT	73	59.8	89	65.9	0.242	0.731(0.433-1.235)

Note: a and b are logistic regression results adjusted for education level, age, dietary taste, constipation and chronic gastritis , P<0.05 is statistically significant.

4.4.7 Interaction between polymorphism at rs34221221 locus of FOXC2 gene and external factors.

In the analysis of the interaction between rs34221221 polymorphism and various external factors on hemorrhoids, it was found that CT genotype had interaction effects with males, non-alcohol consumption, and non-spicy food consumption, all of which could reduce the risk of hemorrhoids(OR=0.404, 95% CI: 0.204-0.719, P=0.009; OR=0.585, 95% CI: 0.361-0.947, P=0.029; OR=0.252, 95% CI: 0.104-0.607, P=0.002). No interaction was found between the polymorphism of rs34221221 locus and women, age, BMI, education level, family income, alcohol consumption, dietary habits, eating chili peppers, pickled food habits, dietary taste, work position, physical activity intensity, number of abortions, and age of first childbirth, as shown in Table 17.

Table 17 The interaction between the rs4221221 polymorphism of FOXC2 gene and various external factors on hemorrhoids

Interaction factors	B	OR	95%CI	p-value
CC*Gender		1		
CT*Male	-0.907	0.404	0.204-0.719	0.009
CT*Female	-0.172	0.842	0.510-1.390	0.501
CC*Age		1		
CT*<40	0.001	1.001	0.985-1.017	0.930
CT*≥40	-0.007	0.993	0.983-1.004	0.202
CC*BMI		1		
CT*<24	-0.358	0.699	0.417-1.172	0.174
CT*≥24	-0.623	0.536	0.279-1.029	0.061
CC*Education level		1		
CT*higher education	-0.342	0.711	0.410-1.233	0.224
CT*Non higher education	-0.501	0.606	0.333-0.101	0.100
CC*Household income (CNY/month)		1		
CT*<4000	-0.538	0.584	0.314-1.086	0.089
CT*≥4000	-0.349	0.708	0.415-1.200	0.198
CC*Drinking situation		1		
CT*Yes	-0.311	0.733	0.346-1.552	0.417
CT*No	-0.537	0.585	0.361-0.947	0.029
CC*Dietary habit		1		
CT*Mainly meat based	-0.586	0.557	0.274-1.130	0.105
CT*Vegetarian oriented	-0.263	0.769	0.441-1.432	0.355
CT*Uniformity of meat and vegetables	-0.663	0.515	0.177-1.501	0.224
CC*Spicy food		1		
CT*Eating regularly	-0.153	0.858	0.408-1.802	0.686
CT*Occasionally eat	-0.160	0.852	0.477-1.522	0.588
CT*Not eating	-1.380	0.252	0.104-0.607	0.002
CC*Pickled food		1		
CT*Yes	-0.186	0.831	0.674-1.023	0.081
CT*No	-0.458	0.633	0.257-1.560	0.320
CC*Dietary taste		1		
CT*Salty	-0.515	0.597	0.327-1.092	0.094
CT*Bland	-0.534	0.586	0.298-1.154	0.122
CT*Moderate	-0.124	0.884	0.345-2.265	0.797
CC*Working position		1		
CT*Sitting position	-0.483	0.617	0.326-1.168	0.138
CT*Non sitting posture	-0.465	0.628	0.371-1.065	0.084
CC*Physical activity		1		
CT*High strength	-1.121	0.326	0.096-1.101	0.071
CT*Medium strength	-0.616	0.54	0.229-1.276	0.540
CT*Low strength	-0.261	0.77	0.463-1.279	0.313
CC*Number of abortions		1		
CT*≤2times	0.145	1.156	0.498-2.679	0.736
CT*≥3times	-0.588	0.556	0.081-3.795	0.549
CC*First childbearing age		1		
CT*≤28years	0.229	1.257	0.576-2.743	0.566
CT*>28years	-0.553	0.575	0.203-1.625	0.296

Note: P<0.05 is statistically significant.

4.5 Establishment of hemorrhoids risk prediction model and evaluation

4.5.1 Establishment of hemorrhoids risk prediction model

The independent risk factors and genotypes related to hemorrhoids obtained from the above analysis were used as independent variables, and whether suffering from hemorrhoids was used as dependent variable for logistic regression (table 18). Then, the regression coefficients of the obtained independent variables were used as weights to establish the hemorrhoids risk prediction model. The final model was: Logit（P）$=-1.4+0.03X_1+0.79X_2-0.71X_3+1.02X_4+0.65X_5-0.45X_6$, X_1 is age, X_2 is education level, X_3 is dietary taste, X_4 is constipation, X_5 is chronic gastritis, X_6 is genotype. The probability of hemorrhoids can be calculated by substituting the relevant information of patients into the model. Model coefficients passed Omnibus test, $\chi^2=31.211$, $P<0.001$, indicating that the modeling was successful. Hosmer-Lemeshow Goodness-Of-Fit test was used to test the constructed model. There was no significant difference between the predicted probability of hemorrhoids and the actual probability（ $\chi^2=8.356$, P=0. 399), suggesting that the model fitting was acceptable.

Table 18 correlation analysis between independent risk factors and hemorrhoids

Risk factor	B	OR	95%CI	p-value
Age	0.03	1.03	1.01-1.05	0.011
Education level	0.79	2.21	1.40-3.49	0.001
Dietary taste	-0.71	0.49	0.27-0.88	0.017
Constipation	1.02	2.76	1.52-5.01	0.001
Chronic gastritis	0.65	1.92	1.12-3.30	0.017
Genotype	-0.45	0.64	0.42-0.97	0.036

Note: P<0.05 is statistically significant.

4.5.2 Evaluation of hemorrhoids risk prediction model

Evaluate the constructed hemorrhoid risk model using ROC curves. Calculate individual predicted values based on the prediction model formula, and plot the ROC curve with the predicted values as the test variable (Figure 11). The results showed that the area under the ROC curve was 0.61 (95% CI: 0.56-0.67, P<0.001).

Because when the sensitivity and specificity are the largest, the Youden index is the largest. It means, at this value, the judgment ability of the method to be evaluated is the strongest. Therefore, this study uses the maximum value of the Youden index to

determine the cut-off value of the prediction model. It means when the maximum value of the Youden index is taken, the corresponding model predictive value is the value to judge whether the predicted person will suffer from hemorrhoids. When the predictive value is greater than this value, it means that the predicted person has the risk of suffering from hemorrhoids. If it is less than this value, there is no risk of suffering from hemorrhoids.

After calculation，when the sensitivity is 0.561 and the specificity is 0.645, the maximum value of the Youden index is 0.21, the corresponding predicted value is 0.6, so the optimal cut-off value is 0.6(All sensitivity, specificity, Youden index, and corresponding predicted values can be found in Appendix 6). This means that a predicted value greater than 0.6 indicates a risk of hemorrhoids, while a value less than 0.6 indicates no risk of hemorrhoids. The Positive and Negative predictive value is 0.76 and 0.90, therefore, the accuracy of this model in predicting the incidence of hemorrhoids is 76%, the accuracy of non hemorrhoid incidence is 90%, and the accuracy of the entire group is 87.8%.

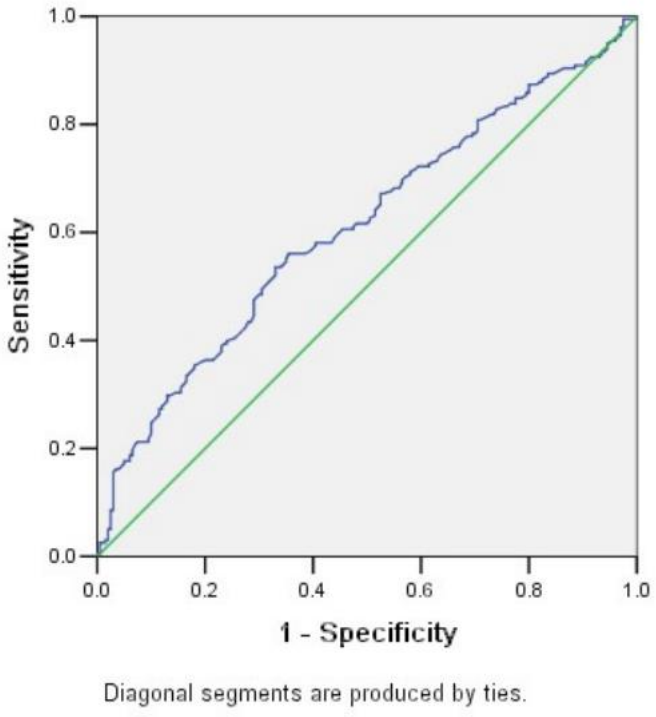


Figure 11 ROC curve of hemorrhoid risk prediction model

CHAPTER V
DISCUSSIONS

In this chapter, the research findings are summarized, discussed, and concluded. Firstly, Section 5.1 provides a summary of the risk factors for hemorrhoids identified in this study. 5.2 is discussion consisting of three parts. Section 5.2.1 discusses the relationship between external factors discovered in research and hemorrhoids. Section 5.2.2 discusses the correlation between internal factors and hemorrhoids. Section 5.2.3 discusses the predictive role of the hemorrhoid risk prediction model in hemorrhoids. Section 5.3 is Summary and suggestions. Section5.4 Strengths and limitations of the study. Section5.5 is a conclusion of this research.

5.1 Summary of research results

At present, there are various research results on the risk factors of hemorrhoids, and many external factors may lead to the occurrence of hemorrhoids. There are also research results indicating that genetic factors are also risk factors for hemorrhoids. This study adopts a case-control research method, and through questionnaire surveys, Mendelian randomization analysis, and genetic polymorphism testing, it is found that age, education level, light diet, constipation, and chronic gastritis are associated with the risk of hemorrhoids. Among them, age, education level, constipation, and chronic gastritis increase the risk of hemorrhoids, while a light diet can reduce the risk of hemorrhoids. This study also demonstrated through Mendelian randomization that there is no causal relationship between BMI and hemorrhoids. In addition, this study also found that the CT genotype at the rs34221221 locus of the FOXC2 gene is associated with susceptibility to hemorrhoids, which can significantly reduce the risk of hemorrhoids. Moreover, the CT genotype also interacts with three factors: male, no alcohol consumption, and no spicy food consumption, all of which can reduce the risk of hemorrhoids. This study also established a hemorrhoid risk prediction model using the 6 hemorrhoid related factors discovered in the study, which has a certain predictive effect on the incidence of hemorrhoids.

5.2 Discussion

5.2.1 The relationship between external factors discovered in the research and hemorrhoids

5.2.1.1 The correlation between education level and hemorrhoids

Education level refers to the level of educational knowledge and abilities acquired by individuals through schools, training institutions, social experience, and other means. Education level is an important indicator of human civilization progress and a crucial condition for participating in social and economic development. In modern society, individuals with higher levels of education generally have more opportunities and advantages in career development, quality of life, and social status. Personal occupation, quality of life, and behavior are related to many diseases, and harmful occupational factors and unhealthy lifestyle behaviors are also risk factors for various diseases.

People with high levels of education are generally engaged in occupations primarily focused on mental labor. Most of these professions require prolonged sitting and minimal physical activity. Long term sitting without movement can slow down gastrointestinal peristalsis, leading to abdominal distension and constipation [188], which has always been considered a risk factor for hemorrhoids. On the other hand, prolonged sitting may lead to slow venous return, stasis of blood in the rectal and anal veins, and may cause venous valve dysfunction, leading to obstruction of venous return and prolonged congestion and dilation of venous plexus[189], ultimately resulting in varicose veins and hemorrhoids. In univariate analysis, this study also found a correlation between a sitting work posture and the risk of hemorrhoids, but the correlation disappeared after adjusting for the factor of education level. This is likely due to the fact that sitting posture is an intermediate factor in the association between education level and hemorrhoids, to the extent that when education level is introduced into the model, its association with hemorrhoids is included in the factor of education level.

On the other hand, compared to those with lower levels of education, those with higher levels of education also have relatively higher economic income. And a higher income level means they have a higher level of consumption. Therefore, they tend to consume high levels of polished rice, noodles, and meat in their diet, while the intake of coarse grains with high dietary fiber is relatively reduced, which slows down food digestion, reduces gastrointestinal motility, and easily causes bloating and

constipation[190], leading to hemorrhoids. In the factor analysis of this study, it was found that higher household income was also associated with the occurrence of hemorrhoids compared to lower household income. However, similar to the sitting position, after adjusting for education level, the correlation between higher family income and hemorrhoids disappeared. This also means that family income is an intermediate factor in the association between education level and hemorrhoids.

5.2.1.2 The correlation between dietary taste and hemorrhoids

Diet plays a crucial role in maintaining life, promoting health, and prolonging lifespan. A reasonable diet can provide the body with the nutrients it needs, such as protein, fat, carbohydrates, vitamins, and minerals, to maintain normal body function, enhance the body's immunity and resistance. In addition to reasonable dietary combinations, dietary taste is also related to certain diseases.

A person's dietary taste is related to taste, which is mediated by receptor cells in the taste buds on the dorsal and posterior surfaces of the tongue, as well as on the surface of the oral and pharyngeal epithelium [191]. Taste receptor cells are also present in the intestine [192]. Saliva plays a crucial role in stimulating food contact with receptor cells. They detect chemical signals that generate taste and stimulate the release of neurotransmitters onto incoming nerve fibers, thereby transmitting signals to the brainstem.

Taste plays a crucial role in food selection, whether one likes or dislikes food for the first time. There are five types of taste, namely bitter, salty, sour, sweet, and fresh[193]. People believe that sweetness can detect high-energy, high calorie, and pleasurable substances, while bitterness perception can lead to avoidance or rejection of toxic or toxic substances. Controlling the intake of Na+through saltiness is crucial for maintaining water balance and blood circulation in the body [194]. Freshness can recognize amino acids, especially glutamic acid [195]. Sourness is an important sensory input that affects the intake of acidic food sources [196].

The latest developments in the field of taste physiology have elucidated the roles of different basic taste patterns and their impact on health and disease [197]. Current research on taste and disease has confirmed that taste is closely related to the risk of certain diseases. For example, studies have shown a relationship between obesity and sweetness [198-199], while saltiness is associated with hypertension. Other taste types,

such as sweetness, freshness, or bitterness, are also associated with changes in blood pressure. And there is convincing evidence that changes in salt sensitivity can be used to predict the onset of hypertension [200]. Li Hanqi et al. used the GeoDetector method to establish associations between seven flavors and multiple chronic diseases from the perspective of spatial stratification heterogeneity. They found that out of 71 chronic diseases, 16 diseases were significantly related to dietary taste. Compared to the impact of individual flavors, the interaction of flavors increases the risk of sixteen diseases, and the multiple combinations of flavors have a non-linear enhancing effect on disease risk [201]. In addition, taste is also related to diabetes, Parkinson's disease and cardiovascular disease [202].

This study found that compared to people with moderate dietary tastes, those with a lighter diet have a significantly reduced risk of developing hemorrhoids. People with a lighter taste tend to choose vegetarian foods, consuming less salt, sugar, and fat in their bodies every day, while consuming a relatively higher proportion of dietary fiber. This not only reduces the burden of high sugar, high fat, and high salt foods on the digestive system, but also promotes the digestive function of the gastrointestinal tract, plays a role in preventing constipation, and thus reduces the risk of hemorrhoids. However, further research is needed to elucidate the reasons and mechanisms in this regard.

5.2.2 Discusses the correlation between internal factors and hemorrhoids.

5.2.2.1 The correlation between age and hemorrhoids

Age is one of the important factors affecting human health. As people age, various organs and systems in the human body will experience varying degrees of degeneration and aging, such as the heart, blood vessels, lungs, kidneys, bones, joints, etc. The function of these organs and systems decreases, and the risk of various chronic or acute diseases increases accordingly.

As age increases, the digestive system function decreases, which may induce abnormalities in the oral cavity, esophagus, stomach, intestines, liver and other organs. As age increases, the secretion of salivary glands decreases, and the burden of food digestion in the lower digestive tract increases; Annual esophageal muscle atrophy, weakened contraction force, reduced esophageal peristalsis, and prolonged passage of food.

Atrophy and degeneration of gastric mucosa and glandular cells, reduced secretion of gastric juice, leading to mechanical damage to the gastric mucosa. The formation obstacle of mucus bicarbonate barrier makes the gastric mucosa susceptible to damage by gastric acid and pepsin, reducing the digestive function of pepsin; The villi of the small intestine become wider and shorter, the smooth muscle layer becomes thinner, the contraction and peristalsis are weak, and the secretion of various digestive enzymes is reduced, greatly reducing the digestive function of the small intestine; Atrophy of colonic mucosa, thickening of muscular layer, and easy formation of diverticula. Slow and weak intestinal peristalsis, weak absorption of water, insufficient filling of the large intestine, inability to cause dilation sensation, ultimately leading to constipation. The main clinical symptoms of constipation are prolonged defecation time, dry stools, and prolonged exertion during defecation, leading to increased abdominal pressure and damage to the connective tissue and submucosal muscles inside the anal pad, causing relaxation and rupture. The anal pad loses its grip and moves downwards, causing it to protrude from the anus and become hemorrhoids [203].

In addition, as age increases, the tension of the valve gradually decreases, making venous return difficult; The elasticity and tension of venous blood vessels will also decrease, further reducing venous reflux, leading to venous filling and elevated venous pressure, resulting in the appearance of dilated and tortuous veins [204]. The varicose veins at the bottom of the rectum and anal mucosal venous plexus form one or more soft vein masses, leading to hemorrhoids. On the other hand, with the increase of age, if there is a long-term high salt, high sugar, high fat diet, lack of exercise, irregular life, etc., the aging of blood vessels begins to accelerate. As time goes on, it can be manifested as the thickening of blood vessel walls or the formation of plaque leading to narrowing of blood vessels, which makes the elasticity of blood vessels gradually decline, while suffering from hypertension, diabetes Patients with diseases such as hyperlipidemia have more significant vascular changes in this area. This change is also the cause of venous congestion, increased venous pressure, and the expansion and tortuosity of the veins, leading to the development of hemorrhoids.

5.2.2.2 The correlation between constipation and hemorrhoids

Constipation refers to poor or difficult bowel movements, usually manifested as prolonged bowel movements, reduced frequency of bowel movements, dry stools, and

difficulty in defecation. People usually attribute the onset of hemorrhoids to constipation and abnormal bowel habits, and believe that constipation is the main culprit. This is consistent with the results of this study. This study found that constipation can significantly increase the risk of hemorrhoids. Possible reasons are as follows: Constipation and excessive force during defecation may increase unnecessary burden on the muscles of the cecum, anus, and pelvic floor, as well as local congestion, leading to the occurrence of diseases. Effortlessly removing dry feces can also cause significant pressure on the anus, leading to a series of pathological changes such as bruising, swelling, and lacerations. At the same time, constipation and prolonged tension during defecation can cause tearing of the elastic support tissue. This may lead to distal displacement of the anal pad and the development of hemorrhoids [51, 52]. In addition, studies have shown that hard stool and increased intra-abdominal pressure can hinder venous return, leading to congestion of the hemorrhoid plexus and arteriovenous anastomosis at the anorectal junction, thereby leading to the development of hemorrhoids [53].

Talley NJ et al.'s study reviewed the relationship between constipation and specific comorbidities [205]. Their data suggests that constipation can have significant clinical consequences. And one consequence is constipation. Their research findings found that both retrospective and prospective case-control studies showed a significant association between constipation and hemorrhoids [206-210], or an increased prevalence of hemorrhoids in populations diagnosed with constipation [211]. Other studies have further demonstrated the association between hemorrhoids and constipation symptoms [212]. Hong YS et al. conducted a cross-sectional study on 194，620 healthy males and females who completed health screening examinations, including colonoscopy, from 2011 to 2017. The study also found that constipation is associated with hemorrhoids [9].

