Polymorphisms analysis for association between ADIPO signaling pathway and genetic susceptibility to T2DM in Chinese Han population

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ABSTRACT

The aim of the present study is to explore the relationship between ADIPO signalling pathway and T2DM, to provide clues for further study of the pathogenesis of T2DM and to determine the possible drug targets. This study employed a case-control study design. Twenty-three single nucleotide polymorphisms (SNPs) of 13 genes in the selected ADIPO signalling pathway were genotyped by SNPsScan kit. All statistical analysis was performed by SPSS 25.0, PLINK 1.07, R 2.14.2, Haploview 4.2, SNPstats, and other statistical software packages. In the association analysis based on a single SNP, rs1044471 had statistical significance in the overdominant model without adjusting covariates. Rs1042531 had statistical significance in the overdominant model. Rs12718444 had statistical significance in the recessive model. There was a linkage disequilibrium between the loci within 9 genes, and the two loci in RXRA gene did not form blocks. Four kernel functions were used for SNPs set analysis based on ADIPO signalling pathway showed that there was no statistical significance whether covariates were added or not, P>0.05. According to our research results, it is found that some single nucleotide polymorphisms (ADIPO2 rs1044471, PCK1 rs1042531, GLUT1 rs12718444) in the adiponectin signalling pathway may be associated with T2DM

1. Introduction

The 9th edition of the Diabetes Map released by the International Diabetes Federation shows that the prevalence of diabetes among adults aged 20–79 in the world reached 9.3% in 2019, indicating that about 463 million adults worldwide suffer from diabetes; China has the largest number of diabetes patients in the world, with an estimated 116.4 million, and is expected to reach 147.2 million by 2045 [1]. T2DM is a metabolic disease caused by the interaction of environmental factors and genetic factors [2]. T2DM not only causes serious psychological and physical pain to patients and nurses, but also brings enormous social and economic pressure to individuals and considerable losses to the global health economy [3].

Adiponectin (ADIPO) is an adipocytokine secreted mainly by adipocytes, first described in 1995 [4], [5]. It is found to be negatively correlated with visceral adiposity [6]. The human ADIPO gene (ADIPOQ) was cloned by sequencing human adipose tissue cDNA library [7]. Human ADIPO consists of 244 amino acids with a relative molecular weight of 30 KD and is located on chromosome 3q27 [8,9]. The human chromosome 3q27 has been shown to be a region carrying a susceptibility gene for T2DM [10]. There are three types of ADIPO receptors (ADIPOR): ADIPOR1 (abundantly expressed in skeletal muscle), ADIPOR2 (expressed in liver tissue), and T-cadherin (predominantly found in the heart and arteries) [11].

Civitarese [12] et al. have revealed that ADIPOR1 and ADIPOR2 isoforms may be important therapeutic targets for improving insulin sensitivity in patients with T2DM or in individuals at risk of developing the disease. ADIPO has a variety of important biological functions, which may improve insulin sensitivity in insulin target tissues, modulate inflammatory responses, and plays a crucial role in oxidative stress, atherosclerotic processes, and the regulation of energy metabolism [13,14].

The molecular signal transduction of ADIPO is activated by AMP-activated protein kinase (AMPK), PPARα, and p38 mitogen-activated protein kinase
(MAPK) signalling pathways [15]. Yoon [16] et al. have provided evidence that ADIPO enhances fatty acid oxidation in muscle cells by stimulating PPAR transcriptional activity via the sequential activation of AMPK and p38MAPK. AMPK is a serine/threonine protein kinase, known as the ‘energy receptor’, which plays a key role in the balance of energy metabolism in body [17,18]. PPARα governs the expression of numerous genes involved in nearly every single aspect of lipid metabolism, including fatty acid uptake, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, and formation and breakdown of triglycerides and lipid droplets [19]. P38MAPK is a type of mitogen activated protein kinases (MAPKs), it consists of 360 amino acids with a molecular weight of 38 KD [20]. The p38MAPK signalling pathway is the junction or common pathway of cellular signalling [21]. There are still many unknown problems in the signal transduction pathway of ADIPO, such as the upstream signal molecules of p38MAPK and AMPK are not clear. Existing studies have shown that adiponectin signalling pathway plays a regulatory role in insulin signalling pathway and can cause insulin resistance [10,22].

