Association of SLC22A1, SLC22A2, SLC47A1, and SLC47A2 Polymorphisms with Metformin Efficacy in Type 2 Diabetic Patients

Peixian Chen¹,‡, Yumin Cao¹,§, Shenren Chen¹, Zhike Liu², Shiyi Chen¹,* and Yali Guo¹,*∥

1 Department of Endocrinology and Metabolism, the Second Affiliated Hospital of Shantou University Medical College, Shantou 515000, China
2 Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, Beijing 100191, China
* Correspondence: 17f2sychen2@stu.edu.cn (S.C.); guoyali@zgkhydhscyygm.onexmail.com (Y.G.)
† These authors contributed equally to this work.
‡ Current affiliation: Department of Endocrinology, Department of Infection Control, Meizhou People’s Hospital, Meizhou 514031, China.
§ Current affiliation: Department of Neurology, Meizhou People’s Hospital, Meizhou 514031, China.
∥ Current affiliation: Department of Endocrinology, University of Chinese Academy of Sciences Shenzhen Hospital, Shenzhen 518000, China.

Abstract: Response to metformin, first-line therapy for type 2 diabetes mellitus (T2DM), exists interindividual variation. Considering that transporters belonging to the solute carrier (SLC) superfamily are determinants of metformin pharmacokinetics, we evaluated the effects of promoter variants in organic cation transporter 1 (OCT1) (SLC22A1 rs628031), OCT2 (SLC22A2 rs316019), multidrug and toxin extrusion protein 1 (MATE1) (SLC47A1 rs2289669), and MATE2 (SLC47A2 rs12943590) on the variation in metformin response. The glucose-lowering effects and improvement of insulin resistance of metformin were assessed in newly diagnosed, treatment-naive type 2 diabetic patients of Han nationality in Chaoshan China (n = 93) receiving metformin. Fasting plasma glucose (FPG), fasting insulin (FINS), glycated hemoglobin A1 (HbA1C), homeostasis model assessment-insulin sensitivity (HOMA-β), and homeostasis model assessment-insulin resistance (HOMA-IR) were the main metformin efficacy measurements. There were significant correlations between both SLC47A1 rs2289669 and SLC47A2 rs12943590 and the efficacy of metformin in individuals with T2DM. In normal weight T2DM patients, significant associations between the AA and GG genotypes of the rs2289669 variant of SLC47A1 and a greater reduction in FINS and HOMA-IR were detected. A significant correlation was observed between the AG genotype of the rs12943590 polymorphism of SLC47A2 and a greater reduction in HOMA-IR. Gene–environment interaction analysis showed that in the FINS interaction model, the second-order of dose30_g-SLC47A2 rs12943590 was statistically significant. The variants of SLC47A1 rs2289669 and SLC47A2 rs12943590 could be predictors of insulin resistance in type 2 diabetic patients treated with metformin. The second-order interaction of dose30_g-SLC47A2 rs12943590 may have a significant effect on FINS in patients with T2DM on metformin treatment. These findings suggest that promoter variants of SLC47A1 and SLC47A2 are important determinants of metformin transport and response in type 2 diabetes mellitus.

Keywords: metformin; single-nucleotide polymorphism; pharmacogenetics; type 2 diabetes mellitus

1. Introduction

Metformin is the first-choice oral drug to control blood glucose, which can be used for monotherapy and combination therapy [1–3]. It can effectively reduce FPG, postprandial blood glucose (PPG), and HbA1C by inhibiting glycogen production in the liver [4–6]. Several genes that can regulate metformin response, such as SLC22A1 [7–10], SLC22A2 [11],
SLC47A1 [12,13], and SLC47A2 [14], have been identified by previous pharmacogenetic studies. Other genes, such as ATM [15–17], are involved in regulating metabolic enzymes.

A large number of new T2DM susceptible loci have been found by genome wide association studies in recent years. Several studies have evaluated the relationship between genetic variations, such as SLC22A1 rs628031, SLC22A1 rs622342, ATM rs11212617, SLC22A2 rs316019, SLC47A1 rs2289669, and SLC47A2 rs12943590, and the pharmacokinetics and clinical consequences of metformin with conflicting results [18–21].

Previous pharmacogenetic studies found metformin was equally effective among type 2 diabetic patients with a different body mass index (BMI) [22–25]. There are great individual differences in genetic polymorphism among different populations. Our previous findings that common variants of ATM rs11212617 and SLC22A1 rs622342 may be associated with the effects of metformin treatment on type 2 diabetic patients of Han nationality in Chaoshan China were published in the journal, Pharmacogenetics and Genomics [26].