Although it is widely believed that constipation is related to hemorrhoids, there are also inconsistent research results. Johnson and Sonnenberg found no association between hemorrhoids and constipation, aging, cirrhosis, and varicose veins[55]. They were also evaluating four large population data files in the United States, England, and Wales when they discovered epidemiological differences in constipation and hemorrhoids (i.e. different prevalence distributions by age, gender, and race). Although these studies suggest that the frequency of hemorrhoids in patients with and without

constipation may be the same, they do not necessarily rule out a causal relationship between symptoms of constipation and hemorrhoids [213].

5.2.2.3 The correlation between chronic gastritis and hemorrhoids

Chronic gastritis refers to various chronic inflammatory lesions of the gastric mucosa caused by different etiologies, and Helicobacter pylori infection is a common cause of chronic gastritis. Animal and human studies have confirmed that Helicobacter pylori infection can cause changes in the microbiota of the stomach or colon [214-217]. Yin et al. found in their experiment that the number of Staphylococcus aureus and Lactobacillus in the stomach and duodenum of Mongolian gerbils decreased after inoculation with Helicobacter pylori [214]. In the same model, Heimesaat et al. demonstrated that mice infected with immunopathological Helicobacter pylori B8 can increase the lumen load of Escherichia coli and enterococcus and Bacteroides/Prevotella in the cecum [207]. Research has found an interactive relationship between dysbiosis of gut microbiota and the onset of diarrhea [218]. Due to the disruption of the gut microbiota, an increase in enterotoxins can lead to symptoms of diarrhea, such as damage or increased sensitivity of the intestinal mucosa. Diarrhea patients have a significantly reduced number of bacteria in their feces compared to healthy individuals, accompanied by an imbalance in the proportion of bacteria. Diarrhea can also cause changes in the number and type of gut microbiota. Therefore, the mutual promotion of the two can easily lead to chronic diarrhea. Long term diarrhea can lead to excessive abdominal pressure during defecation, prolonged increase in abdominal pressure, and affect the blood flow of the rectal anal venous plexus, resulting in blood stasis and venous dilation. Over time, it may form hemorrhoids. In addition, due to the increase in bowel movements and frequent stimulation of the rectum and anus by feces, the local load increases, which is related to the damage to the veins at the rectum and anus. Some existing research results have also found a significant association between diarrhea and hemorrhoids [23, 219], but the mechanism is not yet clear and further research is needed.

In addition, chronic gastritis can lead to weakened gastrointestinal function, resulting in a decrease in the digestive and absorption functions of the gastrointestinal tract towards food. It may also cause difficulty in defecation and result in a defecation surface, which may also be a reason for the association between chronic gastritis and

hemorrhoids.

5.2.2.4The correlation between BMI and hemorrhoids

BMI is calculated by dividing weight (kilograms) by the square of height (meters). The ratio obtained from this formula can to some extent reflect human density. Due to its simple calculation method, it is now widely used to evaluate the nutritional status, obesity or physical development level of the human body. In general, the body mass index of Chinese adults is within the normal range of 18.5-23.9 kg/m^2, 24-27.9 kg/m^2 is considered overweight, 28-32 kg/m^2 is considered obese, and over 32 kg/m^2 is considered super obese.

Currently, research reports suggest that obesity and high body mass index can be considered independent risk factors for hemorrhoids [8-9]. This may be due to increased abdominal pressure caused by high body weight and visceral fat, which is believed to lead to congestion of the distal rectal veins [8, 220]. In addition, obesity can induce the release of inflammatory cytokines and acute phase proteins, ultimately activating the innate immune system and affecting metabolic homeostasis, ultimately leading to the formation of hemorrhoids [216]. Some other studies also support this result. The study by Kibret AA et al. [5] found a significant statistical correlation between BMI≥25kg/m^2 and hemorrhoids, indicating that being overweight increases the risk of developing hemorrhoids. Lee KY et al. also found in their data analysis of 8139 adult women that women with higher body mass index had a higher incidence of hemorrhoids [221]. The study by Rise S et al. also found that body mass index (BMI) has a significant impact on the occurrence of hemorrhoids, and even after correcting for other potential risk factors, an increase in BMI increases the risk of hemorrhoids by 3.5% [8]. However, the research results of Peery AF et al. are opposite, as they believe that overweight or obesity are not related to the presence of hemorrhoids [222]. In addition, Lee JH et al.'s study showed that although multiple logistic regression analysis supports BMI as an independent risk factor for hemorrhoids, there is little difference in BMI between patients with and without hemorrhoids. Therefore, its clinical relevance may be questioned [220].

The inconsistent results of numerous studies on the association between BMI and hemorrhoids may be related to confounding factors, sample size, and different study populations. Because current research on the relationship between the two is

observational, it is inevitable to be influenced by confounding factors. Even randomized controlled trials (RCTs) face high implementation difficulties, significant human and material resources, and ethical issues in reality, making it impossible to conduct unbiased research on a certain factor. Mendelian randomization (MR) is a method developed to address the aforementioned issues. It can effectively utilize the results of existing Genome wide Association Studies (GWAS) data, using genetic variation as instrumental variables (IVs), to explore the causal relationship between risk factors and outcomes [223]. According to Mendelian inheritance law, parental alleles are randomly assigned to offspring, which is equivalent to random grouping in RCT studies. In theory, genetic variation is not affected by common confounding factors such as postnatal environment, and genetic variation occurs earlier than exposure and outcome, ruling out the problem of reverse causality. Therefore, using genetic variation as IVs to analyze causal relationships between variables is gradually being applied in epidemiological studies. At present, with the public publication of large-scale GWAS data, a large number of reliable genetic variations have been provided for MR research, and a large number of studies have used MR methods to explore the causal relationships between multiple traits [224].

At present, there is only one MR LDP (a probabilistic model for MR analysis in identifying the causal effects between risk factors and disease outcomes using GWAS summary statistics in the presence of LD and to properly account for horizontal pleiotropy among genetic variables (MR-LDP)) article reported in the literature on BMI and hemorrhoids, A study in this article confirms a positive causal relationship between BMI and hemorrhoids in the European population [225]. There have been no reports of MR studies on BMI and hemorrhoids in other populations.

This study used a two sample Mendelian randomization method to investigate the causal relationship between BMI and hemorrhoids in East and South Asian populations. The results did not support a causal relationship between BMI and hemorrhoids in both populations, which is consistent with the results of our observational study's univariate analysis. MR is essentially a technique that evaluates the causal effects caused by modifiable non genetic exposure factors through genetic data. The environmental exposure factors measured in observational studies are more or less influenced by behavioral, social, psychological, and other factors, resulting in bias. MR uses genetic

variation as an instrumental variable to estimate the causal relationship between exposure factors and outcomes, and the genetic variation represented by genotype is not influenced by acquired factors. Therefore, compared to other types of research, the results of MR are more reliable. Our study searched for and excluded instrumental variables that may be associated with confounding factors such as gender, age, sedentary behavior, dietary fiber intake, constipation, diarrhea, etc. on Phenoscanner V2 when selecting instrumental variables. At the same time, we conducted heterogeneity tests on the instrumental variables and tested the results for multiple effects and robustness, ensuring the accuracy and reliability of the results.

However, due to the limited research on hemorrhoids GWAS in East and South Asian populations, the sample size for obtaining hemorrhoid GWAS data in this study was relatively small, especially in East Asian populations with only 2658 samples and 228 cases, which had a certain impact on the statistical efficacy of MR. Due to the small sample size, further stratified analysis based on factors such as gender, age, blood type, and occupation was not possible, resulting in a lack of more detailed research results. These are all limitations of this study. When the GWAS data for hemorrhoids becomes more comprehensive, further larger sample size MR analysis can be conducted to obtain more accurate and detailed results.

5.2.2.5 The correlation between the rs34221221 polymorphism of FOXC2 gene and hemorrhoids

FOXC2 is a gene located in the 16q24.1 region of the human chromosome, encoding the forkhead box C2 protein. The FOXC2 gene has been found to be associated with various human diseases. It is an indispensable transcription factor in the occurrence and development of cardiovascular and axial bone. Research has shown that FOXC2, along with TIE2, VEGFR-3, rasa1, KRIT1, MGC4607, PDCD10, Nemo, SOX18, EEG, ACVRLKL, MADH4, NDP, TIMP3, NOTCH3, COL3A1, PTEN, and other important angiogenic regulatory factors, plays an important role in the process of angiogenesis [105]. Before the appearance of the circulatory system, genetic programs first determine the characteristics of arteries and veins. The signal cascade amplification system of FOXC2 with SHH, VEGF, VEGFR2, FOXC1, COUP-TFII, and other factors determines the differentiation of arteries and veins [99]. Animal experiments have confirmed that FOXC2 is highly expressed in mouse embryonic venous valves, and

mutations in the FOXC2 gene can lead to loss of lower limb venous valve function and venous reflux. Studies have shown that FOXC2 is closely related to the pathogenesis of varicose veins [104-107]. Mellor RH, Lim CS, and others have found mutations in the FOXC2 gene in patients with varicose veins [108-109]. The linkage study of twins by Ng et al. confirmed the heritability of FOXC2 mutation in the occurrence and development of primary varicose veins; Further research also suggests that the FOXC2 mutant has evidence of venous reflux during ultrasound examination [110]. The theory of varicose veins in hemorrhoids also suggests the presence of venous congestion and varicose veins in the venous plexus of hemorrhoids. However, there is currently no further research on the relationship between the FOXC2 gene and hemorrhoids in Southeast Asian populations.

This study used the Sanger sequencing method to detect for the first time the difference in the rs34221221 polymorphism of the FOXC2 gene between hemorrhoid and non hemorrhoid patients. The results showed that in the Zhuang population, compared with the CC wild genotype, the carrying rate of CT mutation genotypes in the hemorrhoid population was significantly lower than that in the non hemorrhoid population. This means that the risk of hemorrhoids in Zhuang people carrying the mutated CT genotype was significantly reduced compared to those carrying the wild CC genotype, The mutated CT genotype has a certain protective effect on hemorrhoids in the Zhuang population. In recent years, few researchers have studied the rs34221221 polymorphism of FOXC2 gene, and there are individual studies on diabetes and retinopathy of the eye. However, some studies have also found that the polymorphism of this locus is associated with varicose veins. Surendran et al. found that the FOXC2 gene promoter variant -512C>T (rs34221221) is associated with increased susceptibility to varicose veins[226-227]. Shadrina AS et al. measured the allele, genotype, and haplotype frequencies in 474 samples of patients with primary varicose veins and 478 control samples without a history of chronic venous disease. The haplotype composed of the wild-type allele T and other alleles at the rs34221221 locus polymorphism was found to be associated with an increased risk of varicose veins[228]. Although hemorrhoids themselves are varicose veins, the above conclusions are not consistent with our research findings, which may be related to differences in sample size and the ethnicity of the study subjects. Another possible reason is that humans

interact with the environment, resulting in significant genetic differences between different regions and races, which are designed to adapt to the environment.

Although our study found that mutated CT genotypes can reduce the risk of hemorrhoids in the Zhuang population compared to wild CC genotypes. However, the occurrence of diseases is a complex process that is not only influenced by genetic factors, but also by external environmental and acquired behavioral factors. Hemorrhoids are also a disease influenced by multiple factors, especially behavioral factors. Therefore, after analyzing the relationship between genetic factors and it, we further stratified by gender and behavioral factors to analyze the impact of genetic factors on susceptibility to hemorrhoids in populations of different genders and behaviors. The results showed that after adjusting for confounding factors, compared to the wild CC genotype, individuals with mutated CT genotypes had a significantly reduced risk of hemorrhoids only in males, non drinkers, those who did not eat chili peppers, and those who ate pickled foods. This indicates that certain individual physiological or behavioral factors, and even other environmental factors, have a significant impact on the susceptibility to hemorrhoids, and have masked the protective effect of the rs43221221 polymorphism of the FOXC2 gene on the population with hemorrhoids.

Genetic factors, environmental factors, and acquired behavioral factors can not only affect an individual's susceptibility to diseases alone, but also influence the occurrence and development of diseases through their interaction. Many diseases are affected by the interaction of gene, environment and behavioral factors, such as hypertension, diabetes, cancer, etc.[229-231]. A comprehensive analysis of the interaction between different genotypes of disease susceptibility genes and environmental and behavioral risk factors is crucial for disease prevention. The study of interaction can elucidate the genetic susceptibility of diseases, which helps to effectively prevent diseases by controlling the environmental factors or behavior of individuals carrying susceptibility genes. At the same time, by analyzing and identifying susceptible populations, susceptible populations can be selected from the general population, so as to focus on protecting susceptible populations. Therefore, research on the interaction between genes and the environment has important public health significance. This study analyzed the interaction between genotypes and various environmental or behavioral

factors in individuals carrying mutated CT genotypes. It was found that CT genotypes have an interactive effect on reducing susceptibility to hemorrhoids with gender (male) and two behavioral factors: not drinking alcohol and not eating chili peppers. This result indicates that individuals carrying the CT genotype are at a lower risk of developing hemorrhoids if they are male or have not consumed alcohol or chili peppers. This provides a basis for behavioral prevention of hemorrhoids.

As this study is the first to explore the association between the rs34221221 polymorphism of the FOXC2 gene and hemorrhoids, and also the first to explore the relationship and interaction between this polymorphism and environmental, behavioral factors and hemorrhoids, there are no other research results for comparison. This study used a hospital based case-control study, and the results were inevitably biased. In addition, due to the stratified analysis, the sample size of each layer is small, which has a certain impact on the reliability of the results. These are all limitations of this study. To obtain more reliable results on the impact of genetics and its interaction with environmental and behavioral factors on susceptibility to hemorrhoids, it is necessary to expand the sample size for research.

5.2.3 Effect of Hemorrhoid Risk Prediction Model

Although hemorrhoids are not a fatal disease, they also pose certain risks to the body. Mild hemorrhoids can cause discomfort such as difficulty in defecation, bleeding during defecation, and anal swelling, affecting work and quality of life. Severe hemorrhoids can also cause diseases such as anemia, skin eczema, anal dysfunction, and autonomic nervous system disorders, seriously affecting physical health and quality of life. Moreover, there are various factors that can affect hemorrhoids, with a high incidence rate among the population and a high risk of individual illness. Therefore, We should not underestimate the disease. If the disease risk prediction model can accurately predict the risk of hemorrhoids and take corresponding measures to prevent the occurrence of hemorrhoids, it can not only reduce the pain of people suffering from hemorrhoids, promote physical health, but also reduce the burden of disease. Therefore, this study used logistic regression analysis to establish a hemorrhoid risk prediction model using the factors related to hemorrhoids discovered in this study. The established model has been verified and evaluated to be effective and has a certain degree of accuracy. It can be used for clinical and community prevention of hemorrhoids, as well

as for screening high-risk populations. People can adjust their behavior habits based on the predicted results to achieve the goal of preventing hemorrhoids, especially those who carry genetic genes and have chronic gastritis and constipation.

5.3 Summary, Strengths, limitations and suggestions of the study

Hemorrhoids are likely a hereditary disease, and their onset is likely related to multiple external and internal factors. According to the results of this study, the elderly and highly educated individuals are high-risk groups for hemorrhoids. It is recommended that individuals from these two groups pay special attention to diet and appropriate physical activity, maintain stomach health, avoid chronic gastritis and constipation, and avoid prolonged sitting.

This study is the first to investigate the influencing factors of hemorrhoids in the Zhuang population, and also the first to conduct genetic research on hemorrhoids in this population. At the same time, a hemorrhoid risk prediction model has been established, which provides clues and reference basis for further research on the prevention of hemorrhoids and the pathogenesis of hemorrhoids in this population.

However, in genetic research, this study only investigated the association between a polymorphic site of one gene and hemorrhoids, and the occurrence of diseases is often related to multiple genes. Therefore, more related genes need to be explored in the genetic research of hemorrhoids. Besides, this study used a hospital based case-control study, and the results may inevitably be biased. Moreover, due to the stratified analysis, the sample size of each layer is relatively small, which has a certain impact on the reliability of the results. To obtain more reliable results on the impact of genetics and its interaction with environmental and behavioral factors on susceptibility to hemorrhoids, it is necessary to expand the sample size for research. In addition, because part of the content of the questionnaire is based on the subjective memories of the respondents, the content may not be true enough, and the results may have memory bias. Finally, the risk prediction model constructed in this study has not yet undergone external validation and needs to be further improved and optimized in multiple centers to enhance its predictive ability.

5.4 Conclusion

5.4.1 This study found that factors related to hemorrhoids include age, education level, dietary taste, constipation, and chronic gastritis. Among them, age, education level, constipation, and chronic gastritis are risk factors, while a bland diet is a protective factor. That is, the older the age , the greater the risk of hemorrhoids，and the higher education level， the greater the risk of hemorrhoids; Patients with constipation and chronic gastritis are more likely to develop hemorrhoids; People with a bland diet have a lower risk of developing hemorrhoids.

5.4.2 This study found that there is no causal relationship between BMI and hemorrhoids in both East and South Asian populations.

5.4.3 This study found that the rs34221221 polymorphism of the FOXC2 gene is associated with susceptibility to hemorrhoids, and individuals carrying the mutated CT genotype are less likely to develop hemorrhoids than those carrying the wild-type CC genotype. CT genotype has a protective effect on hemorrhoids in males, those who do not drink alcohol, those who do not eat chili peppers, or eat pickled foods. The CT genotype also interacts with males, those who do not drink alcohol, and those who do not eat chili peppers, making these three populations less susceptible to hemorrhoids.

5.4.4 The risk prediction model for hemorrhoids is： Logit (P)=-1.4+0.03X1+0.79X2-0.71X3+1.02X4+0.65X5-0.45X6, X1 is age, X2 is education level, X3 is dietary stage, X4 is constipation, X5 is chronic gastritis, X6 is genotype. When the predicted value is greater than 0.6, the predicted result indicates the possibility of developing hemorrhoids, and if it is less than 0.6, it indicates the possibility of not developing hemorrhoids.

REFERENCES

Reference

[1] Bingdong Hua, Li Kui, and Ai Hua Wang. Comparative study and prevention of factors associated with hemorrhoids incidence . Hunan Journal of traditional Chinese medicine, 2015, 31 (6): 14-15. (Journal of China)

[2] Sugerman D T. JAMApatient page:Hemorrhoids.Jama,2014,312(24):2698.

[3] Gerjy R , Lindhoff-Larson A , Nyström PO. Grade of prolapse and symptoms of haemorrhoids are poorly correlated:result of a classification algorithm in 270 patients[J].Colorectal Disease,2010,10(7):694-700.

[4] Gallo G, Martellucci J, Sturiale A, et al. Consensus statement of the Italian society of colorectal surgery (SICCR): management and treatment of hemorrhoidal disease. Tech Coloproctol. 2020 Feb;24(2):145-164.

[5] Kibret AA, Oumer M, Moges AM. Prevalence and associated factors of hemorrhoids among adult patients visiting the surgical outpatient department in the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. PLoS One. 2021 Apr 20;16(4):e0249736.

[6] Fox A,Tietze P H,Ramakrishnan K.Anorectal conditions:hemorrhoids.Fp Essent,2014,419(419):11-19.

[7] Kovalev SA, Kotenko KV. Nemedikamentoznye tekhnologii v rannei reabilitatsii bol'nykh posle gemorroidektomii [Non-drug technologies in early rehabilitation of patients after hemorrhoidectomy]. Vopr Kurortol Fizioter Lech Fiz Kult. 2021;98(6. Vyp. 2):65-71.