In this study, we explored the relationship between ADIPO signalling pathway and T2DM, to provide clues for further study of the pathogenesis of T2DM and to determine the possible drug targets.

2. Materials and methods

2.1. Study population

1092 T2DM cases and 1092 health controls were recruited according to the inclusion criteria. The patients came from 8 people’s hospitals including Maoming City, Shaoguan City, Dongguan Houjie, Shenzhen Longhua, Shenzhen Nanshan, Shenzhen Guanlan, Shenzhen Xixiang and Shenzhen Futian, as well as 10 endocrinology departments of Affiliated Hospital of Guangdong Medical College and Dongguan Shilong Boai Hospital. The case group was adopted the 1999 WHO diabetes diagnostic criteria. The control group was consisted of healthy people with non-type 2 diabetes diagnosed by the same diagnostic criteria at the same hospital at the same time as the case group. We matched the case group to the control group by region and age. Selection criteria for control group: (1) Age between 20 and 70, (2) No family genetic history of diabetes, (3) The medical history, physical examination, blood glucose examination and other biochemical results showed no abnormality.

2.2. Information collection and blood sample collection

The subjects were surveyed by qualified professional investigators, including general information such as age and gender. Height and weight are measured to calculate BMI. Blood pressure and heart rate are measured by an electronic sphygmomanometer. Endocrinology nurses collected 5 ml of peripheral blood from healthy subjects and patients respectively in the morning to detect clinical biochemical indicators including FPG, TC, TG, HDL-C, and LDL-C. In addition, 4 ml of peripheral blood of the subjects (2 ml per tube) was taken and anticoagulated with EDTA-k2 and stored at -80°C.

2.3. Data collation and database establishment

All completed questionnaires were uniformly coded, and all participants’ questionnaire information, physical examination, and clinical biochemical examination results were compiled. Use EpiData 3.1 software to build a database and enter data by double input. The entered data is checked by both manual and computer methods to ensure that the data has no logic errors and no entry errors.

2.4. DNA extraction

Subjects need to be fasted for 8 hours before blood collection by a professional nurse. Blood samples were treated with dipotassium dihydrogen ethylenediaminetetraacetate (EDTA-K2). Protease K was used for digestion, and DNA was extracted by salting out method.

2.5. Screening and typing of SNPs

A pathway map of the ADIPO signalling pathway was obtained from the KEGG database to identify 13 major genes. Their upstream and downstream 5kb regions using Hapoloview (ver.4.2). Then use FastSN to select 1–2 high scores tagSNP for each gene. Finally, 23 tagSNPs were selected from 13 genes. The SNPscanTM multiple SNP typing technology was used to classify the selected labelled SNPs. The basic principle of this technique is to use the high specificity of ligase ligation reaction to realize the recognition of SNP locus alleles. Then by introducing non-specific sequences of different lengths at the end of the connection probe and by
ligase addition reaction, the corresponding ligation products of different lengths were obtained. The ligation products were amplified by PCR with labelled fluorescent universal primers. The amplified products were separated by fluorescence capillary electrophoresis. Finally, the genotypes of each SNP site were obtained by electrophoresis analysis. In the Chinese population, the minimum MAF is 0.051 (rs2744537), the maximum is 0.442 (rs4982856), the relevant information of each SNP is shown in Table 1.

### 2.6. Statistical analysis

In the process of comparing all variables between the case group and the control group, the normal quantitative data were expressed as \( \bar{x} \pm s \), and the counting data were expressed as the number of cases or percentage. The differences in continuous variables between the two groups were tested by Student’s \( t \)-test. Comparison of categorical variable data between the two groups was tested by \( \chi^2 \) test. Genotype and allele frequency were compared by \( \chi^2 \) test. Pearson chi-square test, Cochran-Armitage trend test, MAX3 and logistic regression were used to analyse the association between single SNP and T2DM; unconditional logistic regression was used to analyse haplotype in LD block; and SNPs set analysis based on logistic kernel machine regression was used to analyse pathway. All statistical analysis was performed by SPSS25.0, PLINK 1.07, R 2.14.2, Haplview 4.2, SNPstats and other statistical software packages.