OCT1 and OCT2, which are encoded by SLC22A1 and SLC22A2, respectively, involve the transfer of endogenous physiological amino compounds [10]. SLC47A1 and SLC47A2 genes may mediate the transport and excretion of metformin [27]. Some variants of these genes can affect the glycemic response to metformin. Studies in patients showed that rs628031 and rs316019 in the SLC22A1 and SLC22A2 genes, respectively, could exert a significant effect on the distribution and elimination of metformin [28,29]. SLC47A1 rs2289669 and SLC47A2 rs12943590 have been shown to influence the reduction of HbA1c in response to metformin treatment [30]. There are studies reporting the allele and genotype frequency of the four common variants (SLC22A1 rs628031, SLC22A2 rs316019, SLC47A1 rs2289669, and SLC47A2 rs12943590) in other populations; however, so far, there are no data available for the Han nationality in the Chaoshan area of China.

Hence, the current study was mainly aimed to determine the genotype and allele frequency of these four single nucleotide polymorphisms (SNPs), and investigate the relationship between the genetic variants of these four SNPs and the efficacy of metformin in T2DM patients of Chaoshan area Han population.

In addition, type 2 diabetes mellitus is a polygenic genetic disease. It is of great significance to study gene–gene and gene–environment interaction to guide personalized drug use. However, little is known about gene–environment and gene–gene interactions in the efficacy of metformin, such as FPG, HbA1C, FINS, insulin resistance (IR), and β-cell function. Therefore, the purpose of this study was to evaluate whether gene–environment and gene–gene interactions were related to the efficacy of metformin, and explore the predictive function of gene–environment and gene–gene interactions in the individual differences in metformin treatment of patients with type 2 diabetes mellitus.

2. Materials and Methods
2.1. Study Design and Patient Selection

This study was conducted in the Second Affiliated Hospital of Shantou University Medical College. Subjects must meet the following conditions at the same time to be eligible in this study. First, the diagnosis of diabetes mellitus was based on the World Health Organization (WHO) criterion [25]. Second, patients aged 30–65 years with newly diagnosed T2DM were eligible for the study, including both males and females. Third, although the lifestyle was changed, the appropriate fasting blood glucose level (FPG < 7.0 mmol/l) could not be reached. In addition, they had never received hypoglycemic therapy. Fourth, the participants were from the Chaoshan region of China. Fifth, the BMI of the subjects ranged from 18.5 to 30 kg/m². Sixth, patients were excluded if they had diabetes complications, endocrine disorders, chronic gastrointestinal disease, liver dysfunction, renal insufficiency, heart failure, myocardial infarction, systemic inflammatory disease, blood disease, surgery, malignancies, or corticosteroid treatment. Maternal, nursing women, alcoholics, and people who were allergic to metformin were also excluded.

Prior to participating in this study, all the patients provided a written informed consent. The research proposal was approved by the Ethics Committee of this hospital.
After enrolment, all the patients began to receive metformin treatment for two months. In this study, metformin was produced by Zhongmei Shanghai Shiguibao Pharmaceutical Company, located in Shanghai, China. The initial dose of metformin was determined to be 250 mg twice daily or three times daily. If the FPG was greater than 7.0 mmol/L after one month of treatment, the dose of metformin would be adjusted to 500 mg twice or three times daily for the following month.

At the first visit, participants were instructed by a trained physician to complete a questionnaire, which collected information on demographic characteristics, medical history, medication, and lifestyle factors (including sweets, tea drinks, smoking, and alcohol consumption). According to standard protocols, anthropometric parameters, such as height and weight, were measured. The BMI was obtained by dividing the weight (kg) by height squared (m²). An overnight (>10 h) fasting blood sample was used to test FPG, HbA1C, FINS, liver and renal function, and lipid profile. Diabetes education, including some suggestions on diet and exercise, as well as a brief introduction to T2DM, was provided to all the subjects.

During the two-month treatment period, patients underwent four clinical follow-up visits, respectively, on the 15th, 30th, 45th, and 60th days; and four telephone follow-up visits, respectively, on the 7th, 22nd, 37th, and 52nd days. A follow-up questionnaire on medication compliance and adverse reactions was completed by the same physician at each follow-up. FPG, HbA1C, FINS, liver and renal function, and lipid profile were measured at the end of this study, whereas on day 30, FPG was also detected to determine whether the dosage was adjusted or not.