[8] Riss S, Weiser FA, Schwameis K, et al. The prevalence of hemorrhoids in adults. Int J Colorectal Dis. 2012 Feb;27(2):215-20.

[9] Hong YS, Jung KU, Rampal S, et al. Risk factors for hemorrhoidal disease among healthy young and middle-aged Korean adults. Sci Rep. 2022 Jan 7;12(1):129.

[10] Smud D, Kekez T, Majerović M, et al.Hemorrhoids--diagnosis and treatment options. Lijec□nic□ki Vjesnik, 2005, 127 (5-6):129.

[11] Main conclusions and recommendations from an epidemiological survey of common anorectal diseases in Chinese adults [C] / / annual meeting of the anorectal chapter of the Chinese Academy of Chinese medicine and National Conference on flow regulation industries, 2015.

[12] Al-Batayneh KM, Al Battah RM (2008) Genetic variation in the proximal 5′ UTR of FOXC2 gene in varicose veins and hemorrhoids patients. Int J Integr Biol 4:78–80.

[13] Ng MY, Andrew T, Spector TD, et al. Lingage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy, unselected sibling pairs.J Med Genet,2005,42(3):235-239.

[14] Pigot F, Siproudhis L, Allaert FA. Risk factors associated with hemorrhoidal symptoms in specialized consultation. Gastroenterol Clin Biol. 2005 Dec; 29(12): 1270-4.

[15] Huang Yihui, He Jianhui, Cai Le, et al. Analysis of influencing factors on excessive drinking behavior. Journal of Kunming Medical University, 2016,37 (09): 53-57

[16] Xiong Wenyan, Fan Yibing, Feng Xiaowu et al. A case-control study on risk factors for gastric precancerous disease among urban residents in Nanchang City from 2018 to 2019 . Chinese Journal of Cancer Prevention and Treatment, 2021,28 (24):

1856-1861

[17]Doukky R, Mangla A, Ibrahim Z, et al. Impact of physical inactivity on mortality in patients with heart failure. Am J Car diol，2016，117（7）：1135-1143.

[18]Rogerson M C,Le Grande M R,Dunstan D W, et Al. Television viewing time and 13-year mortality in adults with cardio vascular disease:data from the Australian diabetes,obesity and lifestyle study (AusDiab). Heart Lung Circ，2016，25（8）：829-836.

[19]Wu Z, Huang Z, Wu Y, et al.Sedentary time, metabolic abnormalities, and all-cause mortality after myocardial infarction: A media tion analysis. Eur J Prev Cardiol，2019，26（1）：96-104.

[20]Craig CL, Marshall AL, Sjöström M,et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003Aug;35(8):1381-95.

[21]Sharma A, Rao S. Constipation: Pathophysiology and Current Therapeutic Approaches. Handb Exp Pharmacol. 2017;239:59-74.

[22] Thomson WHF. The nature of haemorrhoids. BJS, 1975, 62(7): 542-552.

[23] Sugerman D T. JAMA patient page: Hemorrhoids. Jama,2014,312(24):2698.

[24] Qureshi W A. Office management of hemorrhoids. American Journal of Gastroenterology,2018,378(18):1944.

[25] Gerjy R , Lindhoff-Larson A , Nyström PO. Grade of prolapse and symptoms of haemorrhoids are poorly correlated:result of a classification algorithm in 270 patients. Colorectal Disease,2010,10(7):694-700.

[26] Jiang Wei, Zhang Hongxi, Sui Nan, et al. Epidemiological investigation of common anal and rectal diseases among urban residents in China. China Public Health, 2016, 32 (10): 1293-1296.

[27] Qiao Jinghua, He Jiawei, Zhou Junhui. Exploration of Traditional Chinese Medicine Prevention and Treatment Strategies for Hemorrhoids among Rural Community Residents Based on Epidemiological Survey. Shanghai Journal of Traditional Chinese Medicine, 2019, 53 (6): 14-19

[28] Chen Ping, Tian Zhenguo, Zhou Lu, et al. Epidemiological investigation of anal and rectal diseases among urban residents in Hubei Province . Chinese Medical Science, 2015 (5): 188-191.

[29] Peery AF, Crockett SD, Barritt AS, et al.. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States. Gastroenterology. 2015 Dec;149(7):1731-1741.

[30] Johanson JF, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation. An epidemiologic study. Gastroenterology. 1990 Feb;98(2):380-6.

[31]Gazet JC, Redding W, Rickett JW. The prevalence of haemorrhoids. A preliminary survey. Proc R Soc Med. 1970;63 Suppl(Suppl 1):78-80.
[32] Jacobs, D. Clinical practice:Hemorrhoids.N Engl J Med,2014,371(10):944-951.
[33] Lee J H,Kim H E,Kang J H,et al.Factors Associated with Hemorrhoids in Korean Adults: Korean National Health and Nutrition Examination Survey. Korean Journal of Family Medicine,2014,35(5):227-236.
[34] Riss S,Weiser F A,Schwameis K, et al.The prevalence of hemorrhoids in adults. International Journal of Colorectal Disease,2012,27(2):215-220.
[35] Gallo G, Martellucci J, Sturiale A, et al. Consensus statement of the Italian society of colorectal surgery (SICCR): management and treatment of hemorrhoidal disease. Tech Coloproctol. 2020 Feb;24(2):145-164.
[36] Kibret AA, Oumer M, Moges AM. Prevalence and associated factors of hemorrhoids among adult patients visiting the surgical outpatient department in the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. PLoS One. 2021 Apr 20;16(4):e0249736.
[37] Fox A,Tietze P H,Ramakrishnan K. Anorectal conditions:hemorrhoids. Fp Essent,2014,419(419):11-19.
[38] Kovalev SA, Kotenko KV. Nemedikamentoznye tekhnologii v rannei reabilitatsii bol'nykh posle gemorroidektomii [Non-drug technologies in early rehabilitation of patients after hemorrhoidectomy]. Vopr Kurortol Fizioter Lech Fiz Kult. 2021;98(6. Vyp. 2):65-71.
[39] Idrees JJ, Clapp M, Brady JT, et al. Evaluating the Accuracy of Hemorrhoids: Comparison Among Specialties and Symptoms. Dis Colon Rectum. 2019 Jul;62(7):867-871.
[40] Sengupta N, Tapper EB, Feuerstein JD. Early Versus Delayed Colonoscopy in Hospitalized Patients With Lower Gastrointestinal Bleeding: A Meta-Analysis. J Clin Gastroenterol. 2017 Apr;51(4):352-359.
[41] Bretthauer M, Kaminski MF, Løberg M et al. Nordic-European Initiative on Colorectal Cancer (NordICC) Study Group. Population-Based Colonoscopy Screening for Colorectal Cancer: A Randomized Clinical Trial. JAMA Intern Med. 2016 Jul 1;176(7):894-902.
[42] Mehanna D, Platell C. Investigating chronic, bright red, rectal bleeding. ANZ J Surg. 2001 Dec;71(12):720-2.
[43] Gralnek IM, Neeman Z, Strate LL. Acute Lower Gastrointestinal Bleeding. N Engl J Med. 2017 Jun 8;376(23):e50.
[44] Aoki T, Hirata Y, Yamada A, Koike K. Initial management for acute lower gastrointestinal bleeding. World J Gastroenterol. 2019 Jan 7;25(1):69-84.
[45] Ganz RA. The evaluation and treatment of hemorrhoids: a guide for the gastroenterologist. Clin Gastroenterol Hepatol. 2013 Jun;11(6):593-603.
[46] Chen Ping, Tian Zhenguo, Li October, et al. Epidemiological investigation of anal and rectal diseases among urban residents in Hubei Province . Chinese Medical Science, 2015,5 (05): 188-191.
[47] Tan Naizhi. Analysis of clinical efficacy and related factors of surgical treatment for hemorrhoids . World Latest Medical Information Digest, 2017,17 (64): 124
[48] Tian Yu. Etiological investigation of inpatients with hemorrhoids and observation of postoperative dietary and emotional intervention. Shandong University of Traditional Chinese Medicine, 2014.

[49] Amina Humar, Yan Hong. A study on the relationship between dietary habits and the risk of symptoms related to anal and rectal diseases in adults. Chinese Journal of General Medicine, 2018,21 (08): 975-980.
[50] Zhan Xiaonong, Yu Chengdong, Liu Lian, et al. Related factors and prevention and treatment of hemorrhoids . New Traditional Chinese Medicine, 2007, 39 (8): 8182.
[51] Segre D. [Etiopathogenesis and physiopathology of hemorrhoidal disease. Annali italiani di chirurgia.1995; 66(6):747–50.
[52] Thomson W. The nature of haemorrhoids. British Journal of Surgery. 1975; 62(7):542–52.
[53] Lohsiriwat V. Hemorrhoids: from basic pathophysiology to clinical management. World J Gastroenterol.2012; 18(17):2009–17.
[54]Talley NJ, Lasch KL, Baum CL. A gap in our understanding: chronic constipation and its comorbid conditions. Clin Gastroenterol Hepatol 2009; 7:9 – 19.
[55] Johanson JF, Sonnenberg A. Constipation is not a risk factor for hemorrhoids: a case-control study of potential etiological agents. Am J Gastroenterol 1994;89:1981 – 6.
[56] Chiloiro M , Darconza G , Piccioli E et al. Gastric emptying and orocecal transit time in pregnancy . J Gastroenterol 2001 ; 36 : 538 – 43 .
[57] Fisher RS , Roberts GS , Grabowski CJ et al. Altered lower esophageal sphincter function during early pregnancy . Gastroenterology 1978 ; 74 : 1233 – 7 .
[58] Van Th iel DH, Gavaler JS, Joshi SN et al. Heartburn of pregnancy. Gastroenterology 1977 ; 72 : 666 – 8.
[59] Li Sheng. Overview of the pathogenesis and treatment of hemorrhoids . Chinese Medical Guide, 2014,12 (01): 43-44.
[60] Elderon L, Whooley MA. Depression and cardiovascular disease. Prog Cardiovasc Dis. 2013;55:511 – 523.
[61] Fletcher BC, Kupshik GA, Uprichard S, Shah S, Nash AS. Eating disorders and concurrent psychopathology: a reconceptualisation of clinical need through Rasch analysis. Eur Eat Disord Rev. 2008;16:191 – 198.
[62] Ding Yishan, Causes and Classification of Hemorrhoids. People's Military Medical Journal, 1994, 28 (8:56).
[63] Chen Changxiang, Liu Haijuan, Gao Hongxia, et al. Risk factors and health education for hemorrhoids. Journal of Nurse Continuing Education, 2002, (05): 328-329
[64] Luo Youping, Zhou Wenhao, Liu Ning Risk factors and clinical characteristics analysis of hemorrhoids . Grassroots Medical Forum, 2010, 14 (25): 777-779.
[65] Riss S, Weiser FA, Schwameis K, Riss T, Mittlbock M, Steiner G, et al. The prevalence of hemorrhoids in adults. Int J Colorectal Dis. 2012;27:215 – 220.
[66] Acheson AG，Scholefield JH．Management of haemorrhoids．BMJ，2008，16, 336(7640):380—383．
[67] Haas PA, Haas GP, Schmaltz S,et al. The prevalence of hemorrhoids. Dis Colon Rectum. 1983;26:435–439.
[68] Smud D,Kekez T,Majerović M,et al.Hemorrhoids--diagnosis and treatment options. Lijec · nic · ki Vjesnik,2005,127(5-6):129.

[69] Thomson WH. The nature of haemorrhoids. Br J Surg, 1975, 62 (7): 542-552.

[70] Gass OC, Adams J. Hemosoids: etiology and pathology. Am J Surg, 1950, 79 (1): 40-43.5.

[71] Zhang Xiang, Bai Jingshu. Overview of diagnosis and treatment of hemorrhoids pathogenesis. Chinese Journal of anorectal diseases, 2019, 39 (9): 72-74.

[72] Watts GT. Piles are caused by varicose veins in the anal canal. Lancet. 1986 Jan 18;1(8473):144-5.

[73]Fu Chuangang, Ding Jianhua. A new concept of anatomical physiology of the rectal anal canal. Chinese Journal of modern surgery, 2003,7 (3): 235-239.

[74]Mondal S, Adhikari N, Banerjee S, et al. Matrix metalloproteinase- 9 (MMP-9) and its inhibitors in cancer: a minireview. Eur J Med Chem, 2020, 194: 112260.

[75]Qin Lei, Qin Xin The expression and significance of MMP-9 and VEGFR2 factors in tissues of patients with internal hemorrhoids Chinese Journal of Cell Biology, 2020, 42 (10): 1800-1805.

[76]Dai Hao The effect of modified Shiquan Yuzhen decoction on MMP-7 and MMP-9 in prolapsed internal hemorrhoid tissue Hohhot: Inner Mongolia Medical University, 2020.

[77]Serra R, Gallelli L, Grande R, et al. Hemorrhoids and matrix metalloproteinases: a multicenter study on the predictive role of biomarkers. Surgery, 2016, 159(2): 487-494.10

[78]Yang H, Chen H, Liu F, et al. Up-regulation of matrix metalloproteinases-9 in the kidneys of diabetic rats and the association with neutrophil gelatinase-associated lipocalin. BMC Nephrol, 2021, 22(1): 211.11

[79]Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res, 2006, 69(3): 562- 573.12

[80]Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. Int J Mol Sci, 2020, 21(24): 9739.

[81]Li SL, Jing FY, Ma LL, et al. Myofibrotic malformation vessels: unique angiodysplasia toward the progression of hemorrhoidal disease. Drug Des Devel Ther, 2015, 9: 4649-4656.14.

[82]Feng Dayong, Wang Chunhui, Feng Yuening, etc Exploring the pathogenesis of hemorrhoids from the perspective of cytokine biology. Chinese Medical Journal, 2016, 51 (3): 28-30.

[83]Wang H, Wang L, Xie Z, et al. Nitric oxide (NO) and NO synthases (NOS)-based targeted therapy for colon cancer. Cancers (Basel), 2020, 12(7): 1881.

[84]Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev, 1991, 43(2):109-142.

[85]Han Wei, Wang Zhenjun, Zhao Bo, et al. The mechanism and significance of elastic fiber degeneration and angiogenesis in hemorrhoid tissue. Chinese Journal of Gastrointestinal Surgery, 2005, 8 (1): 56-59.

[86]Lohsiriwat V, Wilson VG, Scholefield JH, et al. Regional distribution of nitric oxide synthase in human anorectal tissue: a pilot study on the potential role for nitric oxide in haemorrhoids. Curr Vasc Pharmacol, 2020, 18(1): 43-49.

[87]Gokce AH, Gokce FS, Durmus S, et al. The effect of nitric oxide, endothelial nitric

oxide synthetase, and asymmetric dimethylarginine in hemorrhoidal disease. Rev Assoc Med Bras (1992), 2020, 66(8): 1128-1133.
[88]di Mola FF, Friess H, Köninger J, et al. Haemorrhoids and transient receptor potential vanilloid 1. Gut, 2006, 55(11): 1665-1666.
[89]Varela-López E, Del Valle-Mondragón L, Castrejón-Téllez V, et al. Role of the transient receptor potential vanilloid type 1 (TRPV1) in the regulation of nitric oxide release in Wistar rat aorta. Oxid Med Cell Longev, 2021, 2021: 8531975.
[90]Correia de Sousa M, Gjorgjieva M, Dolicka D, et al. Deciphering miRNAs' action through miRNA editing. Int J Mol Sci, 2019, 20(24): 6249. doi: 10.3390/ijms20246249.
[91]Song C, Zhou H, Lu H, et al. Aberrant expression for microRNA is potential crucial factors of haemorrhoid. Hereditas, 2020, 157(1): 25.
[92]Wang C, Lu H, Luo C, et al. miR-412-5p targets Xpo1 to regulate angiogenesis in hemorrhoid tissue. Gene, 2019, 705: 167-176.
[93]Liu T, Zhou H, Lu H, et al. MiR-4729 regulates TIE1 mRNA m6A modification and angiogenesis in hemorrhoids by targeting METTL14. Ann Transl Med, 2021, 9(3): 232.
[94]Karaman S, Leppänen VM, Alitalo K. Vascular endothelial growth factor signaling in development and disease. Development, 2018, 145(14): dev151019.
[95]Okada-Ban M, Thiery JP, Jouanneau J. Fibroblast growth factor-2. Int J Biochem Cell Biol, 2000, 32(3): 263-267.
[96]Zhu Huafeng, Wang Chunlan, Zhao Yu. Research progress on the synergistic effect of VEGF and FGF-2 in angiogenesis. Chinese Journal of Plastic Surgery, 2006, 22 (1): 72-75.
[97]Liang Wenlong, Cao Jie, Yang Ping, et al. The distribution characteristics and clinical significance of vascular endothelial growth factor receptor 2 in hemorrhoid mucosa. Journal of Practical Medicine, 2015, 31 (17): 2830-2832.
[98]Wang Qi, Jing Fangyan, Deng Yongjian. Correlation analysis between the expression of VEGF/FGF2 in the mucosa and vascular epithelial cells of internal hemorrhoids and the staging of internal hemorrhoids. Chinese Journal of Clinical Anatomy, 2019, 37 (4): 409-413.
[99]Porwal A, Kundu GC, Bhagwat G, et al. Polygonal formula Anoac-H suppresses the expression of RANTES and VEGF for the management of bleedings and fungi. Mol Med Rep, 2021, 24 (4): 736.
[100] Salnikova LE, Khadzhieva MB, Kolobkov DS. Biological findings from the PheWAS catalog: focus on connective tissue-related disorders (pelvic floor dysfunction, abdominal hernia, varicose veins and hemorrhoids). Hum Genet. 2016 Jul;135(7):779-95.
[101]Chiang CP, Jao SW, Lee SP, et al.Expression pattern, ethanol-metabolizing activities, and cellular localization of alcohol and aldehyde dehydrogenases in human large bowel: association of thefunctional polymorphisms of ADH and ALDH genes with hemorrhoids and colorectal cancer. Alcohol. 2012 Feb;46(1):37-49.
[102] Wang C, Lu H, Luo C, et al. miR-412-5ptargets Xpo1 to regulate angiogenesis in hemorrhoid tissue. Gene. 2019 Jul 15;705:167-176.
[103] Song C, Zhou H, Lu H, et al. Aberrant expression for microRNA is potential crucial factors of haemorrhoid. Hereditas. 2020 Jul 3;157(1):25.