### 3. Results

#### 3.1 The baseline data

After excluding cases with missing information, 1,067 people in the case group and 1,054 people in the control group were included in the analysis. The average age, body mass index (BMI), FPG, TG, and LDL-C of the case group were higher than those of the control group, and the difference was statistically significant \((P < 0.05). See Table 2 for details.

#### 3.2 SNPs typing results

The success rate of 23 SNPs was above 98%, and the minimum allele frequency was 0.016 and the maximum was 0.476. The Hardy-Weinberg equilibrium test shows that each point satisfies the Hardy-Weinberg equilibrium. The results showed that the SNPs loci in this study were representative of the population \((P > 0.01)\). See Table 3 for details.

#### 3.3 Allele association analysis results

The results of allele association analysis are shown in Table 4. There was no significant difference in the sub-allele frequency of each SNP between the case group and the control group. After adding age, BMI, and other covariate corrections, the sub-allele frequency of each SNP in the case group and the control group still had no statistical difference.

| Gene | Chr | Position, 37 | SNP | Region | Allele | MAF |
|------|-----|--------------|-----|--------|--------|-----|
| ADIPOQ | 3 | 186,559,474 | rs266729 | 5'upstream | C | 0.300 |
| ADIPOQ | 3 | 186,561,634 | rs16661205 | intron1 | G | 0.179 |
| ADIPOQ | 1 | 202,914,356 | rs1342387 | intron4 | T | 0.399 |
| ADIPOQ | 1 | 202,922,040 | rs12733285 | intron1 | C | 0.069 |
| ADIPOQ | 12 | 1,889,823 | rs767870 | intron5 | G | 0.084 |
| ADIPOQ | 12 | 1,896,956 | rs1044471 | 3'UTR | C | 0.427 |
| PPARA | 22 | 46,598,307 | rs4823613 | intron4 | A | 0.217 |
| PPARA | 22 | 46,621,994 | rs5767743 | intron7 | T | 0.237 |
| PCK1 | 20 | 56,140,980 | rs1042531 | 3'UTR | T | 0.206 |
| PCK1 | 20 | 56,131,216 | rs11906628 | exon7 | G | 0.230 |
| PCK2 | 14 | 24,569,418 | rs2301336 | exon4 | C | 0.442 |
| PCK2 | 14 | 24,563,212 | rs982856 | intron2 | A | 0.139 |
| G6PC | 17 | 41,056,245 | rs2593959 | intron18 | G | 0.144 |
| ACC2 | 12 | 40,643,645 | rs2268388 | intron2 | A | 0.401 |
| GLUT1 | 1 | 43,399,686 | rs7354219 | intron1 | G | 0.150 |
| GLUT1 | 1 | 43,409,179 | rs12718444 | intron1 | C | 0.352 |
| GLUT4 | 17 | 7,187,123 | rs5435 | exon4 | T | 0.248 |
| CPT-1 | 11 | 68,593,258 | rs11228368 | intron1 | G | 0.368 |
| RXRA | 9 | 137,259,992 | rs11185660 | intron1 | T | 0.144 |
| RXRB | 9 | 137,335,311 | rs1045570 | 3'UTR | T | 0.212 |
| RXRB | 6 | 33,162,215 | rs2744537 | 5'upstream | C | 0.051 |
| RXRB | 6 | 33,166,034 | rs2076310 | intron3 | A | 0.426 |

**Table 1. Basic information of 23 tagSNPs selected from 13 genes in the ADIPO signalling pathway.**

**Abbreviation:** Chr, chromosome number; SNP, single nucleotide polymorphism; MAF, minor allele frequency.
### 3.4 Genotype association analysis results

There was no statistical difference in the genotype distribution of each SNP between the case group and the control group, as shown in Table 5.