2.2. Laboratory Methods

The glucose oxidase method was used to measure plasma glucose. Insulin was determined by radioimmunoassay. The blood lipid, liver function, and kidney function were analyzed by an automatic biochemical analyzer. Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood leukocytes by protein precipitation according to standard operation procedures. According to the technical specifications, the GenomeLab SNP flow genotyping system (Beckman Coulter Inc., Fullerton, California, United States of America) was used to determine the genotypes of SLC22A1 rs628031, SLC22A2 rs316019, SLC47A1 rs2289669, and SLC47A2 rs12943590. The insulin resistance index was calculated as the product of fasting plasma glucose (mmol/L) and fasting insulin (µU/mL), divided by 22.5. The insulin sensitivity index was calculated as 1 divided by the product of fasting plasma glucose (mmol/L) and fasting insulin (µU/mL).

2.3. Definition of Outcomes

Three kinds of outcomes were used to evaluate the relationship between four SNPs and metformin efficacy:

- The first outcome was the decrease in FPG on the 30th day or the 60th day (absolute value of the FPG decrease).
- The second outcome hinged on the decrease in HbA1C on the 60th day (absolute value of the HbA1C decrease).
- The third outcome was the decrease in FINS or HOMA-IR, or the increase in HOMA-IS on the 60th day (absolute value of the FINS or HOMA-IR reduction, or HOMA-IR increase).

2.4. Statistical Analysis

Continuous variables presented as mean and standard deviation (SD) were compared using t-test analysis among the groups. Categorical variables of which the frequency distribution was expressed in numbers (proportions) were compared by Pearson χ²-test among the groups. When the theoretical frequency was less than 5 or the sample size was less than 40, categorical variables were analyzed using the Fisher exact test. The Pearson χ²-test was used to assess the Hardy–Weinberg equilibrium for variants of SLC22A1 rs628031, SLC22A2 rs316019, SLC47A1 rs2289669, and SLC47A2 rs12943590. Regression analysis and t-test analysis...
were applied in genetic associations. Meanwhile, the interference of potential confounders, such as gender, BMI, FPG, FINS, and HbA1c, were adjusted. A generalized multifactor dimensionality reduction (GMDR) was used to analyze the correlation between gene–environment and gene–gene interactions, and various indicators of diabetes, such as FPG, HbA1c, FINS, HOMA-IR, and HOMA-IS. The changes of FPG, HbA1c, FINS, HOMA-IR, and HOMA-IS between the sixtieth day and the first day were expressed as \( \Delta FPG, \Delta HbA1c, \Delta FINS, \Delta HOMA-IR, \) and \( \Delta HOMA-IS \), respectively. The decline of FPG between the thirtieth day and the first day was represented with \( \Delta FPG \). Moreover, \( \Delta (60-30)FPG \) represented the change of FPG between the sixtieth day and the thirtieth day. There were several non-normally distributed variables, including \( \Delta FPG, \Delta (60-30)FPG, \Delta FINS, \Delta HbA1c, \Delta HOMA-IR, \) and \( \Delta HOMA-IS \); however, the residual errors of them were approximately normal distribution. All the statistical analyses used the SAS Institute’s statistical software (that is, SAS) for Windows version 9.1 (SAS Institute Inc., Cary, CA, USA). A two-tailed \( p \) value of less than 0.05 was considered statistically significant in the \( t \)-test, Pearson \( \chi^2 \)-test, and GMDR. According to multiple testing, significance thresholds of \( p < 0.0125 \) or \( p < 0.025 \) were used, which were related to the number of experimental groups. When the number of experimental groups was two, the significant threshold was 0.025. If the number of experimental groups was three, the significant threshold would be 0.0125.

3. Results

In total, 93 patients with type 2 diabetes mellitus were included in this study. Their baseline demographic, anthropometric, biochemical, and genetic characteristics were showed in Table 1. Seven patients were unable to continue the study due to adverse reactions, such as abdominal pain, abdominal distension, and diarrhea. Due to poor compliance, four patients who missed metformin for more than ten days withdrew from the study. A total of 82 T2DM patients completed a two-month treatment with metformin in this study. There were 38 males and 44 females having a mean age of 49.80 ± 12.18 years. The participants had a mean FPG of 10.6 ± 3.2 mmol/L, a mean HbA1c of 8.4 ± 2.1%, and a mean FINS of 12.4 ± 9.3 µU/mL. The SNPs were in Hardy–Weinberg equilibrium \((p > 0.05)\). Details on the studies can be found in the Supplementary Material, Table S1.