[104] Al-Batayneh K M, Al Battah R M. Genetic variation in the proximal 5’ UTR of FOXC2 gene in varicose veins and hemorrhoids patients. Int J Integ Biol, 2008, 4(2): 78-80.
[105] Lamont RE,Childs S.MAPping out arteries ang veins.Sci STKE,2006, 2006 (355):39-45.
[106] Timur AA,Driscoll DJ,Wang Q. Biomedicine and diseases:the Klippel-Trenaunay syndrome,vascular anomalies and vascular morphogenesis. Cell Mol Life Sci,2005, 62(13):1434-1447.
[107] Ng MY,Andrew T,Spector TD, et al. Lingage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy,unselected sibling pairs. J Med Genet,2005,42(3):235-239.
[108] Mellor RH, Brice G, Stanton AW, et al. Mutations in FOXC2 are strongly associated with primary valve failure in veins of the lower limb. Circulation, 2007, 115(14):1912–1920.
[109] Lim CS, Davies AH. Pathogenesis of primary varicose veins. BrJ Surg, 2009, 96(11):1231–1242.
[110] Ng MY, Andrew T, Spector TD, et al. Linkage to the FOXC2 region of chromosome 16 for varicose veins in otherwise healthy, unselected sibling pairs. J Med Genet, 2005, 42(3):235–239.
[111] Valet P，TavernierG，Castan-Laurell I，et al. Understanding adipose setissue development from mtransgenic animal models. J. Lpid Res，2002，43:835-860.
[112] PajukanraP，AllayeeH，KrassKL，etal. Combined analysis of gnome scans of Dute hand Finnish families reveals a susceptibility locus for high-density lipoprotein cholesterol on chromosome16q. Am J Hum Genet,2003,72:903-917.
[113] Carlsson E, Groop L, Ridderstråle M. Role of the FOXC2 -512C>T polymorphismin type 2 diabetes: possible association with the dysmetabolic syndrome. Int J Obes (Lond). 2005 Mar; 29(3):268-74.
[114] Herold A. Differenzialdiagnose des Hämorrhoidalleidens [Differential diagnosis of hemorrhoidal disease]. Hautarzt. 2020 Apr;71(4):269-274. German.
[115] Hollingshead JR, Phillips RK. Haemorrhoids: modern diagnosis and treatment. Postgrad Med J. 2016 Jan;92(1083):4-8.
[116] Mott T, Latimer K, Edwards C. Hemorrhoids: Diagnosis and Treatment Options. Am Fam Physician. 2018 Feb 1;97(3):172-179.
[117] Chinese Guidelines for the Diagnosis and Treatment of Hemorrhoids (2020). Colorectal and Anorectal Surgery, 2020,26 (05): 519-533
[118] Marino Martin. Anorectal Surgery Hemorrids . Dis Colon Rectum，1980，23：211-222.
[119] Labidi Asma，Maamouri Feriel，Letaief-Kson-Tini Feriel，et al. Dietary Habits Associated With Internal Hemorrhoidal Disease: A Case-Control Study. Tunis Med, 2019, 97(4): 572-578.
[120] Alonso-Coello P, Mills E, Heels-Ansdell D, et al. Fiber for the treatment of hemorrhoids complications: systematic review and meta-analysis. Am J Gastroenterol, 2006, 101(1): 181-188.
[121] Zagriadskiĭevgeny A,Bogomazov Alexey M, Golovko Evgeny B.Conservative Treatment ofHemorrhoids: Results of an Observational Multicenter Study. Springer Nature, 2018, 35(11): 1979-1992.
[122] An Zhiying, Dong Changxia, Tang Yugen. Clinical observation of Bai Zhi San

Huang Tang in the treatment of internal hemorrhoids. Chinese Medical Innovation, 2012, 9 (14): 39

[123] Na Yunlang, Fu Yuxiang, Su Zhenyu,et al. Comparison of therapeutic effects between Huaihua Powder and Huaijiao Pill in the treatment of stage I internal hemorrhoid bleeding. Chinese and Foreign Medical Journal, 2015, 34 (15): 152-153

[124] Quijano Ce, Abalos E. Conservative management of sympto-matic and/or complicated haemorrhoids in pregnancy and the puerperium. Cochrane Database Syst Rev, 2005, 20(3): CD004077.

[125] Treatment of Hemorrhoids with Ligation by the Professional Committee of Colorectal and Anorectal Diseases in China, Integrated Traditional Chinese and Western Medicine Expert Group. Chinese Expert Consensus on Hemorrhoid Ligation Treatment (2015 Edition). Chinese Journal of Gastrointestinal Surgery, 2015, 18 (12): 1183-1185

[126] Fahmi Omer Aram. Rubber Band Ligation for Hem Orrhods: an Office Experience . Indian J Surg, 2016, 78 (4): 271-274.

[127] Yang Liang. Clinical effect of endoscopic negative pressure ligation in the treatment of internal hemorrhoids . Jian Kang Bidu, 2020, 1 (3): 266.

[128] Abiodun Adekunle Adedapo, Alatise Oluse Gun Isaac, Okereke Chukwuma Eze, et al. Comparative study of endoscopic band ligations versus in projection microscopy with 50% dextrose in water, in symptomatic internal haemorhaoids. The Nigerian postgraduates medical journal, 2020, 1 (27): 13-20.

[129] Xiao Zhaoxue. Clinical efficacy analysis of automatic hemorrhoid ligation (RPH) in the treatment of grade II to IV internal hemorrhoids . Inner Mongolia Traditional Chinese Medicine, 2018, 8 (37): 49.

[130] Nisarpasha J, Scholefieldjohn H. Managing Haemorrhoids. Br MedJ, 2003327 (7419): 847-851.

[131] Peng Huabin. Observation of the effect of automatic hemorrhoid ligation and stapler hemorrhoid mucosal circumcision in the treatment of mainly internal hemorrhoids and annular mixed hemorrhoids . Henan Journal of Surgery, 2018, 3 (24): 12-14.

[132] Tomikl Yuichi, Seigo Ono, Aokijun, et al. Treatment of Internal Hemorrhoids by Endoscopic Sclerotherapy with Aluminum Potassium Sulfate and Tannic Acid. Diagnostic and Therapeutic Endoscopy, 2015, 7 (1): 1-7.

[133] Huang Changxi, Zhu Zhihua, Chen Yaocheng. Study on the application of colonoscopic injection of poly (cinnamyl alcohol) in the treatment of internal hemorrhoid sclerosis. Chinese Health Standard Management, 2016, 7 (13): 89-90

[134] Liang Qishou, Liu Chengwei. Clinical observation on the treatment of hemorrhagic internal hemorrhoids by injection of polyoxymethylene alcohol under anal endoscopy. Chinese Journal of Practical Medicine, 2015, 10 (11): 26-27.

[135] Huang Junjie. Clinical comparative study on the treatment of hemorrhagic internal hemorrhoids by injection of poly (cinnamyl alcohol) into the hemorrhoid area [D]. Nanning: Guangxi Medical University, 2017

[136] Kodak. Study on Colonoscopic Polyacrylamide Injection for the Treatment of Grade II and III Internal Hemorrhoids. Journal of Chinese and Foreign Medical

Research, 2020, 18 (10): 5
[137]Liu Xians, Hou Yanping, Zhang Yanhua. Clinical observation on the treatment of stage II and III internal hemorrhoids by injection of colonoscopic poly (cinnamyl alcohol) sclerosant. Modern Digestive and Interventional Diagnosis and Treatment, 2015, 20 (3): 250-252
[138]Fu Xinyao, Huang Guilin, Ma Hui, et al. Clinical efficacy observation of local injection of poly (cinnamyl alcohol) in the treatment of stage III internal hemorrhoids. Journal of Clinical Medical Literature, 2018, 5 (92): 94-95
[139] Pu Wanyun, Fu Haiping, Gai Changyue, et al. Clinical observation of automatic hemorrhoid ligation stapler combined with 50% glucose injection in the treatment of internal hemorrhoids. Heilongjiang Medical Journal, 2019, 32 (6): 1482-1484
[140] Zhu Yong. Clinical efficacy of automatic hemorrhoid ligation (RPH) combined with sclerotherapy injection in the treatment of internal hemorrhoids. Medical Information, 2016, 29 (36): 130-131
[141] Zeng Lusun, Huang Zhihuan, Xu Xinping. Clinical observation on the treatment of elderly internal hemorrhoids with the combination of internal hemorrhoid ring ligation and polyoxymethylene injection. Practical Chinese and Western Medicine Summary, 2017, 17 (3): 120-126
[142] Zhu Zhanqiu. Clinical effect analysis of sclerosing agent injection combined with automatic hemorrhoid ligation in the treatment of internal hemorrhoids. Surgical Research and New Technology, 2017, 6 (2): 102-104
[143] Li Kangping, Zhang Lufang, Yu Dengming, et al. Clinical efficacy of negative pressure rubber ring ligation combined with injection of internal hemorrhoid hardening agent in the treatment of mild to moderate internal hemorrhoids. Contemporary Medicine, 2014, 20 (14): 52-53
[144] Linares Santiago E, Gomz Parra M, Men Doza Olivares Fj, et al. Effectiveness of hemoral treatment by rubber band ligation and infrared photocoating. Rev Esp Enferm Dig, 2001, 93 (4): 238-247
[145] Marques Cf, Nahas Sc, Nahas Cs, et al. Early results of the treatment of internal heterogeneous disease by infrared coagulation and elastic banding: a prospective randomized cross over trial. Tech Colombol, 2006, 10 (4): 312-317
[146] Lu Can Province, Shi Jian, Zhang Xinlong, et al. Clinical efficacy and mechanism research on the treatment of internal hemorrhoid bleeding by pricking the Jiaojiao acupoint. Journal of Anhui University of Traditional Chinese Medicine, 2017, 36 (5): 41-44
[147] Wei-Liang Loh, Shaun Tan, Ming Soen Ngooi, et al. Endoscopic monopolar Coagulation of internal haemonrhoids: a large one's experience of the first hunted cases.Colorectal Dis, 2017, 19 (1): 86-89
[148] Iyer Vs, Shrier I, Gordon Ph. Long term out come of rubber band alignment for symptomatic primary and current internal hospitals. Dis Colon Rec-um, 2004, 47 (8): 1364-1370
[149]Paulo Bourini, Lucas Rodrigues Bourini, Paulo De Azeredo Passos Candelaria, et al. Laser hemorheological deartialization. Journal of Colo Proctology, 2017, 37 (1): 38-43
[150] Bhatti Mi, Sajid Ms, Baid Mk. Milligan-Morgan (open)versus Ferguson haemorrhoidectomy(closed): a systematic review and meta-analysis of published ran-domized, controlled trials. World J Surg, 2016, 40(6): 1509-1519

[151] Argov S, Levandovsky O, Yarhi D. Milligan-Morgan hemorrhoidectomy under local anesthesia an old operation that stood the test of time-Asingle-team experience with 2280 operations. International journal of Colorectal Disease，2012, 27(7): 981-985.
[152]Adrian Medina-Gallardo,Yuhamy Curbe Lo-Peña, Xavier De Castro, et al. Is the severe Pay after Milligan Morgan hematology still curcurrently remaining a major posterior problem aspire being one of the old surgical techniques described? A case series of 117 consecutive patients . International Journal of surgery case reports, 2017, 30:73-75.
[153]Bilgin Yusuf, hot semih, Barras İ Lhami Soykan, et al. Short-and long-term results of harmonic scalpel hematology versus stapler hematoidopexy in treatment of hematological disease. Asian Journal of surgery, 2015, 38 (1): 214-219
[154]Nienhuijs S, DE hingh I. Conventional verse Liga-Sure hemorrhoidotomy for patients with symptomatic hemorrhoids . Cochrane Database syst rev, 2009 (1): cd006761
[155]Leeako-chao, chenahong-hwa, Chun Gkuan-chih, et al. International Journal of surgery, 2013, 11 (9): 914-918
[156]Tsunoda a, sada h, Sugimoto T, et al. Random Sized controlled trial of bipolar diathermy vs ultrastronic scalpel for closed hemosidotomy. World J gastrointest surg, 2011, 3 (10): 147-152.
[157]Sameh Hany Email, Hossam Elfeki1, Ahmad Sakrl, et al. Transanalytic hemosiderial dearialization (THD) versus stabilized hemosiderosis (SH) in treatmentment of internal hemosideroids: a systematic review and meta analysis of randomized clinical trials. International Journal of colorectal disease, 2019, 34 (1): 1-11
[158]Sheng Liuqing, Zhang Heng. Retrospective study of PPH in the treatment of stage Ⅱ~Ⅳ internal hemorrhoids. Chinese Journal of anorectal diseases, 2017, 37 (9): 72-73
[159]Liang MingChao , Liang Shengzhi, Wu Yuerui, et al. Open external stripping and internal ligation hemorrhoidectomy Comparison of clinical efficacy of surgery and PPH in the treatment of grade Ⅲ and Ⅳ internal hemorrhoids . Modern diagnosis and treatment, 2017, 28 (23): 4324-4326.
[160]Kam MH, ng KH, Lim JF, et al. Results of 7302 sta Pled Haier idea operation in a single centre: a seven year reviewed follow up questionnaire survey. ANZ J Surg, 2011, 81 (4): 253-256
[161] Sakr MF, Moussa MM. Ligasure hemorhoidectomy Versus stapled hemosidopexy: a prospective, ran localized clinical trial. Dis colon rectum, 2010, 53 (8): 1161-1167
[162] Li Guobin, Yuan Weitang, Sun Xiantao, et al. Starr procedure for the treatment of severe mixed Retrospective analysis of the effect of hemorrhoids combined with rectal mucosal relaxation. Chinese Journal of practical medicine, 2014, (11): 45-46.
[163] Stuto a, Renzi a, carriero a, et al. Stapledtrans anal rectal result (STARR) in thesurgical treatment of the observed defectionsymdrome:results of Starr Italian Registry. Surginnov, 2011, 18 (3): 248-253.
[164] Lin HC, Ren Di, he QL, et al. Parial stapled hemor Rhoidopexy versus cicularly stapled hepatopexy for grade Ⅲ-Ⅳprolapsing hemosides:a two year Pro-

prospectecontrolled study. Tech coloproctol, 2012, 16 (5): 337-343
[165] Liu h, Yang C, Chen B, et al.clinical outcomes of Doppler guided hemorrhagic artery ligation: a meta analysis. Int J Clin exp Med, 2015, 8 (4): 4932-4939
[166] Brown Sr, Tienan JP, Watson AJM, et al. Hubble study team Haemorrhoidal artery ligation versus rubber band ligation for the management of symptomatic second-degree and third-degree haemorrhoids (hubble): a multicentre, open label, randomized controlled trial. Lancet, 2016388 (10042): 356-364.
[167] Liu You, Mao Xiaowei, Lai Jing. Hemorrhoid artery ligation guided by Doppler ultrasound Clinical observation on the treatment of mixed hemorrhoids with internal hemorrhoids encircling ligation. Chinese Journal of medicine and clinical, 2019, 19 (12): 2051-2053.
[168] Vidal a, Louisa g, J M Bartoli, et al. Emboliza Ion of the hematopoietic arteries (the emborhoid technology unique): a new concept and challenge for interventional radiology. Diagnostic and interactive imaging, 2014, 95 (3): 307-315.
[169] Zakharchenko A , Kaitoukov Y , Vinnik Y, et al. Safety and efficiency of super recent apartment embossment with particles and metallic coils for the treatment of hemorroids (embossoid Technology). Diagnostic and interactive imaging, 2016, 97 (11): 1079-1084
[170]Wang Tianyun. Discussion on the prevention and nursing of hemorrhoids.Inner Mongolia Traditional Chinese Medicine, 2014, 33 (04): 163-164
[171]Meng Wei. The formation, treatment, and prevention of hemorrhoids. Modern Drug Applications in China, 2011, 5 (07): 126-127
[172]Chen Gang. Community prevention and treatment of hemorrhoids. Modern Drug Applications in China, 2010, 4 (21): 225-226
[173]Li Shuang, Zheng Zhonghua, Jing Ying. Community doctors should attach importance to the prevention, education, and scientific and reasonable treatment of hemorrhoids. Chinese Community Physician (Medical Major), 2012, 14 (18): 365
[174]Hu Chunjie .Prevention and health care of hemorrhoids. Modern Distance Education of Traditional Chinese Medicine in China, 2010, 8 (03): 131-132
[175]Lin Xingming. The formation, treatment, and prevention of hemorrhoids. Electronic Journal of Clinical Medical Literature, 2017, 4 (14): 2634+2636
[176]Visscher PM，Wray NR ，Zhang Q，et al. 10 Years of GWAS Discovery:Biology，Function，and Translation. Am J Hum Genet， 2017，101(1):5-22.
[177]Greenland S. An introduction to instrumental variables for epidemiologists. Int J Epidemiol，2018，47(1):358.
[178]Lawlor DA，Harbord RM，Sterne JA，et al. Mendelian randomization:using genes as instruments for making causal inferences in epidemiology. Statist Med，2008，27:1133-1163.
[179]Katan MB. Apolipoprotein E isoforms，serum cholesterol，and cancer. Lancet，1986，1(8479):507-508.
[180]Guo Fang, Tian Linwei. Correlation and causal association. Chinese Medical Journal, 2019, (10): 790-795
[181]Smith GD，Ebrahim S. Data dredging，bias，or confounding. BMJ， 2002，325(7378):1437-1438.

[182]Harrison RK. PhaseIIand phaseIIIfailures:2013-2015. Nat Rev Drug Discov，2016，15(12):817-818.

[183]Smith GD，Ebrahim S.′ Mendelian randomization′:can genetic epidemiology contribute to understanding environmental determinants of disease?. Int J Epidemiol，2003，32(1):1-22.

[184]Cohen JC，Boerwinkle E，Mosley TH Jr，et al. Sequence variations in PCSK9，low LDL，and protection against coronary heart disease. N Engl J Med，2006，354(12):1264-1272.

[185]Benn M，Nordestgaard BG. From genome-wide association studies to Mendelian randomization:novel opportunities for understanding cardiovascular disease causality，pathogenesis，prevention，and treatment. Cardiovasc Res，2018，114(9):1192-1208.

[186] Sekula P，Del Greco M F，Pattaro C，et al. Mendelian Randomization as an Approach to Assess Causality Using Observational Data. J Am Soc Nephrol，2016，27(11):3253-3265.

[187] Relton CL，Davey Smith G. Two-step epigenetic Mendelian randomization:a strategy for establishing the causal role of epigenetic processes in pathways to disease. Int J Epidemiol，2012，41(1):161- 176.

[188]De Marco S, Tiso D. Lifestyle and Risk Factors in Hemispheric Disease Fron Surg. 2021 Aug 18; 8: 729166.

[189]Liu Jinxiong. Clinical effects of bloodletting therapy based on lower limb venous ultrasound observation on primary superficial varicose veins in the lower limbs. Fujian University of Traditional Chinese Medicine, 2023

[190]Gong Lingxiao, Liu Feiyue, Fang Fang, Wang Jing. Research progress on the role of dietary fiber in gastrointestinal dysfunction syndrome. Chinese Journal of Food Science, 2023,23 (06): 385-401

[191]Hendricks SJ, Brunjes PC, Hill DL. Taste bud cell dynamics during normal and sodium-restricted development. J Comp Neurol. 2004 Apr 26;472(2):173-82.

[192]Sugita M. Taste perception and coding in the periphery. Cell Mol Life Sci. 2006 Sep;63(17):2000-15.

[193]Stewart JE, Seimon RV, Otto B, et al. Marked differences in gustatory and gastrointestinal sensitivity to oleic acid between lean and obese men. Am J Clin Nutr. 2011 Apr;93(4):703-11.

[194]Bolhuis DP, Costanzo A, Newman LP, Keast RS. Salt Promotes Passive Overconsumption of Dietary Fat in Humans. J Nutr. 2016 Apr;146(4):838-45.

[195]Keast RS, Azzopardi KM, Newman LP, Haryono RY. Impaired oral fatty acid

chemoreception is associated with acute excess energy consumption. Appetite. 2014 Sep;80:1-6.

[196] Heinze JM, Costanzo A, Baselier I, et al. Detection thresholds for four different fatty stimuli are associated with increased dietary intake of processed high-caloric food. Appetite. 2018 Apr1;123:7-13.