To further confirm whether each SNP is associated with T2DM, whether the probability of disease increases with the increase of the number of risk alleles in the genotype, we have made Cochran-Armitage trend test under different genetic models (additive model, codominant model, dominant model, recessive model and overdominant model). Rs1044471 was statistically significant in the overdominant model, $P_{obs} = 0.030$, and the OR of genotype CT relative to TT-CC was 1.21, 95% CI (1.02–1.43). Rs1042531 was statistically significant in the overdominant model, $P_{obs} = 0.038$, and the OR of genotype GT relative to TT-GG was 1.20, 95% CI (1.02–1.44). In the recessive model of rs12718444, TT genotype was a protective factor compared with GG-GT genotype, $P_{obs} = 0.043$, OR = 0.56, 95% CI (0.32–0.99). The results were shown in Table 6.

To control for confounding factors, covariates (Age, BMI, Sex, and FPG) were added to the different genetic models for adjusting (Table 7). Rs1044471 was not statistically significant under the five models. Rs1042531 was still statistically significant in the overdominant model, $P_{adj} = 0.044$, and the OR of genotype GT relative to TT-GG was 1.21, 95% CI (1.02–1.45), under the codominant model, TG genotype was a protective factor compared with TT genotype, $P_{adj} = 0.044$, OR = 1.21, 95% CI (1.01–1.45), and genotype GG was not statistically significant relative to genotype TT, $P_{adj} = 0.101$, OR = 0.98, 95% CI (0.71–1.36).

| Gene  | SNP      | MinorAllele | MajorAllele | Call Rate (%) | MAF | $P_{HWE}$ |
|-------|----------|-------------|-------------|---------------|-----|-----------|
| ADIPOR1 | rs16681205 | A | G | 98.37 | 0.157 | 0.741 |
| ADIPOR2 | rs12733285 | C | T | 98.37 | 0.078 | 0.833 |
| PPARA  | rs4823613  | G | A | 98.37 | 0.137 | 0.023 |
| PKC1   | rs1042531  | G | T | 98.37 | 0.281 | 0.038 |
| GLUT1  | rs11908628 | A | G | 98.34 | 0.282 | 0.261 |
| G6PC   | rs4982856  | C | T | 98.37 | 0.218 | 0.999 |
| ACC2   | rs2268388  | A | G | 98.29 | 0.241 | 0.058 |
| GLUT4  | rs5435     | C | T | 98.37 | 0.366 | 0.643 |
| CPT-1  | rs11228368 | G | A | 98.37 | 0.150 | 0.028 |
| RXRA   | rs11185660 | C | T | 98.37 | 0.218 | 0.059 |
| RXRB   | rs1045570  | G | T | 98.37 | 0.173 | 0.731 |

Abbreviation: SNP, single nucleotide polymorphism; MAF, minor allele frequency; $P_{HWE}$, values of the Hardy-Weinberg test for each SNP.
3.5 Linkage disequilibrium analysis and association analysis based on haplotype

Linkage disequilibrium (LD) analysis was performed between different sites within the same gene using Haplovie 4.2 software. It was found that there was a linkage disequilibrium between the sites within 9 genes such asADIPOQ, and the two loci in RXRA gene did not form blocks. Figure 1 shows the composition of the LD blocks of these 10 genes in turn.

Unconditional logistic regression analysis of haplotypes in LD blocks were performed using SNPstats online software. The analysis results were shown in Table 9. There were no statistically significant positive results for haplotypes in LD blocks in each gene.

3.6 SNPs – SNPs interaction results

We uploaded 13 genes from the ADIPO signalling pathway to the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) tool. The interaction between proteins encoded by these genes was analysed and the results were shown in Figure 2.

3.7 Pathway analysis results

Four kernel functions, such as linear, linear-weighted, identical-by-state (IBS), and IBS-weighted, were used for SNPs set analysis based on ADIPO signalling pathway showing that there was no statistical significance whether covariates were added or not, \( P > 0.05 \), the results were shown in Table 10. The empirical \( P \) value obtained by the bootstrap method had no statistical significance.