Table 1. Genotypes of SLC47A1 rs2289669 on the effect of metformin efficacy in subgroup analysis.

| Variables \( ^a \) | Normal Weight Group | Overweight Group |
|---------------------|---------------------|-----------------|
|                     | AA/AG              | AG              | F Value | \( p \) Value | AA/AG | AG | F Value | \( p \) Value |
| \( \Delta FPG \)    | \(-0.3 ± 0.2,(8)\) | \(-0.2 ± 0.2,(27)\) | 1.87    | 0.1805 | \(-0.2 ± 0.2,(9)\) | \(-0.3 ± 0.2,(35)\) | 1.08 | 0.3051 |
| \( \Delta (60-30)FPG \) | \(-0.4 ± 0.2,(8)\) | \(-0.3 ± 0.1,(27)\) | 0.73 | 0.3990 | \(-0.4 ± 0.2,(9)\) | \(-0.4 ± 0.2,(35)\) | 0.42 | 0.5200 |
| \( \Delta FINS \) | \(-0.035 ± 0.126,(8)\) | \(-0.057 ± 0.159,(27)\) | 1.33 | 0.2559 | \(-0.145 ± 0.146,(9)\) | \(-0.122 ± 0.175,(35)\) | 0.13 | 0.7155 |
| \( \Delta HOMA-IR \) | \(-0.113 ± 0.182,(8)\) | \(-0.149 ± 0.208,(29)\) | 0.20 | 0.6585 | \(-0.222 ± 0.143,(9)\) | \(-0.218 ± 0.159,(36)\) | 0.01 | 0.9418 |
| \( \Delta (60)HOMA-IR \) | \(-0.555 ± 0.258,(6)\) | \(-0.010 ± 0.456,(27)\) | 7.87 | 0.0086 | \(-0.123 ± 0.500,(8)\) | -0.035 ± 0.0,534,(35) | 0.21 | 0.6518 |
| \( \Delta (60)HOMA-IS \) | \(-0.923 ± 0.436,(6)\) | \(-0.250 ± 0.494,(27)\) | 9.44 | 0.0044 | \(-0.486 ± 0.487,(8)\) | \(-0.442 ± 0.516,(35)\) | 0.05 | 0.8202 |
| \( \Delta (60)HOMA-IS \) | 0.141 ± 0.294,(6) | 0.460 ± 0.815,(27) | 0.87 | 0.3574 | 0.496 ± 0.631,(8) | 0.679 ± 0.720,(30) | 0.43 | 0.5139 |

\( ^a \) Continuous variables were expressed as mean ± SD, \( (N) \) and analyzed using a \( t \)-test. Genetic associations were tested using regression analysis.

Considering that some factors, such as sex, age, education, smoking, alcohol drink, tea drink, sweet, and biochemical characteristics, might affect the treatment response, we conducted the following analysis presented in Tables S2 and S3. There were no significant differences among the genotypes of the four SNPs at baseline \((p > 0.0125, 0.025, \) respectively). Some suggestions on diet, including alcohol drinks, sweets, and tea drinks, were given to all the patients.

Tables S4–S6 and Table 1 show the relationship between different genotypes and metformin efficacy in the subgroup analysis, which was performed to divide the patients into a normal weight group \((\text{BMI} \geq 18.5 \text{ and } < 25 \text{ kg/m}^2)\) and an overweight group \((\text{BMI} \geq 25 \text{ and } < 30 \text{ kg/m}^2)\) according to BMI. There were no significant differences in the \( \Delta FPG, \Delta (60)FPG, \Delta (60-30)FPG, \Delta HbA1c, \Delta FINS, \Delta HOMA-IR, \) and \( \Delta HOMA-IS \) among the different genotype groups of SLC22A1 rs628031, SLC22A2 rs316019, and SLC47A2 rs12943590.
For **SLC47A1 rs2289669**, the reduction in \(\Delta 60 FINS\) and \(\Delta 60 HOMA-IR\) after a two-month treatment with metformin was significantly different in the normal weight group (\(p = 0.0086, 0.0044\), respectively). The results showed that there were no differences in the others among the genotypes of **SLC47A1 rs2289669** (\(p > 0.025\)).