[197]Khan AS, Keast R, Khan NA. Preference for binary fat: From detection to disease. Prog Lipid Res. 2020 Apr; 78:101032

[198]Berthoud HR, Zheng H. Modulation of tax response and food preference by objectivity and weight loss. Physical Behav 2012 Nov 5; 107 (4): 527-32

[199]Nasser J. Taste, food intake and obesity. Obes Rev. 2001 Nov; 2 (4): 213-8

[200]Mattes RD. Salt Taste and Hypertension: a critical review of the culture. J Chronic Dis 1984; 37 (3): 195-208

[201]Li H, Jia P, Fei T. Associations between treat preferences and chronic diseases: a population based exploratory study in China. Public Health Nutr June 2021; 24 (8): 2021-2032

[202]Precone V, Beccari T, Stuppia L, et al. Genetic Project Taste, olfactory and texture related genes and food choices: implications on health status. Eur Rev Med Pharmacol Sci 2019 Feb; 23 (3): 1305-1321

[203]Shen Ying, Liu Hua. Clinical Summary of Elderly Renal Diseases. Yunnan Science and Technology Press, April 2012

[204]Jacobs BN, Andraska EA, Obi AT, Wakefield TW. Pathophysiology of varicose veins. J Vasc Surg Venous Lymphat Disord. 2017 May;5(3):460-467.

[205]Talley NJ, Lasch KL, Baum CL. A gap in our understanding: chronic conditioning and its combined conditions. Clin Gastroenterol Hepatol 2009 Jan; 7 (1): 9-19

[206]Singh G. Kahler K. Bharathi V. et al. Constipation in adults: compilations and comorbidities. Gastroenterology 2005; 128: A154

[207]Singh G. Vadhavkar S. Wang H. et al. Complications and comorbidities of constipation in adults Gastroenterology 2007; 132: A458

[208]Mitra D. Davis K.L. Baran R.W. Healthcare costs and clinical sequelae associated with conditioning in a managed care population. Am J Gastroentol 2007; 102:2

[209]Delco F. Sonnenberg A. Associations between heroes and other diagnoses. Dis Colon Rectum 1998; 41: 1534-1542

[210]Pigot F. Siproudhis L. Allaert F.A. Risk factors associated with epidemiological symptoms in specialized consultation. Gastroenterol Clin Biol. 2005; 29: 1270-1274

[211]Brook R.A. Talley N.J. Kleinman N.L. et al. Functional gastrointestinal disorder comorbidities: comparisons of validity and costs in the 6 months before and after diagnoses of constitution (C) and irreversible bowel syndrome and constitution (IBS+C). Am J Gastroentol 2007; 102:2

[212]Dehn T.C. Kettlewell M.G. Haemorhaids and defaecatory hats Lancet 1989; 1: 54-55

[213]Johanson J.F. Sonnenberg A. The prevalence of hemorrhoids and chronic constipation. An epidemiologic study. Gastroenterology. 1990 Feb;98(2):380-6.

[214]Yin Y. N., Wang C.L., Liu X.W.et al. Gastric and duodenum microflora analysis after long term Helicobacter pylori infection in Mongolian Gerbils. Helicobacter 2011; 16: 389-397

[215]Heimesaat M.M., Fischer A., Lickert R.et al. Helicobacter pylori induced gastric immunopathology is associated with distinct microbiota changes in the large intentions of long term affected Mongolian gerbils. PLoS ONE 2014; 9: 15

[216]Bik E.M., Eckburg P.B., Gill S.R., et al. Molecular analysis of the bacterial microbiota in the human population. Proc Natl Acad Sci USA 2006; 103:732-737.

[217]Maldonado Contraras A., Goldfarb K.C., Godoy Vitorino F.,et al. Dominguez Bello M.G. Structure of the human gastric bacterial community in relation to Helicobacter pylori status ISME J. 2011; 5: 574-579.

[218]BinP, TangZY, LiuSJ, et al. Internal microbiota mediates Enterotoxigenic Escherichiacoli induced diarrarheinpiglets . BMCVetRes, 2018, 14 (1): 385

[219]Johanson JF, Sonnenberg A. Constipation is not a risk factor for heroes: a case control study of potential ecological agents. Am J Gastroentol 1994 Nov; 89 (11): 1981-6

[220]Lee JH, Kim HE, Kang JH, et al. Factors associated with heroes in Korean adults: Korean national health and nutrition examination survey. Korean J Fam Med. 2014 Sep; 35 (5): 227-36.

[221]Lee KY, Lee JI, Park YY,et al. Hemohods Are Associated with Urinary Incontinence. J Women Health (Larchmt) 2020 Nov; 29 (11): 1464-1468

[222]Peer AF, Sandler RS, Galanko JA, et al. Baron JA Risk Factors for Hemorrhoids on Screening Colonoscopy. PLoS One 2015 Sep 25; 10 (9): e0139100.

[223]Hemani G, Zheng J, Elsworth B, et al. The MR Base platform supports systemic cause interference across the human phenome. Elife May 30, 2018; 7: E34408

[224]Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomization studies: a guide, vocabulary, and checklist for clinicians. BMJ 2018 Jul 12; 362: k601

[225]Cheng Q, Yang Y, Shi X, et al. MR LDP: a two sample Mendelian randomization for GWAS summary statistics accounting for linkage disequilibrium and horizontal pleiotropy NAR. Genom Bioinformation 2020 May 4; 2 (2): lqaa028

[226]Surendran S, Girijamma A, Nair R,et al. Forkhead box C2 promoter variant -512C>T is associated with increasing susceptibility to chronic diseases. PLoS One 2014 Mar 7; 9 (3): e90682

[227]Surendran S, S Ramegowda K, Suresh A, et al. Arterialization and anomalous vein wall remodelling in variose veins is associated with regulated FoxC2-Dll4 pathway. Lab Investment 2016 Apr; 96 (4): 399-408

[228]Shadrina AS, Smetanina MA, Sokolova EA, et al. Association of polymorphisms near the FOXC2 gene with the risk of variant vectors in European. Russians Phlebology 2016 Oct; 31 (9): 640-8

[229]Singh M, Mensah GA, Bakris G. Pathogenesis and clinical physiology of tension. Cardiol Clin 2010 Nov; 28 (4): 545-59

[230]Grarup N, Andersen G. Gene environment interactions in the pathogenes of type 2 diabetes and metabolism. Curr Opin Clin Nutr Metab Care 2007Jul; 10 (4): 420-6

[231]Simonds NI, Ghazarian AA, Pimentel CB, et al. Gillanders EM, Mechanical LE Review of the Gene Environment Interaction Literature in Cancer: What Do We Know? Genet Epidemiol 2016 Jul; 40 (5): 356-65

Appendix 1 Ethics Review Certificate

MAHASARAKHAM UNIVERSITY ETHICS COMMITTEE FOR RESEARCH INVOLVING HUMAN SUBJECTS

Certificate of Approval

Approval number: 208-075/2023

Title : Association between FOXC2 gene polymorphism and susceptibility to hemorrhoids

Principal Investigator : Huabei Wu
Responsible Department : Faculty of Public Health
Research site : Provincial General Hospital in Guangxi, China

Review Method : Expedited Review

Date of Manufacture : 30 May 2023 **expire :** 29 May 2024

This research application has been reviewed and approved by the Ethics Committee for Research Involving Human Subjects, Mahasarakham University, Thailand. Approval is dependent on local ethical approval having been received. Any subsequent changes to the consent form must be re-submitted to the Committee.

.....

(Associate Vorapoj Promasatayaprot)
Vice Chairman

Approval is granted subject to the following conditions: (see back of this Certificate)

All approved investigators must comply with the following conditions:

1. Strictly conduct the research as required by the protocol;
2. Use only the information sheet, consent form (and recruitment materials, if any), interview outlines and/or questionnaires bearing the Institutional Review Board's seal of approval ; and return one copy of such documents of the first subject recruited to the Institutional Review Board (IRB) for the record (if applicable);
3. Report to the Institutional Review Board any serious adverse event or any changes in the research activity within five working days;
4. Provide reports to the Institutional Review Board concerning the progress of the research upon the specified period of time or when requested;
5. If the study cannot be finished within the expire date of the approval certificate, the investigator is obliged to reapply for approval at least two month before the date of expiration.
6. All the above approved documents are expired on the same date of the previously approved protocol (Protocol Number.....)

* A list of the Institutional Review Board members (names and positions) present at the meeting of Institutional Review Board on the date of approval of this study has been attached (per requested). All approved documents will be forwarded to the principal investigator.

Appendix 2 Questionnaire

075/64

NO._____

Consent Form

Association Between FOXC2 Gene Polymorphism and Susceptibility to Hemorrhoids

I agree to participate the research project titled association between FOXC2 gene polymorphism and susceptibility to hemorrhoids conducted by Huabei Wu, who has discussed the research project with me.

I have received, read and kept a copy of the information letter/plain language statement. I have had the opportunity to ask questions about this research and I have received satisfactory answers. I understand the general purposes, risks and methods of this research.

I consent to participate in the research project and the following has been explained to me:

the research may not be of direct benefit to me
my participation is completely voluntary
my right to with draw from the study at any time without any implications to me
the risks including any possible inconvenience, discomfort or harm as a consequence of my participation in the research project
the steps that have been taken to minimize any possible risks
public liability insurance arrangements
what I am expected and required to do
whom I should contact for any complaints with the research or the conduct of the research
security and confidentiality of my personal information.

In addition, I consent to:
publication of results from this study on the condition that my identify will not be revealed.

Name: _____ (please print)

Signature:

_____

Date: _____

Filling requirements

1. Please use a signing pen or ball point pen to fill in the form. The handwriting should be correct and clear, and should not be illegible or altered at will.
2. Words or figures shall be filled in the designated horizontal line.
3. Except for special instructions, the options that do not need to be investigated logically do not need to fill in any symbols.
4. Correction method after filling in errors: first cross out the wrong words or figures with double horizontal lines, and fill in the correct words or figures above the lines. Do not alter the original figures or words.

1. Basic information

Age (birth date on ID card) _____

Height (cm)_____, Weight (kg)_____

Current address (write down the street or village name)_____

1.1 Gender: 1) male 2) female

1.2 Nationality

1.21 Your nationality is (indicated on the ID card): 1) Han 2) Zhuang 3) Others_____

1.22 Your father's nationality is: 1) Han 2) Zhuang 3) Others_____

1.23 Your mother's nationality is: 1) Han 2) Zhuang 3) Others_____

1.3 Current occupation

1) Government Official 2) Manager 3) Chairman 4) Teacher 5) Doctor 6) Engineer 7) Lawyer 8) Artist 9) Accountant 10) Business Staff 11) Civil Servant 12) Secretary 13) Staff 14) Attendants 15) Security personnel 16) Agriculture, forestry, animal husbandry, fishing and other labor personnel 17) Production and transportation equipment operators and related personnel 18) Military police personnel 19) Retired personnel 21) Other employees inconvenient to classify

1.4 Marital status:

1) Unmarried 2) Married 3) Widowed 4) Separation 5) Divorce

1.5 Your education level:

1) Below primary school 2) Primary school 3) Middle school

4) High school or technical secondary school 5) College and university

6) Postgraduate and above

1.6 The average monthly income of your family is:

1) Less than 2000 yuan 2) 2000-3999 yuan 3) 4000-5999 yuan

4) more than 6000 yuan

1.7 Menstruation and fertility (female only)

1.71 Your menarche is _____years old;

Have you stopped menstruation? 1) No; 2) Yes. If yes, menopause age is_____years old.

1.72 Do you have regular menstruation?

1) <21 days/cycle 2) 21-35 days/cycle 3)>35 days/cycle 4) None

1.73 The age of first birth is _____years, the number of existing children is _____, the number of abortions is _____

2.Hemorrhoidal condition

2.1 Type of hemorrhoids

1) No hemorrhoids 2) Internal hemorrhoids 3) External hemorrhoids

4) Mixed hemorrhoids

2.2 How long has you got hemorrhoids?（Please write down the time of hemorrhoids）

_____

2.3 What treatment have you had after hemorrhoids?

1) surgical treatment 2) Oral medication 3) External drug treatment

3. Drinking (Note: Drinking more than 50ml at a time is considered as drinking)

3.1 Do you drink now?

1) Drink 2) Don't drink

3) Drink before, but don't drink now (turn to 3.4)

3.2 How often do you drink now (or before)?

1) Daily or almost daily 2) 3-4 times a week

3) 1-2 times a week 4) 1-3 times a month 5) less than once a month

3.3 Your first drinking age_____ year

3.4 If you have given up drinking, when will you start to give up drinking?

1) 1 month ago 2) 1-6 months ago 3) 1 year ago

4) Two years ago 5) Quit drinking_____ Year 6) Unclear

3.5 What kind of wine do you choose to drink on most occasions?

1) Bai Wine 2) Red wine 3) Beer

4) Rice Wine 5) Others_____

4. Eating habits

4.1 Your usual eating habits tend to

1) More meat and less vegetable 2) Even meat and vegetables 3) More vegetables and less meat 4) Others

4.2 Your habits for staple food

1) Mainly rice 2) Mainly pasta 3) Mainly miscellaneous cereals

4) Rice, noodles and miscellaneous grains

4.3 Do you eat chilli?

1) Regular 2) Occasionally 3) No

4.4 Do you eat pickled vegetables or meat?

1) Regular 2) Occasionally 3) No

4.5 What is your usual taste?

1) Slightly salty 2) Slightly light 3) Moderate

5. What is your working position?

1) Standing for a long time (more than 4 hours a day)

2) Sitting upright for a long time (more than 4 hours a day)

3) All kinds of postures

6. Physical activity

6.1 Do you often do strong physical activities? (Powerful physical activity refers to the activity that takes strenuous physical load and makes you breathe more quickly than normal, at least 3 times a week, at least 10 minutes each time.)

1) Yes 2) No

6.2 Do you often do some moderate physical activities? (Moderate activity refers to the activity with moderate body load and making your breath more laborious

than normal, at least 3 times a week, at least 10 minutes each time).

1) Yes 2) No

6.3 How much time do you spend walking every day, including walking at work, at home, from one place to another, for entertainment, games or leisure.

1) Walk for more than 1 hour every day

2) Walk for dozens of minutes every day

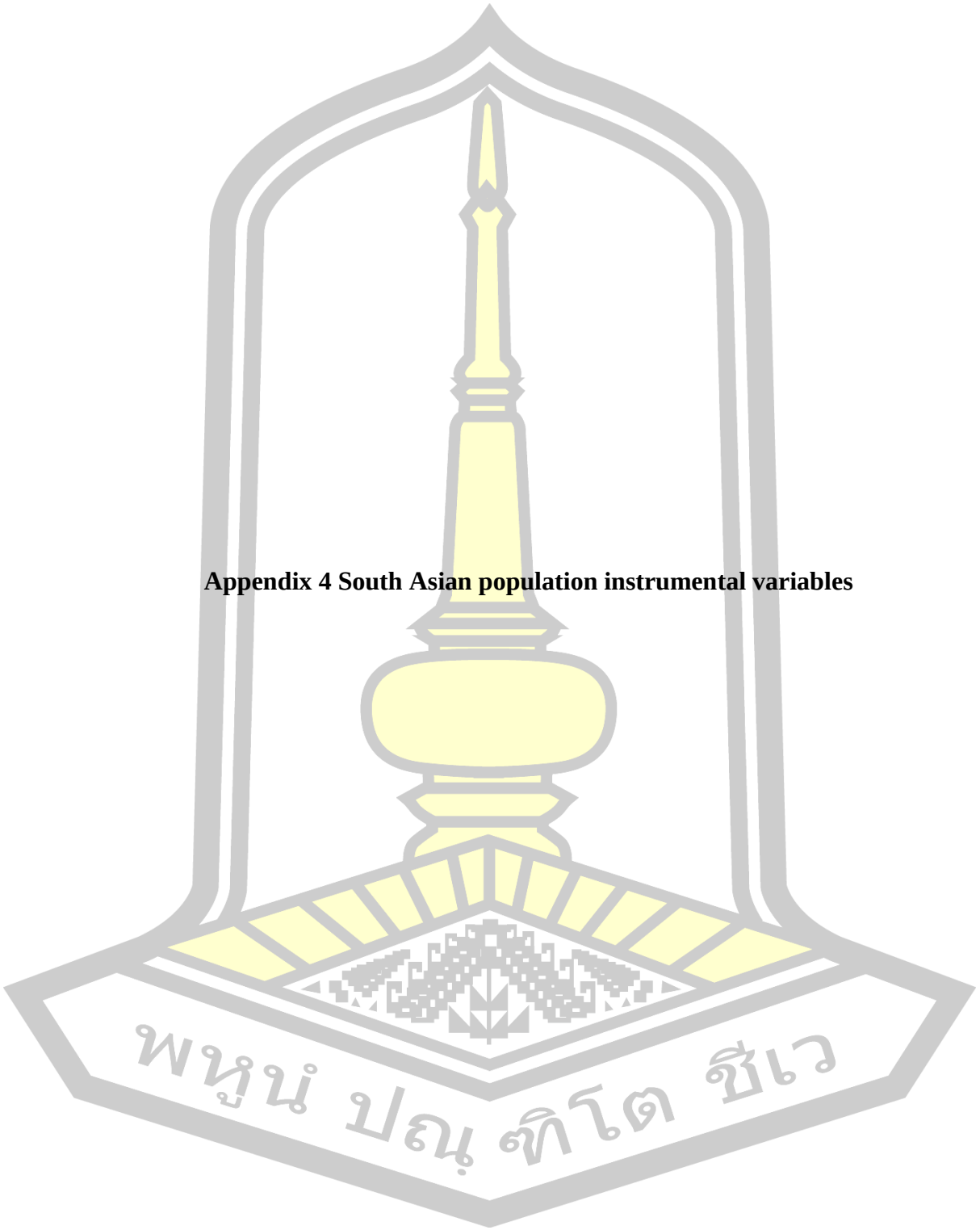
3) Walk for less than 10 minutes every day

7. Disease information (Note: "Yes" should be checked in the blank, and "No or uncertain" should not be filled in)

disease	oneself	Parents	brothers and sisters
7.01 hypertension	☐ F01a	☐ F01b	☐ F01c
7.02 Diabetes	☐ F02a	☐ F02b	☐ F02c
7.03 Hyperlipidemia	☐ F03a	☐ F03b	☐ F03c
7.04 Chronic gastritis	☐ F04a	☐ F04b	☐ F04c
7.05 Chronic gastric ulcer	☐ F05a	☐ F05b	☐ F05c
7.06 Chronic diarrhea	☐ F06a	☐ F06b	☐ F06c
7.07 Constipation	☐ F07a	☐ F07b	☐ F07c
7.08 Pancreatitis	☐ F08a	☐ F08b	☐ F08c
7.09 Colon cancer	☐ F09a	☐ F09b	☐ F09c
7.10 Gastric cancer	☐ F10a	☐ F10a	☐ F10a
7.11 Pancreatic cancer	☐ F11a	☐ F11b	☐ F11c
7.12 Liver cancer	☐ F12a	☐ F12b	☐ F12c
7.13 Hemorrhoids	☐ F13a	☐ F13b	☐ F13c
7.14 Varicosity	☐ F14a	☐ F14b	☐ F14c