4. Discussion

In recent years, T2DM susceptibility and gene polymorphisms have been widely studied. Multiple gene SNPs in the adipocytokine signalling pathway have been shown to be significantly associated with the risk of developing T2DM, for example, 3 SNPs (rs10789038, rs2796498, and rs2746342) of the PPARA gene [23,24], 3 SNPs (rs1800206, rs4253776 and rs4253778) of the PPARC gene in the ADIPOQ signalling pathway [25] and 5 SNPs (rs1501299, rs17300539, rs2241766, rs266729 and rs16861194) of the ADIPOQ [26–29]. More and more evidences show that the study of gene polymorphisms is beneficial to the clinical diagnosis and treatment of diseases. Anna Maria Jung found that two SNPs (SOS1 rs2888586 and CDK4 rs2069502) were significantly associated with response to recombinant human growth hormone (rhGH) treatment [30]. Genetic variations are potentially suitable as predictive markers of rhGH treatment response in growth hormone deficiency. There is a study that has found an association between SNPs of some risk genes and the effect to antipsychotic therapy [31]. In the future, this means that patients may be able to select the most appropriate antipsychotic drug after testing these SNPs. At the same time, gene polymorphisms may
provide clues for further study of the pathogenesis of T2DM and search for new drug targets.

Rs1042531 is located in the 3’UTR of PCK1 gene on chromosome 20. PCK1, also known as cytoplasmic phosphoenolpyruvate pyruvate (PEPCK-C), is a multifunctional gene related to glycogen isogenesis, glycerol isogenesis, reproduction and female fertility, obesity and diabetes [32]. PCK1 gene is highly expressed in adipocytes, and a radioactive imprint study indicates that PCK1 in white adipose tissue is involved in glycerol xenobiotics [33,34]. Due to the lack of glycerol kinase in adipocytes, glycerol released by triglyceride degradation cannot be phosphorylated, and 3-phosphoglycerol necessary for free fatty acid re-esterification is a precursor substance derived from gluconeogenesis. Devine [35] et al. believe that PCK1 is also the rate-limiting enzyme in glycerol xenobiotics. Overexpression of PCK1 gene in adipocytes may be associated with obesity and insulin resistance. PCK1 gene may be one of the important susceptibility genes related to T2DM. Any abnormality in the kinase product produced at the transcriptional or translational level may lead to diabetes. Vimalaswaran [36] et al. have found that PCK1 gene polymorphism is not associated with obesity in European adolescents. Rees [37] et al. have discovered that rs1042531 is not associated with T2DM in South Asian populations. However, Jablonski [38] et al. have found that rs1042531 is associated with T2DM through GWAS research. This suggests that the locus is highly heterogeneous and varies by race or even by country. In this study, the association between PCK1 rs1042531 and T2DM was further studied in Chinese Han population samples. Since the microRNA binds to the 3’UTR of the gene, the expression of the gene is regulated, and the rs1042531 site is located at the 3’UTR of the PCK1 gene. We performed target microRNA prediction on the position of the rs1042531 site of the PCK1 gene by the online software of miRNASNP (http://www.bioguo.org/miRNASNP/). We found that when the rs1042531 site T is mutated to a G base, the A base of the miR-1178 seed sequence region cannot be matched, thereby affecting the binding of miR-1178 to the PCK1 gene and regulating the expression of the PCK1 gene. Therefore, in the next functional experimental study, we will verify by experimental methods such as the construction of luciferase reporter vector.