The association between the genotypes of these four SNPs and the efficacy of metformin was researched by regression analysis, which was adjusted for several variables, including sex, age, BMI, metformin dosage, education, tea drink, smoking, and sweets. There were no significant differences in \(\Delta 60 FINS\) and \(\Delta 60 HOMA-IR\) among the different genotypes of **SLC22A1 rs628031**, **SLC22A2 rs316019**, and **SLC47A1 rs2289669** (\(p > 0.0125, 0.025\), respectively). Compared with the GG and AA genotypes, patients with the AG genotype of **SLC47A2 rs12943590** had a greater reduction in \(\Delta 60 HOMA-IR\) (\(p = 0.00748\)). No significant difference was observed in \(\Delta 60 FINS\) among the different genotypes of **SLC47A2 rs12943590** (\(p > 0.0125\)). See more details in Table 2.

Table 2. Genotypes of four SNPs on the effect of metformin efficacy in regression analysis.

| Variables \(^a\) | Genotype | Mean ± SD (n) | BETA BETA | Crude | \(p\) Value | Adjusted | \(p\) Value |
|-----------------|----------|--------------|-----------|-------|-------------|----------|-------------|
| **\(\Delta 60 FINS\)** | **SLC22A1 rs628031** | | | | | | |
| | GG | \(-0.178 ± 0.430\) (44) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | GA/AA | \(0.061 ± 0.561\) (32) | 0.238 | 0.03364 | 0.228 | 0.03300 |
| | **SLC22A2 rs316019** | | | | | | |
| | AC | \(-0.170 ± 0.456\) (35) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | CC | \(0.002 ± 0.527\) (41) | 0.172 | 0.12611 | 0.228 | 0.03371 |
| | **SLC47A1 rs2289669** | | | | | | |
| | AA/GG | \(-0.312 ± 0.456\) (14) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | AG | \(-0.024 ± 0.498\) (62) | 0.287 | 0.04491 | 0.248 | 0.05860 |
| | **SLC47A2 rs12943590** | | | | | | |
| | GG | \(0.090 ± 0.475\) (27) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | AG | \(-0.190 ± 0.461\) (37) | \(-0.280\) | 0.02129 | \(-0.273\) | 0.01884 | |
| | AA | \(-0.105 ± 0.603\) (12) | \(-0.195\) | 0.24290 | \(-0.242\) | 0.12332 | |
| **\(\Delta 60 HOMA-IR\)** | **SLC22A1 rs628031** | | | | | | |
| | GG | \(-0.448 ± 0.461\) (44) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | GA/AA | \(-0.373 ± 0.600\) (32) | 0.075 | 0.53045 | 0.083 | 0.47461 | |
| | **SLC22A2 rs316019** | | | | | | |
| | AC | \(-0.511 ± 0.475\) (35) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | CC | \(-0.337 ± 0.551\) (41) | 0.174 | 0.13896 | 0.202 | 0.07673 | |
| | **SLC47A1 rs2289669** | | | | | | |
| | AA/GG | \(-0.675 ± 0.501\) (14) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | AG | \(-0.359 ± 0.512\) (62) | 0.316 | 0.03378 | 0.274 | 0.04756 | |
| | **SLC47A2 rs12943590** | | | | | | |
| | GG | \(-0.208 ± 0.482\) (27) | 0.000 | 0.000 | 0.000 | 0.000 | |
| | AG | \(-0.543 ± 0.472\) (37) | \(-0.335\) | 0.00730 | \(-0.323\) | 0.00748 | |
| | AA | \(-0.499 ± 0.636\) (12) | \(-0.291\) | 0.08887 | \(-0.364\) | 0.02586 | |

\(^a\) Continuous variables were expressed as mean ± SD, (N) and analyzed using a \(t\)-test. Genetic associations were tested using regression analysis. Several variables were adjusted, including sex, age, BMI, metformin dosage, education, tea drink, smoking, and sweets.

GMDR was used to analyze the correlation between the gene–environment interaction and FINS in Table 3. Gene–environment interaction analysis showed that in the FINS interaction model, the second-order of the dosage of metformin on day 30 (dose30_g)-
SLC47A2 rs12943590 was statistically significant ($p = 0.0107$). The balance accuracy and cross-validation consistency were 0.7167 and 5/10, respectively. However, there was no difference in the four-order of dose30_g, SLC22A1 rs628031, SLC22A2 rs316019, and SLC47A1 rs2289669 ($p = 0.206$).