Appendix 3 East Asian population instrumental variables

ID	EA	NEA	eaf	beta	se	p-value	n_samples	R2	F值
rs10077638	G	T	0.481818	0.017211	0.003179	1.70E-09	2658	0.010905	29.28172
rs1011939	G	A	0.67463	0.022501	0.003456	8.90E-11	2658	0.015695	42.35065
rs10220751	T	G	0.503941	0.017234	0.00319	4.70E-08	2658	0.010858	29.15534
rs10460960	G	A	0.295705	-0.01984	0.00347	7.90E-09	2658	0.012151	32.66955
rs10504374	T	C	0.597736	0.020317	0.003276	2.50E-10	2658	0.014264	38.43231
rs10795945	T	C	0.446737	-0.02008	0.003302	4.20E-10	2658	0.013719	36.94324
rs10811660	G	A	0.566393	-0.03335	0.003213	3.30E-25	2658	0.038961	107.6747
rs10835389	T	C	0.407781	0.020796	0.003237	1.30E-09	2658	0.015295	41.25561
rs10993170	G	C	0.702622	0.020526	0.003545	3.40E-08	2658	0.012454	33.49521
rs11065983	C	A	0.401218	0.020049	0.003336	1.80E-09	2658	0.013403	36.08132
rs11191021	A	G	0.554408	-0.0193	0.003197	4.60E-10	2658	0.013517	36.39333
rs11191425	C	T	0.730832	-0.02423	0.003575	6.10E-10	2658	0.016992	45.91175
rs11642015	C	T	0.796593	-0.07739	0.003949	9.80E-79	2658	0.126237	383.7272
rs11992893	G	A	0.511201	0.017491	0.003175	2.70E-08	2658	0.011287	30.31967
rs12440611	C	T	0.784487	0.023638	0.003886	1.30E-09	2658	0.013733	36.98161
rs12597682	C	A	0.823583	0.030829	0.004186	4.10E-13	2658	0.020002	54.21079
rs1292079	A	G	0.698259	0.01886	0.00347	2.00E-08	2658	0.01099	29.51265
rs13019219	T	C	0.600084	-0.0191	0.003279	2.30E-08	2658	0.012596	33.88065
rs13419498	G	A	0.461279	-0.01943	0.003175	1.50E-10	2658	0.013889	37.40756
rs1568079	T	A	0.646554	0.021867	0.003326	2.40E-11	2658	0.016005	43.20161
rs16903285	T	C	0.519083	-0.02537	0.00319	2.90E-14	2658	0.023231	63.16821
rs16937956	A	G	0.436821	0.019149	0.003202	1.20E-09	2658	0.013276	35.73417
rs185956466	C	T	0.733963	-0.02414	0.003799	1.20E-10	2658	0.014967	40.35594
rs1899444	G	A	0.363124	0.020684	0.003304	9.80E-10	2658	0.014533	39.16957
rs1907240	G	A	0.302364	0.02527	0.003452	4.20E-13	2658	0.019759	53.53871
rs191569181	G	A	0.864925	0.024324	0.004687	2.00E-08	2658	0.010033	26.91662
rs1996023	T	G	0.289436	0.029896	0.003506	7.70E-16	2658	0.026631	72.66731
rs2112347	T	G	0.438402	0.023655	0.003196	1.30E-12	2658	0.020199	54.75422
rs2206271	T	A	0.648646	-0.02885	0.003326	9.30E-17	2658	0.027528	75.18504
rs2237897	C	T	0.62387	-0.04225	0.003335	2.90E-39	2658	0.056945	160.3785
rs2240920	C	T	0.513297	-0.02022	0.003185	6.00E-10	2658	0.014933	40.26243
rs2297775	T	C	0.231635	0.024792	0.003767	2.30E-10	2658	0.016034	43.28015
rs2611742	T	C	0.606303	-0.03547	0.003251	6.60E-25	2658	0.042872	118.9689
rs2920286	G	A	0.368536	0.020637	0.00331	1.10E-09	2658	0.014411	38.83399
rs3104546	T	C	0.459587	0.018117	0.003199	2.30E-09	2658	0.011922	32.04643
rs329124	A	G	0.619871	0.018806	0.003281	2.40E-08	2658	0.012207	32.82329
rs3811801	G	A	0.275387	0.024907	0.003741	1.00E-10	2658	0.016404	44.29687
rs4385321	G	A	0.392974	-0.01984	0.003242	2.80E-09	2658	0.0139	37.43846
rs4686392	A	G	0.663187	0.028955	0.00336	7.00E-18	2658	0.027173	74.18863
rs4713762	G	A	0.868813	-0.03215	0.004705	5.10E-13	2658	0.017263	46.65647
rs5015480	C	T	0.165453	-0.02816	0.004264	4.20E-11	2658	0.016139	43.56948
rs545943172	C	T	0.854935	-0.0288	0.004916	3.70E-09	2658	0.012748	34.29626
rs62116681	A	T	0.753288	0.022152	0.003685	2.40E-09	2658	0.013416	36.11768
rs633715	T	C	0.774585	-0.04486	0.003795	7.50E-33	2658	0.049944	139.6235
rs6475738	C	G	0.231396	-0.01983	0.003776	4.40E-08	2658	0.010269	27.55652
rs6504551	T	G	0.26655	-0.02222	0.003611	1.20E-09	2658	0.014048	37.84211
rs6567160	T	C	0.783178	-0.04857	0.003856	1.80E-34	2658	0.056348	158.5976
rs6575334	C	T	0.650123	-0.01776	0.003365	2.10E-08	2658	0.010371	27.83305
rs6722025	G	A	0.513584	0.016553	0.003171	1.00E-08	2658	0.010152	27.23905
rs6727262	G	A	0.568971	-0.01891	0.003301	6.60E-09	2658	0.012187	32.76814
rs6947395	A	T	0.801718	-0.0299	0.003978	1.00E-13	2658	0.020806	56.43613
rs7089228	A	G	0.661346	0.018799	0.003355	6.80E-10	2658	0.011672	31.36807
rs7120413	A	G	0.634239	-0.02273	0.003442	4.70E-11	2658	0.016146	43.58653
rs7132908	G	A	0.712054	-0.01999	0.003505	4.40E-08	2658	0.012094	32.51431
rs7199766	G	A	0.65046	-0.02162	0.003382	2.70E-09	2658	0.015142	40.83462
rs7350906	G	A	0.349639	-0.01789	0.003396	2.30E-08	2658	0.010334	27.7326
rs7576635	C	T	0.095708	-0.05033	0.005384	1.30E-19	2658	0.031837	87.33867
rs75766425	G	C	0.869451	-0.03109	0.004738	8.50E-11	2658	0.015942	43.02794
rs76179989	T	G	0.759418	-0.02523	0.003749	1.50E-11	2658	0.016751	45.24975
rs77154330	T	C	0.924939	0.033176	0.006269	4.70E-08	2658	0.010426	27.98429
rs79663428	C	G	0.894576	-0.03278	0.005626	2.50E-08	2658	0.012608	33.91365
rs80234489	A	C	0.804546	0.029459	0.004054	1.70E-11	2658	0.019479	52.76496
rs8101149	G	A	0.753244	-0.02893	0.004395	2.90E-10	2658	0.016037	43.28879
rs879620	C	T	0.668447	-0.02322	0.003378	1.10E-10	2658	0.017462	47.2037
rs9266627	A	G	0.724929	0.021896	0.003551	2.70E-10	2658	0.014101	37.98713
rs9268949	C	T	0.526696	0.022082	0.003216	1.50E-13	2658	0.017433	47.12322
rs9368219	C	T	0.583612	0.037145	0.003238	1.50E-30	2658	0.047186	131.5324
rs9568867	G	A	0.776045	-0.02923	0.003824	1.50E-13	2658	0.021507	58.37952



Appendix 4 South Asian population instrumental variables

ID	EA	NEA	eaf	beta	se	p-value	n_samples	R2	F值
rs10077638	G	T	0.481818	0.017211	0.00318	1.70E-09	8640	0.00338018	29.29699
rs1011939	G	A	0.67463	0.022501	0.00346	8.90E-11	8640	0.00488144	42.37273
rs10220751	T	G	0.503941	0.017234	0.00319	4.70E-08	8640	0.00336564	29.17054
rs10460960	G	A	0.295705	-0.01984	0.00347	7.90E-09	8640	0.00376978	32.68658
rs10504374	T	C	0.597736	0.020317	0.00328	2.50E-10	8640	0.00443181	38.45235
rs10795945	T	C	0.446737	-0.02008	0.0033	4.20E-10	8640	0.00426083	36.9625
rs10811660	G	A	0.566393	-0.03335	0.00321	3.30E-25	8640	0.01231811	107.7309
rs10835389	T	C	0.407781	0.020796	0.00324	1.30E-09	8640	0.00475582	41.27712
rs10993170	G	C	0.702622	0.020526	0.00355	3.40E-08	8640	0.00386469	33.51267
rs11065983	C	A	0.401218	0.020049	0.00334	1.80E-09	8640	0.00416183	36.10013
rs11191021	A	G	0.554408	-0.0193	0.0032	4.60E-10	8640	0.00419767	36.4123
rs11191425	C	T	0.730832	-0.02423	0.00357	6.10E-10	8640	0.00528973	45.93568
rs11642015	C	T	0.796593	-0.07739	0.00395	9.80E-79	8640	0.04255491	383.9273
rs11992893	G	A	0.511201	0.017491	0.00318	2.70E-08	8640	0.00349957	30.33547
rs12419948	T	A	0.592799	0.035092	0.00323	1.50E-26	8640	0.0134545	117.805
rs12440611	C	T	0.784487	0.023638	0.00389	1.30E-09	8640	0.00426523	37.0009
rs12597682	C	A	0.823583	0.030829	0.00419	4.10E-13	8640	0.00623994	54.23905
rs1292079	A	G	0.698259	0.01886	0.00347	2.00E-08	8640	0.00340674	29.52804
rs13019219	T	C	0.600084	-0.0191	0.00328	2.30E-08	8640	0.00390898	33.89831
rs13419498	G	A	0.461279	-0.01943	0.00318	1.50E-10	8640	0.00431415	37.42706
rs1568079	T	A	0.646554	0.021867	0.00333	2.40E-11	8640	0.00497904	43.22413
rs16903285	T	C	0.519083	-0.02537	0.00319	2.90E-14	8640	0.0072635	63.20114
rs16937956	A	G	0.436821	0.019149	0.0032	1.20E-09	8640	0.00412195	35.7528
rs185956466	C	T	0.733963	-0.02414	0.0038	1.20E-10	8640	0.0046526	40.37698
rs1899444	G	A	0.363124	0.020684	0.0033	9.80E-10	8640	0.00451644	39.18999
rs1907240	G	A	0.302364	0.02527	0.00345	4.20E-13	8640	0.00616306	53.56662
rs191569181	G	A	0.864925	0.024324	0.00469	2.00E-08	8640	0.00310801	26.93066
rs1996023	T	G	0.289436	0.029896	0.00351	7.70E-16	8640	0.00834665	72.7052
rs2112347	T	G	0.438402	0.023655	0.0032	1.30E-12	8640	0.0063021	54.78276
rs2206271	T	A	0.648646	-0.02885	0.00333	9.30E-17	8640	0.00863334	75.22423
rs2237897	C	T	0.62387	-0.04225	0.00333	2.90E-39	8640	0.01823752	160.4621
rs2240920	C	T	0.513297	-0.02022	0.00319	6.00E-10	8640	0.00464186	40.28342
rs2297775	T	C	0.231635	0.024792	0.00377	2.30E-10	8640	0.00498804	43.30271
rs2611742	T	C	0.606303	-0.03547	0.00325	6.60E-25	8640	0.01359261	119.0309
rs2920286	G	A	0.368536	0.020637	0.00331	1.10E-09	8640	0.00447792	38.85424
rs3104546	T	C	0.459587	0.018117	0.0032	2.30E-09	8640	0.00369814	32.06314
rs329124	A	G	0.619871	0.018806	0.00328	2.40E-08	8640	0.00378745	32.8404
rs35560038	A	T	0.380392	0.050017	0.0033	4.50E-49	8640	0.02594768	230.1068
rs4385321	G	A	0.392974	-0.01984	0.00324	2.80E-09	8640	0.00431769	37.45797
rs4686392	A	G	0.663187	0.028955	0.00336	7.00E-18	8640	0.0085199	74.22731
rs4713762	G	A	0.868813	-0.03215	0.0047	5.10E-13	8640	0.00537507	46.6808
rs5015480	C	T	0.165453	-0.02816	0.00426	4.20E-11	8640	0.00502122	43.5922
rs545943172	C	T	0.854935	-0.0288	0.00492	3.70E-09	8640	0.00395675	34.31414
rs62116681	A	T	0.753288	0.022152	0.00368	2.40E-09	8640	0.00416601	36.13651
rs633715	T	C	0.774585	-0.04486	0.0038	7.50E-33	8640	0.01591492	139.6963
rs6504551	T	G	0.26655	-0.02222	0.00361	1.20E-09	8640	0.00436404	37.86184
rs6567160	T	C	0.783178	-0.04857	0.00386	1.80E-34	8640	0.01803865	158.6802
rs6575334	C	T	0.650123	-0.01776	0.00337	2.10E-08	8640	0.00321348	27.84756
rs6722025	G	A	0.513584	0.016553	0.00317	1.00E-08	8640	0.00314512	27.25325
rs6727262	G	A	0.568971	-0.01891	0.0033	6.60E-09	8640	0.00378111	32.78522
rs6947395	A	T	0.801718	-0.0299	0.00398	1.00E-13	8640	0.00649443	56.46556
rs7089228	A	G	0.661346	0.018799	0.00336	6.80E-10	8640	0.00362014	31.38443
rs7120413	A	G	0.634239	-0.02273	0.00344	4.70E-11	8640	0.00502318	43.60926
rs7132908	G	A	0.712054	-0.01999	0.0035	4.40E-08	8640	0.00375193	32.53126
rs7199766	G	A	0.65046	-0.02162	0.00338	2.70E-09	8640	0.00470752	40.85591
rs7350906	G	A	0.349639	-0.01789	0.0034	2.30E-08	8640	0.00320192	27.74706
rs75447473	C	T	0.988943	0.105011	0.01531	4.60E-12	8640	0.00541723	47.04887
rs7576635	C	T	0.095708	-0.05033	0.00538	1.30E-19	8640	0.01001494	87.3842
rs76179989	T	G	0.759418	-0.02523	0.00375	1.50E-11	8640	0.00521386	45.27334
rs77154330	T	C	0.924939	0.033176	0.00627	4.70E-08	8640	0.00323089	27.99888
rs80234489	A	C	0.804546	0.029459	0.00405	1.70E-11	8640	0.00607453	52.79247
rs8101149	G	A	0.753244	-0.02893	0.0044	2.90E-10	8640	0.00498903	43.31136
rs879620	C	T	0.668447	-0.02322	0.00338	1.10E-10	8640	0.00543777	47.22831
rs9266627	A	G	0.724929	0.021896	0.00355	2.70E-10	8640	0.00438069	38.00693
rs9268949	C	T	0.526696	0.022082	0.00322	1.50E-13	8640	0.00542855	47.14779
rs9368219	C	T	0.583612	0.037145	0.00324	1.50E-30	8640	0.0150065	131.601
rs9568867	G	A	0.776045	-0.02923	0.00382	1.50E-13	8640	0.00671656	58.40995

Appendix 5 Concentration and purity of sample DNA

Sample number	volume (ul)	DNA concentration (ng/ul)	A260/A280	A260/A230
1	30	50.90	1.943	1.919
2	30	41.45	1.793	1.847
3	30	56.41	1.824	1.873
4	30	37.85	1.894	1.802
5	30	49.20	1.898	1.844
6	30	72.31	1.886	1.803
7	30	57.05	1.921	1.847
8	30	71.05	1.937	1.885
9	30	51.50	1.856	1.836
10	30	42.20	1.759	1.855
11	30	44.85	1.785	1.913
12	30	70.50	1.843	1.928
13	30	53.50	1.787	1.958
14	30	62.80	1.826	1.867
15	30	37.35	1.765	1.811
16	30	49.75	2.014	1.906
17	30	56.25	2.003	1.969
18	30	62.25	1.855	1.912
19	30	51.60	1.945	2.039
20	30	79.85	1.951	1.954
21	30	51.65	1.899	1.965
22	30	62.05	1.839	1.831
23	30	43.95	1.917	1.926
24	30	47.05	1.910	1.837
25	30	42.95	1.858	1.889
26	30	39.60	1.868	1.885
27	30	51.10	1.932	1.849
28	30	45.20	1.906	1.848
29	30	55.50	1.983	1.837
30	30	46.60	1.943	1.821
31	30	53.70	1.992	1.840
32	30	71.65	2.028	2.012
33	30	46.45	1.889	2.018
34	30	47.20	1.914	1.899
35	30	46.95	1.910	1.838
36	30	58.05	1.984	2.051
37	30	89.90	1.973	1.983

38	30	60.20	1.869	1.880
39	30	44.70	1.904	1.875
40	30	61.45	1.919	1.979
41	30	88.25	1.990	2.071
42	30	139.30	1.964	2.167
43	30	62.00	1.956	1.880
44	30	55.30	1.914	1.932
45	30	73.20	1.994	2.033
46	30	37.35	1.855	1.869
47	30	139.20	2.010	2.147
48	30	50.15	1.938	1.842
49	30	41.20	1.865	1.892
50	30	63.80	1.926	1.869
51	30	41.10	1.916	1.915
52	30	36.65	1.841	1.890
53	30	41.25	1.897	1.804
54	30	50.95	1.821	1.832
55	30	42.65	1.849	1.825
56	30	111.95	1.991	1.902
57	30	63.40	1.918	1.927
58	30	36.30	1.765	1.838
59	30	54.45	1.890	1.896
60	30	48.20	1.822	1.894
61	30	139.45	1.984	1.879
62	30	213.20	1.931	2.025
63	30	81.80	1.943	1.849
64	30	41.85	1.740	1.847
65	30	53.60	1.894	1.945
66	30	48.90	1.875	1.836
67	30	56.80	1.923	1.849
68	30	82.75	1.949	1.834
69	30	45.30	1.832	1.838
70	30	69.30	1.992	1.871
71	30	76.95	1.941	1.842
72	30	74.30	1.958	1.834
73	30	81.40	1.963	1.858
74	30	146.40	1.965	2.006
75	30	58.15	1.906	1.849
76	30	78.75	1.908	1.833
77	30	82.50	1.935	1.850
78	30	143.15	1.987	2.026

79	30	105.65	1.954	1.950
80	30	73.65	1.879	1.914
81	30	93.65	1.900	1.881
82	30	37.50	1.736	1.798
83	30	64.80	1.929	1.848
84	30	39.85	1.744	1.804
85	30	43.20	1.830	1.852
86	30	40.80	1.787	1.836
87	30	44.65	1.803	1.832
88	30	54.60	1.807	1.874
89	30	83.05	1.810	1.902
90	30	37.90	1.802	1.871
91	30	51.15	1.811	1.838
92	30	146.75	1.909	1.981
93	30	313.50	1.929	2.175
94	30	51.35	1.761	1.829
95	30	33.15	1.729	1.867
96	30	43.75	1.757	1.844
97	30	35.70	1.725	1.838
98	30	65.25	1.760	1.848
99	30	44.60	1.773	1.875
100	30	62.05	1.830	1.840
101	30	34.35	1.873	1.844
102	30	56.85	1.869	1.962
103	30	73.55	1.885	1.864
104	30	60.30	1.968	1.929
105	30	34.05	1.810	1.853
106	30	32.70	1.789	1.837
107	30	48.25	1.969	1.884
108	30	35.55	1.784	1.890
109	30	34.60	1.724	1.824
110	30	33.30	1.758	1.855
111	30	35.25	1.763	1.878
112	30	42.80	1.774	1.879
113	30	38.90	1.721	1.824
114	30	62.10	1.845	1.939
115	30	36.65	1.732	1.831
116	30	36.25	1.734	1.853
117	30	48.85	1.818	1.888
118	30	72.40	1.870	1.986
119	30	34.40	1.902	1.793