Rs12718444 is located in the first intron region of GLUT1 gene on chromosome 1. GLUT1 is an important member of the GLUTs family, providing many cells with their basic glucose requirements, and it is a major transporter across the blood–brain barrier [39]. Because T2DM is characterized by persistent and abnormal extracellular hyperglycaemia [40], the relationship between them may be very close. Up to now, there is no report on rs12718444, so it needs to be validated by an independent population. Because the rs12718444 locus is located in the intron region of the gene, its function is unknown, it may be linked with other nearby gene SNPs or may affect the splicing of mRNA, thus affecting the function of proteins, which need to be further verified in subsequent studies. ADIPOQ has a protective effect on liver dysfunction in obesity, T2DM, and other insulin resistance states, and

Table 5. Comparison of genotype frequencies between case group and control group in the ADIPO signalling pathway.

| Gene   | SNP          | Genotype | non-diabetic controls | T2DM patients | \( \chi^2 \) | P-value |
|--------|--------------|----------|-----------------------|---------------|-------------|---------|
| ADIPOQ | rs266729     | CC/CG/GG | 591/402/61            | 580/422/65    | 0.636       | 0.728   |
|        | rs16861205   | GG/GA/AA | 735/293/26            | 774/273/20    | 2.418       | 0.299   |
| ADIPOR1| rs1342387    | CC/CT/TT | 456/486/112           | 434/506/127   | 1.809       | 0.405   |
|        | rs12733285   | CC/CT/TT | 896/153/5             | 912/146/9     | 1.369       | 0.504   |
| ADIPOR2| rs767870     | AA/AG/GG | 800/226/28            | 796/254/17    | 4.253       | 0.119   |
|        | rs1044471    | AA/AG/GG | 396/480/178           | 371/336/160   | 4.781       | 0.092   |
| PPARA  | rs4823613    | AA/AG/GG | 622/366/66            | 622/393/52    | 1.535       | 0.464   |
|        | rs5767743    | TT/TC/CC | 636/366/52            | 651/386/53    | 0.117       | 0.943   |
| PCK1   | rs1042531    | TT/TT/GG | 563/396/95            | 533/448/86    | 4.393       | 0.111   |
|        | rs11908628   | AA/AG/GG | 532/445/77            | 525/460/81    | 0.328       | 0.849   |
| PCK2   | rs2301336    | AA/AG/GG | 656/353/45            | 626/388/53    | 2.929       | 0.231   |
|        | rs4982856    | CC/CT/TT | 303/526/223           | 269/569/229   | 3.665       | 0.160   |
| G6PC   | rs2539395    | AA/AG/GG | 741/292/21            | 723/313/31    | 2.831       | 0.243   |
| ACC2   | rs2268388    | GG/GA/AA | 628/354/69            | 619/385/63    | 1.517       | 0.468   |
| GLUT1  | rs3754219    | AA/AC/CC | 419/485/150           | 438/476/153   | 0.456       | 0.796   |
|        | rs12718444   | GG/CT/TT | 773/248/33            | 772/276/19    | 5.187       | 0.075   |
| GLUT4  | rs5435       | CC/CT/TT | 429/509/116           | 428/513/126   | 0.350       | 0.839   |
|        | rs16956647   | CC/CT/TT | 558/436/58            | 583/415/68    | 1.767       | 0.413   |
| CPT-1  | rs1122368    | AA/AG/GG | 568/420/66            | 582/423/62    | 0.226       | 0.693   |
| RXRA   | rs11185660   | TT/TC/CC | 725/307/22            | 736/303/28    | 0.749       | 0.687   |
| RXRB   | rs2744537    | CC/CA/AA | 1019/35/0             | 1036/30/1     | 0.457       | 0.499   |
|        | rs2076310    | GG/GA/AA | 425/487/142           | 422/509/135   | 0.606       | 0.739   |
ADIPOR2 is mainly expressed in liver [41]. The common SNPs in ADIPOR2 (rs1044471) were associated with differences in liver function in the population. The human body may be able to increase circulating ADIPO through some negative regulation, thereby ameliorating the ADIPOR2 gene variant (rs1044471) resulting in a decrease in insulin sensitivity [42]. Our findings also proved that ADIPOR2 rs1044471 may be related to the occurrence and development of T2DM, which further supported the research results of Martine Vaxillaire [43].

The premise of this study is that existing studies have found that ADIPO is closely related to energy metabolism and susceptibility to type 2 diabetes, while the specific function of ADIPO signal transduction pathway in T2DM is still unclear. According to our research results, it is found that some single nucleotide polymorphisms (ADIPOR2 rs1044471, PCK1 rs1042531, GLUT1...
rs12718444) in the adiponectin signalling pathway may be associated with T2DM. Linkage disequilibrium analysis and haplotype-based association analysis showed that there was a linkage disequilibrium between the two loci in 9 genes such as ADIPOQ in the pathway. This is a preliminary independent sample verification for Chinese Han population, and its results can provide clues to whether ADIPO has a difference in correlation with T2DM due to ethnic heterogeneity. Therefore, it provides a partial research basis for further studying the pathogenesis of T2DM and looking for possible drug targets. We will also analyse the molecular mechanisms in subsequent studies to clarify the pathogenesis of diabetes from a genetic point of view.

In this study, 1067 subjects were included in the case group and 1054 subjects in the control group. The sample size is medium. In consideration of bias, cases from ten different hospitals were selected, and the samples were representative. However, the

| Gene   | SNP        | $\chi^2$ | P-value |
|--------|------------|----------|---------|
| ADIPOQ | rs266729   | 0.794    | 0.694   |
|        | rs16861205 | 1.542    | 0.223   |
| ADIPO1 | rs1342387  | 1.345    | 0.326   |
| ADIPO2 | rs767870   | 1.700    | 0.190   |
|        | rs1044471  | 1.342    | 0.332   |
| PPARA  | rs4823613  | 1.395    | 0.321   |
|        | rs5767743  | 0.316    | 0.943   |
| PCK1   | rs1042531  | 1.600    | 0.215   |
|        | rs1908628  | 0.564    | 0.843   |
| PCK2   | rs2301336  | 1.688    | 0.188   |
|        | rs4982856  | 1.835    | 0.144   |
| G6PC   | rs2593595  | 1.541    | 0.242   |
| ACC2   | rs2268388  | 0.813    | 0.638   |
| GLUT1  | rs3754219  | 0.608    | 0.794   |
|        | rs12718444 | 2.010    | 0.097   |
| GLUT4  | rs5433     | 0.582    | 0.816   |
|        | rs16956647 | 0.842    | 0.652   |
| CPT-1  | rs11228368 | 0.437    | 0.903   |
|        | rs11185660 | 0.815    | 0.700   |
| RXRA   | rs1045570  | 1.547    | 0.240   |
| RXRB   | rs2744537  | 0.994    | 0.997   |
|        | rs2076310  | 0.552    | 0.828   |

Figure 1. Results of LD analysis of 10 genes in ADIPO signalling pathway. LD analysis showed that the probability of co-existence of two alleles in the target gene was greater than the probability of co-occurrence due to random distribution in the population. Figure 1 shows the LD block composition of these 10 genes in turn, and two loci in RXRA gene did not form LD block.
Table 9. Results of haplotype unconditional logistic regression analysis of 9 genes LD block in ADIPO signalling pathway.