120	30	56.10	1.798	1.887
121	30	115.70	1.851	1.824
122	30	37.70	1.768	1.878
123	30	40.10	1.730	1.827
124	30	57.65	1.872	1.942
125	30	38.35	1.737	1.856
126	30	108.30	1.879	1.990
127	30	41.40	1.765	1.905
128	30	42.45	1.780	1.830
129	30	35.85	1.722	1.799
130	30	43.75	1.765	1.888
131	30	36.95	1.767	1.846
132	30	44.05	1.922	1.872
133	30	41.35	1.883	1.907
134	30	86.15	1.888	1.952
135	30	39.20	1.769	1.824
136	30	47.00	1.770	1.860
137	30	65.45	1.895	1.995
138	30	57.35	1.802	2.122
139	30	31.80	1.724	1.826
140	30	58.35	1.803	2.126
141	30	152.15	1.948	2.142
142	30	81.45	1.870	2.130
143	30	277.25	2.004	2.154
144	30	145.75	1.909	2.151
145	30	155.15	1.931	2.101
146	30	86.40	1.871	1.992
147	30	58.80	1.841	1.889
148	30	298.45	1.933	2.137
149	30	44.60	1.731	1.914
150	30	31.45	1.908	1.857
151	30	100.40	1.944	1.971
152	30	114.80	1.949	2.110
153	30	98.70	1.928	1.986
154	30	42.20	1.732	1.905
155	30	34.60	1.880	1.890
156	30	106.25	1.875	1.986
157	30	157.70	2.015	1.936
158	30	133.30	1.983	1.879
159	30	214.45	1.998	2.090
160	30	109.85	1.905	2.133

161	30	46.35	1.733	1.836
162	30	54.50	1.825	1.880
163	30	39.95	1.814	1.899
164	30	89.65	1.867	2.047
165	30	223.05	1.906	2.115
166	30	197.05	1.965	2.149
167	30	253.90	1.983	2.038
168	30	182.25	1.933	2.210
169	30	173.00	1.964	2.097
170	30	64.30	1.808	1.905
171	30	72.25	1.849	2.095
172	30	179.20	1.954	2.058
173	30	237.55	1.965	2.161
174	30	194.10	1.983	2.144
175	30	112.35	1.901	2.064
176	30	131.30	1.902	1.947
177	30	60.55	1.949	1.815
178	30	166.90	1.893	2.166
179	30	190.40	1.955	1.862
180	30	101.85	1.886	1.867
181	30	169.15	1.943	2.040
182	30	53.30	1.960	1.836
183	30	58.55	1.972	2.022
184	30	83.55	1.961	2.021
185	30	61.90	1.908	1.789
186	30	51.80	1.928	1.792
187	30	60.60	1.936	1.809
188	30	143.65	1.946	1.977
189	30	137.85	1.932	2.102
190	30	478.35	1.940	2.151
191	30	81.40	1.933	2.001
192	30	108.05	1.854	1.813
193	30	182.15	1.953	2.049
194	30	51.25	1.850	1.787
195	30	26.35	1.729	1.788
196	30	95.80	1.837	2.023
197	30	93.60	1.896	1.836
198	30	43.70	1.742	1.784
199	30	39.65	1.879	1.793
200	30	150.75	1.832	1.782
201	30	142.51	1.835	1.966

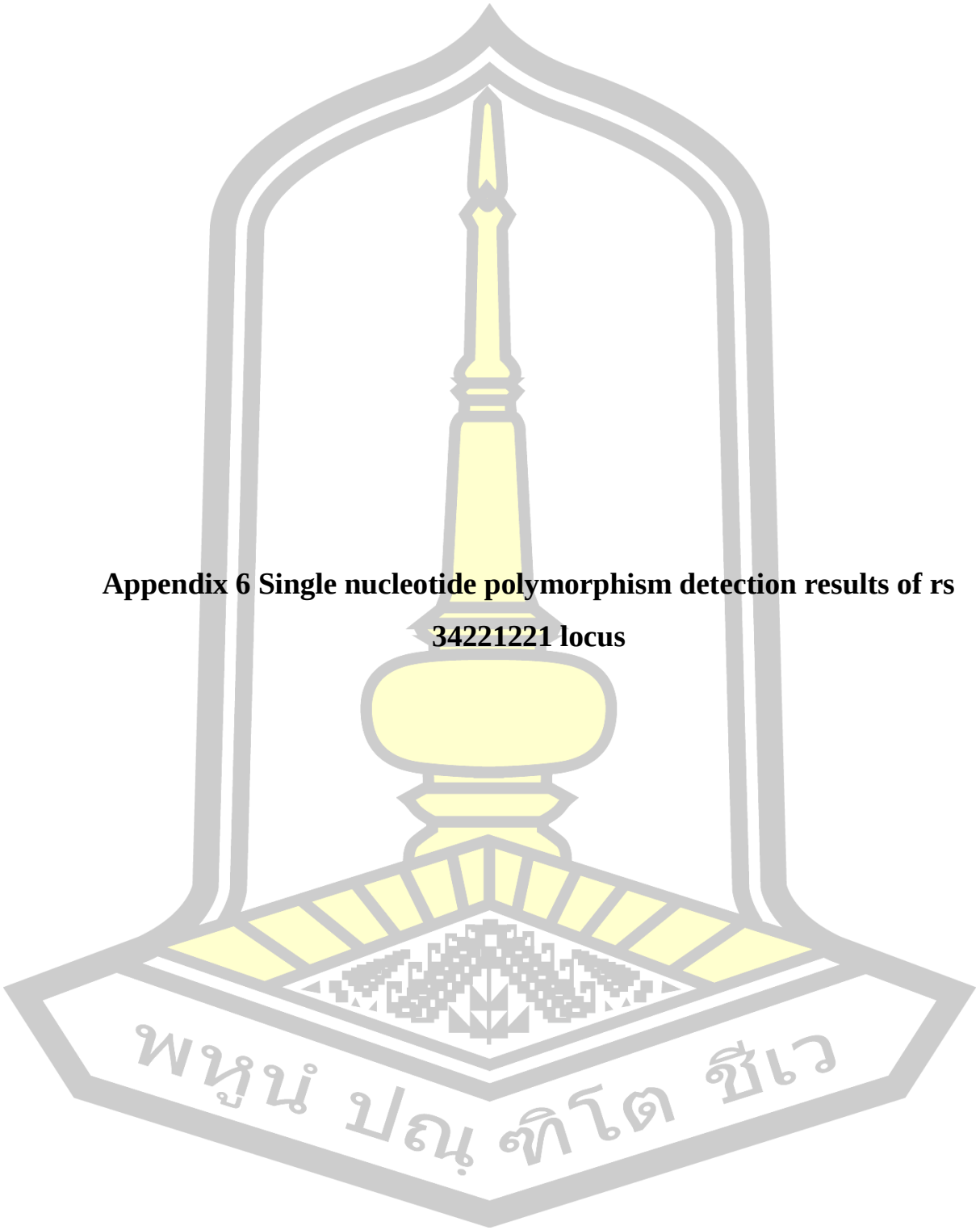
202	30	113.84	1.862	1.903
203	30	61.90	1.833	1.822
204	30	102.00	1.840	1.830
205	30	49.45	1.838	1.804
206	30	102.65	1.881	1.870
207	30	77.05	1.853	2.049
208	30	87.20	1.940	2.000
209	30	95.25	1.900	1.783
210	30	178.95	1.822	1.954
211	30	103.00	1.899	1.964
212	30	140.90	1.938	1.800
213	30	375.65	1.977	2.085
214	30	63.10	1.898	1.785
215	30	114.95	1.875	1.950
216	30	108.10	1.876	1.833
217	30	82.45	1.894	2.161
218	30	97.60	1.954	1.849
219	30	87.95	1.957	2.099
220	30	60.35	1.919	1.985
221	30	116.00	1.910	1.866
222	30	76.75	1.940	2.103
223	30	78.85	1.941	2.033
224	30	133.95	1.894	1.808
225	30	47.00	1.973	2.164
226	30	85.60	1.923	2.073
227	30	105.85	1.915	1.827
228	30	132.00	1.924	1.856
229	30	83.15	1.941	2.060
230	30	140.30	1.936	2.138
231	30	175.30	1.899	1.875
232	30	89.80	1.912	1.789
233	30	51.70	1.962	2.057
234	30	46.50	1.915	1.836
235	30	79.75	1.837	1.785
236	30	137.30	1.894	1.903
237	30	161.70	1.912	1.834
238	30	131.65	1.961	2.027
239	30	127.90	1.923	2.070
240	30	117.65	1.905	1.788
241	30	99.20	1.940	2.024
242	30	261.70	1.883	1.984

243	30	97.30	1.904	2.114
244	30	85.45	1.822	1.795
245	30	87.10	1.948	1.859
246	30	112.55	1.909	1.957
247	30	47.60	1.936	2.100
248	30	63.85	1.895	1.792
249	30	96.75	1.884	2.027
250	30	110.75	1.844	2.087
251	30	54.95	1.921	1.799
252	30	66.65	1.698	1.791
253	30	73.70	1.954	1.829
254	30	51.00	1.966	1.992
255	30	31.10	1.910	2.048
256	30	70.85	1.886	1.833
257	30	91.20	1.734	1.841
258	30	75.15	1.900	1.959
259	30	70.90	1.909	1.816
260	30	104.30	1.932	1.837
261	30	138.70	1.912	1.931
262	30	175.10	1.836	1.822
263	30	86.95	1.909	2.006
264	30	69.65	1.857	1.809
265	30	72.20	1.918	1.876
266	30	53.45	1.866	1.786
267	30	66.80	1.809	1.805
268	30	112.35	1.967	1.886
269	30	163.25	1.965	2.001
270	30	90.05	1.989	2.138
271	30	91.25	1.855	1.912
272	30	131.30	1.928	1.826
273	30	47.50	1.907	1.818
274	30	84.60	1.886	1.803
275	30	66.80	1.900	1.872
276	30	47.70	1.912	2.032
277	30	46.95	1.955	2.102
278	30	118.25	1.913	1.870
279	30	94.65	1.930	2.134
280	30	137.50	1.950	1.828
281	30	71.90	1.743	1.807
282	30	102.19	1.944	1.924
283	30	131.05	1.898	1.820

284	30	56.90	1.974	2.054
285	30	77.70	1.882	2.038
286	30	84.45	1.914	1.821
287	50	45.60	1.987	2.141
288	50	109.30	1.944	2.037
289	50	120.70	1.930	2.001
290	50	90.75	1.922	2.103
291	50	125.60	1.984	2.118
292	50	170.00	1.936	1.809
293	50	130.70	1.907	2.184
294	50	78.10	1.896	2.074
295	50	78.80	1.951	2.014
296	50	128.90	1.800	1.892
297	50	85.80	1.933	2.017
298	50	145.10	1.956	2.096
299	50	76.10	1.976	2.131
300	50	97.15	1.950	1.878
301	50	73.00	1.945	1.981
302	50	146.70	1.914	1.965
303	50	127.50	1.966	2.105
304	50	74.50	1.848	1.854
305	50	81.05	1.854	2.059
306	50	66.55	1.864	2.196
307	50	137.95	1.820	1.940
308	50	122.90	1.953	2.130
309	50	180.80	1.947	1.902
310	50	71.70	1.878	2.015
311	50	109.40	1.864	2.006
312	50	60.05	1.870	2.191
313	50	53.40	1.850	1.961
314	50	44.05	1.835	2.045
315	50	55.60	1.907	2.115
316	50	103.00	1.953	2.003
317	50	99.70	1.855	2.117
318	50	78.35	1.955	2.080
319	50	99.95	1.898	1.802
320	50	103.00	1.932	1.835
321	50	114.50	1.850	2.087
322	50	98.75	1.814	2.173
323	50	47.90	1.825	1.862
324	50	135.53	1.727	1.804

325	50	37.90	1.860	2.160
326	50	46.45	1.839	1.999
327	50	72.70	1.847	2.161
328	50	74.20	1.992	2.086
329	50	126.90	1.823	2.035
330	50	107.00	1.975	2.140
331	50	108.50	1.800	1.966
332	50	52.20	1.854	2.144
333	50	49.50	1.956	2.099
334	50	127.70	1.943	2.030
335	50	77.50	1.994	2.148
336	50	126.55	1.812	1.873
337	50	130.60	1.802	1.810
338	50	43.30	1.814	1.936
339	50	83.80	1.878	2.129
340	50	47.05	1.847	2.057
341	50	89.50	1.946	1.886
342	50	89.90	1.992	1.839
343	50	102.60	1.924	2.003
344	50	137.30	1.933	2.033
345	50	106.00	1.936	2.064
346	50	161.30	1.912	1.920
347	50	250.01	1.845	1.892
348	50	79.55	1.863	2.158
349	50	118.70	1.955	2.155
350	50	57.80	1.877	1.811
351	50	92.80	1.875	2.054
352	50	154.60	1.947	1.914
353	50	123.70	1.933	1.927
354	50	72.75	1.885	2.192
355	50	119.20	1.882	1.841
356	50	47.50	1.867	2.159
357	30	143.45	1.946	1.966
358	30	36.05	1.918	1.808
359	30	155.75	1.964	2.007
360	30	185.25	1.997	1.837
361	30	33.05	1.701	1.806
362	30	141.35	1.960	1.934
363	30	135.75	1.950	1.862
364	30	235.76	1.932	2.099
365	30	86.85	1.911	1.812

366	30	270.10	1.966	2.081
367	30	108.35	1.894	1.807
368	30	191.50	1.966	1.955
369	30	242.85	1.958	2.122
370	30	102.90	1.775	1.825
371	30	141.70	1.997	2.187
372	30	66.80	1.921	2.077
373	30	205.20	1.970	2.002
374	30	119.85	1.935	1.838
375	30	100.90	1.935	1.807
376	30	34.40	1.846	1.814
377	30	81.95	1.881	1.861
378	30	35.25	1.812	1.805
379	30	130.65	1.914	1.846
380	30	123.35	1.729	2.018
381	30	38.65	1.810	1.805
382	30	176.85	1.968	1.946
383	30	139.10	1.926	1.808
384	30	104.80	1.934	1.874
385	30	67.05	1.880	1.804
386	30	36.35	1.780	1.816
387	30	122.90	1.916	1.851
388	30	134.00	1.913	1.833
389	30	205.55	1.961	2.014
390	30	124.15	1.915	1.824
391	30	110.00	1.852	1.804
392	30	63.75	1.949	1.925
393	30	79.70	1.835	1.810
394	30	59.20	1.768	1.816
395	30	91.35	1.825	1.809
396	30	128.75	1.950	1.827
397	30	124.15	1.886	1.851
398	30	74.50	1.828	1.833
399	30	81.50	1.857	1.840
400	30	145.35	1.968	2.045



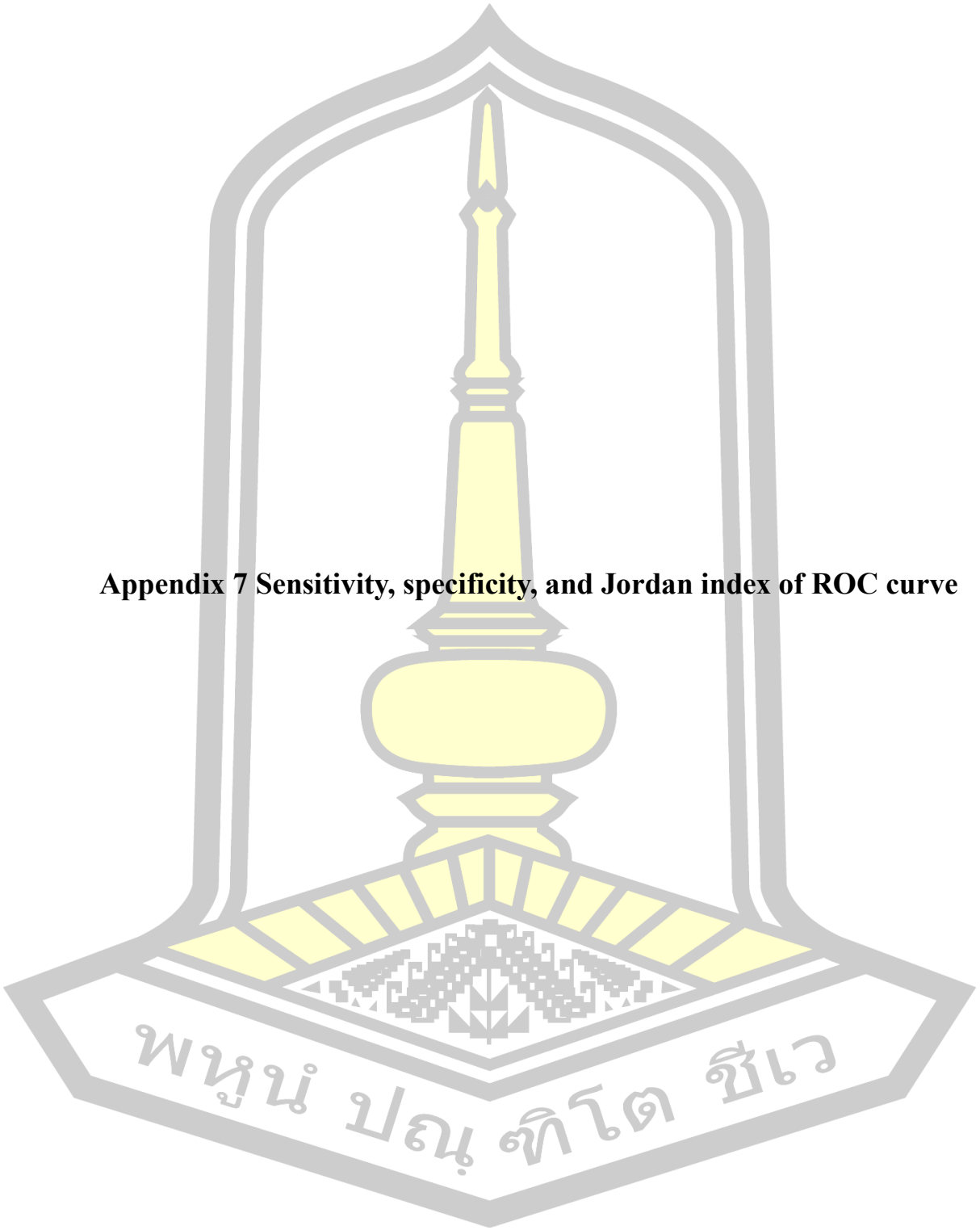
Appendix 6 Single nucleotide polymorphism detection results of rs 34221221 locus

Sample number	genotype	Sample number	genotype	Sample number	genotype
1	CT	41	CC	81	CT
2	CT	42	CC	82	CC
3	CC	43	CC	83	CT
4	CC	44	CT	84	CC
5	CC	45	CC	85	CT
6	CC	46	CT	86	CT
7	CT	47	CC	87	CT
8	CC	48	CT	88	CC
9	CC	49	CT	89	CC
10	CT	50	CC	90	CT
11	CT	51	CT	91	CC
12	CT	52	CT	92	CT
13	CC	53	CT	93	CC
14	CC	54	CT	94	CT
15	CC	55	CT	95	CC
16	CC	56	CT	96	CT
17	CC	57	CT	97	CT
18	CC	58	CC	98	CT
19	CC	59	CT	99	CT
20	CT	60	CC	100	CT
21	CT	61	CC	101	CT
22	CC	62	CT	102	CT
23	CT	63	CC	103	CC
24	CC	64	CC	104	CT
25	CT	65	CT	105	CT
26	CT	66	CT	106	CC
27	CT	67	CT	107	CT
28	CC	68	CT	108	CC
29	CC	69	CT	109	CC
30	CC	70	CC	110	CC
31	CT	71	CT	111	CT
32	CT	72	CT	112	CT
33	CC	73	CC	113	CC
34	CT	74	CT	114	CT
35	CC	75	CC	115	CT
36	CC	76	CT	116	CT
37	CC	77	CC	117	CT
38	CC	78	CC	118	CC
39	CT	79	CT	119	CT
40	CT	80	CT	120	CC
121	CT	161	CC	201	CC
122	CC	162	CC	202	CC
123	CC	163	CT	203	CT

124	CC	164	CT	204	CT
125	CC	165	CC	205	CT
126	CT	166		206	CT
127	CC	167	CT	207	CT
128	CC	168	CT	208	CT
129	CT	169	CT	209	CT
130	CC	170	CT	210	CT
131	CC	171	CT	211	CC
132	CT	172	CC	212	CC
133	CT	173	CC	213	CT
134	CC	174	CT	214	CC
135	CT	175	CC	215	CC
136	CT	176	CC	216	CC
137	CT	177	CT	217	CT
138	CC	178	CT	218	CT
139	CC	179	CT	219	CT
140	CC	180	CT	220	CC
141	CT	181	CT	221	CC
142	CC	182	CT	222	CC
143	CC	183	CC	223	CC
144	CC	184	CC	224	CT
145	CT	185	CT	225	CC
146	CT	186	CT	226	CT
147	CC	187	CT	227	CT
148	CT	188	CC	228	CT
149	CT	189	CC	229	CT
150	CT	190	CC	230	CC
151	CT	191	CT	231	CT
152	CT	192	CC	232	CT
153	CT	193	CT	233	CT
154	CT	194	CT	234	CC
155	CC	195	CC	235	CC
156	CC	196	CC	236	CT
157		197	CC	237	CT
158	CC	198	CT	238	CT
159	CC	199	CT	239	CT
160	CC	200	CC	240	CT
241	CT	**281**	CC	**321**	CT
242	CT	**282**	CT	**322**	CT
243	CT	**283**	CT	**323**	CC
244	CC	**284**	CT	**324**	CT
245	CC	**285**	CT	**325**	CT
246	CC	**286**	CT	**326**	CT
247	CT	**287**	CC	**327**	CC
248	CT	**288**	CT	**328**	CT

249	CT	**289**	CT	**329**	CT
250	CT	**290**	CT	**330**	CT
251	CT	**291**	CT	**331**	CC
252	CC	**292**	CC	**332**	CT
253	CT	**293**	CT	**333**	CT
254	CC	**294**	CT	**334**	CC
255	CT	**295**	CT	**335**	CT
256	CT	**296**	CT	**336**	CC
257	CT	**297**	CT	**337**	CC
258	CC	**298**	CT	**338**	CC
259	CC	**299**	CC	**339**	CT
260	CT	**300**	CC	**340**	CT
261	CC	**301**	CT	**341**	CT
262	CC	**302**	CC	**342**	CT
263	CT	**303**	CT	**343**	CT
264	CC	**304**	CC	**344**	CC
265	CT	**305**	CT	**345**	CT
266	CT	**306**	CC	**346**	CC
267	CT	**307**	CC	**347**	CT
268	CT	**308**	CT	**348**	CT
269	CT	**309**	CT	**349**	CC
270	CT	**310**	CC	**350**	CT
271	CC	**311**	CC	**351**	CC
272	CT	**312**	CC	**352**	CT
273	CC	**313**	CT	**353**	CT
274	CT	**314**	CC	**354**	CT
275	CT	**315**	CT	**355**	CC
276	CT	**316**	CT	**356**	CT
277	CT	**317**	CC	**357**	CT
278	CT	**318**	CT	**358**	CC
279	CT	**319**	CC	**359**	CC
280	CC	**320**	CC	**360**	CT
361	CC	**371**	CT	**381**	CC
362	CT	**372**	CT	**382**	CC
363	CT	**373**	CT	**383**	CT
364	CC	**374**	CT	**384**	CC
365	CT	**375**	CT	**385**	CT
366	CT	**376**	CC	**386**	CC
367	CT	**377**	CC	**387**	CT
368	CT	**378**	CT	**388**	CT
369	CC	**379**	CT	**389**	CC
370	CC	**380**	CT	**390**	CT
391	CT	**395**	CT	**399**	CC
392	CT	**396**	CC	**400**	CT
393	CC	**397**	CT	**398**	CT

394	CT



Appendix 7 Sensitivity, specificity, and Jordan index of ROC curve

The coordinates of the curve
Test result variable: predicted value

Positive when greater than or equal to this value	Sensitivity	1- specificity	Youden index
-3.59	1	1	0
-2.53	0.995	1	-0.005
-2.46	0.995	0.995	0
-2.4	0.995	0.99	0.005
-2.34	0.995	0.98	0.015
-2.31	0.995	0.975	0.02
-2.275	0.985	0.975	0.01
-2.25	0.98	0.975	0.005
-2.22	0.98	0.97	0.01
-2.165	0.97	0.97	0
-2.11	0.965	0.97	-0.005
-2.065	0.965	0.965	0
-2.03	0.96	0.965	-0.005
-2.015	0.96	0.96	0
-2	0.955	0.96	-0.005
-1.985	0.955	0.955	0
-1.975	0.949	0.945	0.004
-1.96	0.944	0.945	-0.001
-1.925	0.939	0.945	-0.006
-1.89	0.934	0.94	-0.006
-1.86	0.934	0.935	-0.001
-1.835	0.929	0.935	-0.006
-1.825	0.924	0.93	-0.006
-1.81	0.924	0.915	0.009
-1.8	0.924	0.915	0.009
-1.78	0.914	0.905	0.009
-1.75	0.909	0.905	0.004
-1.735	0.909	0.895	0.014
-1.73	0.909	0.895	0.014
-1.72	0.909	0.885	0.024
-1.705	0.904	0.885	0.019
-1.69	0.904	0.88	0.024
-1.675	0.904	0.865	0.039
-1.66	0.894	0.845	0.049
-1.645	0.894	0.84	0.054
-1.635	0.894	0.835	0.059
-1.625	0.889	0.835	0.054
-1.62	0.889	0.83	0.059

-1.615	0.884	0.825	0.059
-1.605	0.879	0.825	0.054
-1.595	0.879	0.82	0.059
-1.59	0.879	0.82	0.059
-1.585	0.874	0.815	0.059
-1.575	0.874	0.81	0.064
-1.565	0.874	0.8	0.074
-1.555	0.869	0.8	0.069
-1.545	0.864	0.8	0.064
-1.535	0.859	0.8	0.059
-1.525	0.859	0.795	0.064
-1.51	0.854	0.795	0.059
-1.5	0.854	0.795	0.059
-1.495	0.848	0.785	0.063
-1.485	0.848	0.775	0.073
-1.47	0.838	0.775	0.063
-1.45	0.838	0.765	0.073
-1.435	0.833	0.76	0.073
-1.415	0.833	0.755	0.078
-1.4	0.833	0.755	0.078
-1.395	0.828	0.74	0.088
-1.39	0.823	0.74	0.083
-1.385	0.818	0.735	0.083
-1.375	0.818	0.73	0.088
-1.37	0.813	0.72	0.093
-1.365	0.808	0.71	0.098
-1.355	0.808	0.705	0.103
-1.345	0.793	0.705	0.088
-1.335	0.788	0.705	0.083
-1.325	0.783	0.7	0.083
-1.31	0.783	0.695	0.088
-1.29	0.778	0.695	0.083
-1.265	0.778	0.69	0.088
-1.245	0.778	0.685	0.093
-1.235	0.773	0.68	0.093
-1.23	0.773	0.68	0.093
-1.225	0.768	0.675	0.093
-1.22	0.763	0.675	0.088
-1.215	0.758	0.67	0.088
-1.205	0.758	0.665	0.093
-1.195	0.758	0.66	0.098
-1.19	0.758	0.66	0.098

-1.185	0.753	0.655	0.098
-1.165	0.747	0.645	0.102
-1.15	0.747	0.645	0.102
-1.145	0.742	0.635	0.107
-1.135	0.732	0.63	0.102
-1.125	0.727	0.615	0.112
-1.11	0.722	0.615	0.107
-1.095	0.722	0.61	0.112
-1.085	0.722	0.595	0.127
-1.08	0.722	0.595	0.127
-1.065	0.717	0.59	0.127
-1.045	0.712	0.58	0.132
-1.035	0.707	0.58	0.127
-1.025	0.702	0.57	0.132
-1.02	0.702	0.57	0.132
-1.015	0.697	0.565	0.132
-1.005	0.692	0.565	0.127
-1	0.687	0.565	0.122
-0.985	0.682	0.56	0.122
-0.955	0.682	0.55	0.132
-0.935	0.677	0.545	0.132
-0.93	0.677	0.545	0.132
-0.92	0.672	0.53	0.142
-0.905	0.672	0.525	0.147
-0.9	0.662	0.525	0.137
-0.895	0.657	0.525	0.132
-0.885	0.652	0.525	0.127
-0.88	0.652	0.52	0.132
-0.865	0.646	0.52	0.126
-0.85	0.646	0.52	0.126
-0.845	0.641	0.515	0.126
-0.84	0.636	0.515	0.121
-0.835	0.631	0.515	0.116
-0.83	0.631	0.515	0.116
-0.825	0.626	0.505	0.121
-0.815	0.621	0.505	0.116
-0.81	0.616	0.505	0.111
-0.805	0.616	0.5	0.116
-0.795	0.616	0.495	0.121
-0.785	0.616	0.49	0.126
-0.775	0.616	0.48	0.136
-0.765	0.611	0.475	0.136

-0.755	0.606	0.475	0.131
-0.75	0.606	0.47	0.136
-0.745	0.606	0.46	0.146
-0.735	0.606	0.455	0.151
-0.725	0.601	0.45	0.151
-0.72	0.601	0.445	0.156
-0.715	0.591	0.44	0.151
-0.7	0.581	0.435	0.146
-0.685	0.581	0.43	0.151
-0.675	0.581	0.425	0.156
-0.67	0.581	0.42	0.161
-0.665	0.581	0.415	0.166
-0.655	0.581	0.405	0.176
-0.645	0.576	0.405	0.171
-0.64	0.571	0.395	0.176
-0.635	0.566	0.395	0.171
-0.63	0.561	0.39	0.171
-0.625	0.561	0.385	0.176
-0.615	0.561	0.37	0.191
-0.605	0.561	0.365	0.196
-0.6	0.561	0.36	0.201
-0.595	0.561	0.355	0.206
-0.59	0.556	0.35	0.206
-0.585	0.551	0.35	0.201
-0.575	0.545	0.35	0.195
-0.565	0.54	0.345	0.195
-0.555	0.535	0.34	0.195
-0.545	0.535	0.33	0.205
-0.54	0.53	0.33	0.2
-0.535	0.52	0.33	0.19
-0.53	0.52	0.325	0.195
-0.52	0.515	0.325	0.19
-0.505	0.51	0.32	0.19
-0.495	0.505	0.315	0.19
-0.49	0.505	0.315	0.19
-0.485	0.5	0.31	0.19
-0.48	0.5	0.31	0.19
-0.475	0.495	0.305	0.19
-0.46	0.485	0.305	0.18
-0.445	0.485	0.3	0.185
-0.435	0.475	0.29	0.185
-0.425	0.47	0.29	0.18

-0.42	0.465	0.29	0.175
-0.415	0.46	0.29	0.17
-0.4	0.455	0.29	0.165
-0.375	0.449	0.29	0.159
-0.355	0.444	0.29	0.154
-0.345	0.434	0.285	0.149
-0.335	0.434	0.28	0.154
-0.33	0.434	0.275	0.159
-0.33	0.429	0.275	0.154
-0.325	0.424	0.275	0.149
-0.32	0.424	0.275	0.149
-0.32	0.414	0.265	0.149
-0.32	0.414	0.265	0.149
-0.305	0.409	0.26	0.149
-0.285	0.404	0.255	0.149
-0.28	0.404	0.255	0.149
-0.275	0.399	0.24	0.159
-0.26	0.394	0.24	0.154
-0.245	0.394	0.235	0.159
-0.235	0.389	0.23	0.159
-0.23	0.384	0.23	0.154
-0.22	0.379	0.23	0.149
-0.21	0.374	0.23	0.144
-0.205	0.374	0.225	0.149
-0.195	0.364	0.215	0.149
-0.18	0.364	0.21	0.154
-0.165	0.364	0.205	0.159
-0.15	0.364	0.2	0.164
-0.14	0.359	0.2	0.159
-0.14	0.359	0.195	0.164
-0.13	0.354	0.18	0.174
-0.115	0.348	0.18	0.168
-0.11	0.348	0.17	0.178
-0.11	0.343	0.17	0.173
-0.095	0.338	0.17	0.168
-0.075	0.333	0.165	0.168
-0.065	0.323	0.165	0.158
-0.06	0.318	0.165	0.153
-0.06	0.318	0.16	0.158
-0.055	0.313	0.155	0.158
-0.045	0.308	0.155	0.153
-0.035	0.303	0.155	0.148

-0.025	0.303	0.145	0.158
-0.02	0.303	0.14	0.163
-0.02	0.303	0.135	0.168
-0.015	0.298	0.135	0.163
0.005	0.298	0.13	0.168
0.03	0.288	0.13	0.158
0.04	0.288	0.125	0.163
0.045	0.278	0.12	0.158
0.06	0.273	0.12	0.153
0.075	0.273	0.115	0.158
0.08	0.268	0.115	0.153
0.09	0.263	0.115	0.148
0.1	0.263	0.11	0.153
0.115	0.253	0.105	0.148
0.13	0.253	0.1	0.153
0.135	0.247	0.1	0.147
0.15	0.242	0.1	0.142
0.16	0.237	0.1	0.137
0.185	0.232	0.1	0.132
0.215	0.227	0.1	0.127
0.22	0.222	0.1	0.122
0.225	0.217	0.095	0.122
0.235	0.212	0.095	0.117
0.245	0.212	0.09	0.122
0.265	0.212	0.085	0.127
0.285	0.212	0.075	0.137
0.29	0.212	0.07	0.142
0.295	0.207	0.07	0.137
0.32	0.197	0.065	0.132
0.34	0.192	0.065	0.127
0.36	0.187	0.065	0.122
0.385	0.187	0.06	0.127
0.4	0.182	0.06	0.122
0.42	0.177	0.06	0.117
0.44	0.177	0.055	0.122
0.455	0.177	0.05	0.127
0.47	0.172	0.05	0.122
0.48	0.167	0.05	0.117
0.485	0.167	0.045	0.122
0.495	0.162	0.04	0.122
0.515	0.162	0.035	0.127
0.53	0.162	0.035	0.127

0.54	0.157	0.03	0.127
0.56	0.141	0.03	0.111
0.575	0.136	0.03	0.106
0.585	0.131	0.03	0.101
0.595	0.126	0.03	0.096
0.63	0.121	0.03	0.091
0.675	0.116	0.03	0.086
0.695	0.111	0.03	0.081
0.71	0.106	0.03	0.076
0.725	0.101	0.03	0.071
0.75	0.091	0.03	0.061
0.775	0.086	0.03	0.056
0.795	0.086	0.025	0.061
0.815	0.081	0.025	0.056
0.83	0.076	0.025	0.051
0.855	0.071	0.025	0.046
0.87	0.066	0.025	0.041
0.915	0.056	0.025	0.031
0.98	0.051	0.025	0.026
1.01	0.051	0.02	0.031
1.02	0.04	0.02	0.02
1.04	0.035	0.02	0.015
1.065	0.03	0.02	0.01
1.09	0.03	0.015	0.015
1.145	0.025	0.015	0.01
1.19	0.025	0.01	0.015
1.26	0.025	0.005	0.02
1.385	0.02	0.005	0.015
1.525	0.015	0.005	0.01
1.67	0.01	0.005	0.005
1.845	0.005	0.005	0
2.205	0.005	0	0.005
3.46	0	0	0

BIOGRAPHY

NAME	Ms.Huabei Wu
DATE OF BIRTH	10/02/1983
PLACE OF BIRTH	Guangxi Zhuang Autonomous Region - China
ADDRESS	Room 702, Building 11, Yunxing Qianlong Tianxia, No. 26-2, Zhuxi Avenue, Qingxiu District, Nanning, Guangxi Zhuang Autonomous Region, China
POSITION	Master
PLACE OF WORK	Guangxi Medical University, No. 22 Shuangyong Road, Qingxiu District, Nanning, Guangxi Zhuang Autonomous Region, China
EDUCATION	2003 bachelor degree of medicine Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 2010 master degree of medicine Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 2024 Doctor degree of Public Health Mahasarakham University, Thailand
Research grants & awards	1. Guangxi Higher Education Undergraduate Teaching Reform Project: exploration and practice of building online and offline hybrid teaching mode under the background of "Golden Course", project number:2020JGB156, funding: 10000 yuan, under research. 2. The teaching ability development project for young teachers of Guangxi Medical University: the research and practice of building an online and offline hybrid "golden course" based on MOOC platform, project number: 02506220036X, funding: 3000 yuan, under research. 3. Key teaching material construction project of Guangxi Medical University: community medicine, project number: 02605004065X, fund: 5000 yuan, under research. 4. The youth fund project of Guangxi Medical University, project number: GXMUYSF201537, S100A8 and S100A9, and the association study of essential hypertension, fund: 20000 yuan, completed. 5. Education and teaching reform project of Guangxi Medical University, project No.: 2016XJGA09, discussion and evaluation on teaching scheme of community diagnosis practice course for general medical students, 2000 yuan, completed.

Research output

[1] Wu Huabei, Li H, Zhang HY. Expression and significance of vascular cell adhesion factor 1 in peripheral blood of patients with essential hypertension Journal of Cardiopulmonary Vascular Diseases, 2017,36 (5): 338-340 (Chinese domestic journals)

[2] Wu Huabei, Li Hong Evaluation and result analysis of community diagnosis practice teaching by general practice medical students Guangxi Education 2017,01(12):67-68 (Chinese domestic journals)

[3]Ouyang Y, Wu H, Tan A,et al.E-selectin gene polymorphism (A561C) and essential hypertension Meta-analysis in the Chinese population[J]. Herz, 2015,40 Supply 2 (1): 197-202. (Co-first author)

[4] Wu Huabei Study on the relationship between CTLA-4, mRNA expression, rs231775 polymorphism and essential hypertension [D]. Guangxi Medical University, 2013 (Chinese domestic journals)

[5] Wu Huabei, Zhang Haiying, Yang Hong, Tan Aihua, Lin Baojing, He Yunyan, Hu Yanling, Qin Qiulan, Li Hong. Expression and significance of cytotoxic T lymphocyte associated antigen 4 in peripheral blood of patients with primary hypertension [J]. Shandong Medicine, 2012, 52 (29): 7-9 (Chinese domestic journals)

[6] Wu Huabei, Li Hong, Chen Enran, Wei Siyu, Zuo Yanli, Application and evaluation of online and offline hybrid teaching mode based on SPOC [J]. China's higher medical education, accepted (Chinese domestic journals)

[7] Yin Shijie, Wu Wei, Qin Guanbin, Cai Yuanchun, Zhuo Jinyi, Wu Huabei. The effect of phased teaching model in clinical anesthesia practice teaching [J]. Clinical Medical Research and Practice, 2020,5 (04): 182-183. (Corresponding author) (Chinese domestic journals)

[8] Wu Huabei, Li Hong, Wei Siyu, Chen Enran, Kong Yan, Zuo Yanli. Exploration and Practice on the construction of first-class undergraduate courses guided by the competency of grassroots general practitioners [J]. Progress in education, 2023, 13 (7): 4473-4478 Doi: 10.12677/ae.2023.137703(Chinese domestic journals)

[9] Wu H , Yin S .Relationship of Toll-Like Receptors 2 and 4 Gene Polymorphisms with Essential Hypertension in Chinese Han Population[J].Bioscience and medicine, 2023, 11(2):53-63.