| Gene     | SNP         | SNP         | Freq | OR (95% CI)       | P-value |
|----------|-------------|-------------|------|-------------------|---------|
| ADIPOQ   | rs16861205  | G           | rs266729 | C | 0.591 | 1.00   | –  |
|          |             | G           | rs266729 | C | 0.254 | 1.05 (0.91–1.22) | 0.510 |
|          |             | A           | rs1342387 | C | 0.155 | 0.90 (0.75–1.07) | 0.241 |
| ADIPOR1  | rs12733285  | C           | rs1342387 | C | 0.652 | 1.00   | –  |
|          |             | T           | rs1342387 | T | 0.271 | 1.09 (0.95–1.26) | 0.221 |
|          |             | T           | rs1342387 | T | 0.076 | 1.08 (0.85–1.37) | 0.542 |
| ADIPOR2  | rs1044471   | C           | rs767870  | A | 0.468 | 1.00   | –  |
|          |             | T           | rs767870  | A | 0.398 | 1.01 (0.88–1.15) | 0.933 |
| PPARA    | rs4823613   | A           | rs576743  | T | 0.714 | 1.00   | –  |
|          |             | A           | rs576743  | G | 0.134 | 1.00 (0.83–1.21) | 0.982 |
|          |             | C           | rs576743  | C | 0.170 | 0.97 (0.83–1.15) | 0.761 |
|          |             | G           | rs576743  | T | 0.065 | 0.99 (0.76–1.28) | 0.910 |
| PCK1     | rs1042531   | T           | rs11908628 | A | 0.450 | 1.00   | –  |
|          |             | G           | rs11908628 | G | 0.266 | 1.13 (0.96–1.32) | 0.151 |
|          |             | T           | rs11908628 | T | 0.262 | 1.15 (0.98–1.34) | 0.087 |
|          |             | A           | rs11908628 | A | 0.051 | 1.04 (0.78–1.38) | 0.811 |
| PCK2     | rs2301336   | A           | rs4982856 | C | 0.525 | 1.00   | –  |
|          |             | T           | rs4982856 | T | 0.254 | 1.05 (0.90–1.22) | 0.522 |
|          |             | G           | rs4982856 | G | 0.218 | 1.12 (0.95–1.31) | 0.171 |
| GLUT1    | rs12718444  | G           | rs3754219 | A | 0.486 | 1.00   | –  |
|          |             | T           | rs3754219 | A | 0.366 | 0.97 (0.85–1.11) | 0.691 |
| GLUT4    | rs16956647  | C           | rs5435   | C | 0.389 | 1.00   | –  |
|          |             | T           | rs5435   | T | 0.351 | 1.03 (0.89–1.19) | 0.733 |
|          |             | C           | rs5435   | C | 0.256 | 0.98 (0.84–1.15) | 0.821 |
| RXRB     | rs2076310   | G           | rs2744537 | C | 0.634 | 1.00   | –  |
|          |             | A           | rs2744537 | C | 0.350 | 0.97 (0.85–1.10) | 0.610 |
|          |             | A           | rs2744537 | A | 0.016 | 0.92 (0.56–1.50) | 0.733 |

Figure 2. The interaction map of 13 genes in adipo signalling pathway.

(1) Network nodes represent proteins: Each node in the figure represents a protein due to variable splicing and post-transcriptional modification in eukaryotes. The letter marked on the node is the gene symbol of the corresponding gene. (2) Edges represent protein-protein associations: The line between nodes represents an interaction between two proteins. Associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function. Different colours correspond to different types of interactions. As you can see from the figure, there is more than one line between the two proteins, indicating that there are multiple interactions between the two proteins. Among all the correlations, there are both experimental verification and data prediction results. The width of the lines represents the degree of interaction between proteins. The bold lines represent greater interactions between proteins. The value (combined score) is larger.
heterogeneity of different races was considered because the sample of this study is the only the Han population in Guangdong Province. The following cases of different races can be selected and the sample size can be increased to improve the credibility of the conclusion.

5. Conclusions

According to our research results, it is found that some single nucleotide polymorphisms (ADIPOR2 rs1044471, PCK1 rs1042531, GLUT1 rs12718444) in the adiponectin signalling pathway may be associated with T2DM.

Ethics Statement

This study passed the ethical review (Medical Ethics Committee of the Affiliated Hospital of Guangdong Medical University, No.PJ2012079, China.) All surveys and samples were obtained with the consent of participants in advance, and the informed consent forms were legally consented.

Disclosure statement

The authors have declared that there is no conflict of interest in the article.

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Author Contributions

Haibing Yu and Wei Hu contributed equally to this study. Wei Hu, Haibing Yu, Hao Liu, and Chen Yang participated in the collection of clinical biochemical data. Lin Xu, Chunwen Lin, Jialiu Huang, Ling Luo, and Rong Chen did data analysis. Yuanlin Ding and Danli Chen designed and directed the experiment.

Data Availability Statement

The data that support the findings of this study are openly available in 'figshare' at https://doi.org/10.6084/m9.figshare.15104412. The more detail of this study is available from the corresponding author upon reasonable request.

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