**Table 3.** Gene–environment interaction model for fasting insulin by GMDR method.

| Model                      | Test Accuracy | The $p$ Value of GMDR | CVC | $p$ Value |
|----------------------------|---------------|-----------------------|-----|-----------|
| dose30_g, SLC47A2 rs12943590 | 0.7167        | 9 ($p = 0.0107$)      | 5   | 0.007     |
| dose30_g, SLC22A1 rs628031, SLC22A2 rs316019, SLC47A1 rs2289669 | 0.5983        | 7 ($p = 0.1719$)      | 8   | 0.206     |

The correlation between gene–environment interactions and various indicators of diabetes were tested using GMDR. CVC, cross-validation consistency.

4. Discussion

Genetic determinants and environmental factors play an important role in the development of type 2 diabetes, which is a complex metabolic disease [31]. Fewer than two-thirds of T2DM patients achieved appropriate control of FPG [32]. At present, metformin has been recommended as the first choice of treatment drug for T2DM by multinational guidelines. In this study, we studied the correlation between SLC22A1, SLC22A2, SLC47A1, and SLC47A2 variants and the risk of T2DM in Han Chinese from Chaoshan, China; and explored the effect of genotype on FPG, HbA1c, FINS, HOMA-IR, and HOMA-IS in T2DM patients receiving metformin treatment.

In this prospective cohort study, we investigated the relationship between SLC22A1 rs628031, SLC22A2 rs316019, SLC47A1 rs2289669, and metformin efficacy in T2DM patients. A country-specific point has been established for Asian populations (such as Chinese), and it is recommended that the cutoff point of overweight is 24 kg/m$^2$ [33]. Nevertheless, this study adopted the World Health Organization criteria to define normal weight as a BMI $\geq 18.5$ and <25 kg/m$^2$ and overweight as a BMI $\geq 25$ and <30 kg/m$^2$, which made it easy to compare studies on different populations [34].

The MATE 1 protein, which is encoded by the SLC47A1 gene, affects the excretion of metformin into the bile and urine, thereby affecting metformin efficacy [27,35]. In this study, patients were from the Chaoshan area of China and the frequencies of the genotype of SLC47A1 rs2289669 were 0.061, 0.793, and 0.146 for AA, AG, and GG, respectively. Another study conducted in the Han population in southeast China, including 110 patients treated with metformin for 90 days, reported that the frequencies of the SLC47A1 rs2289669 genotype in AA, AG, and GG were 0.227, 0.557, and 0.216, respectively [12]. The reason the gene frequency distribution was different may be the regional difference, which may lead to different research results.

To the best of our knowledge, two other studies carried out in a Scottish population and a Chinese population separately reported a positive result, which was that they found a significant effect of SLC47A1 rs2289669 on the efficacy of metformin. In Scottish individuals, a study involving 148 T2DM patients who received metformin treatment for six months found that SLC47A1 rs2289669 had a significant genotype-specific effect on the response to metformin, and HbA1c in patients with the homozygous SLC47A1 rs2289669 A-allele had a significantly lower reduction [8]. The other study showed that among 180 newly diagnosed type 2 diabetic patients with the SLC47A1 rs2289669 AA genotype, the change of HbA1c level was significantly different [21]. However, no significant difference was found in the HbA1c reduction among the different genotype groups of SLC47A1 rs2289669.
in the study. The reason for the two different results may be related to the differences in gene frequency distribution between regions. Compared with carriers of the AG genotype, normal weight T2DM patients with the SLC47A1 rs2289669 AA or GG genotype had a better effect in lowering FINS and HOMA-IR after a two-month metformin monotherapy. However, regression analysis showed that there was no significant difference in FINS and HOMA-IR between patients with the SLC47A1 rs2289669 AG genotype and those with the AA/GG genotype, which showed that this SNP was not associated with metformin efficacy in 82 patients.

The MATE2 transporter is encoded by the SLC47A2 gene [36]. SLC47A2 rs12943590, which is one of the most relevant clinical gene variants in the SLC47A2 gene, affects metformin depuration [37]. Using the statistical method of regression analysis, our study showed that SLC47A2 rs12943590 played a role in the interindividual variability of metformin efficacy. In the present study, we showed that the AG genotype of SLC47A2 rs12943590 correlated with a significant reduction in HOMA-IR, whereas the genotypes of SLC47A2 rs12943590 were not associated with a significant reduction in HbA1c. Significant associations between the AG genotype of SLC47A2 rs12943590 and a greater reduction of HbA1c were detected, whereas no significant effect of this polymorphism on HOMA-IR was found [30]. Our results were inconsistent with the research conclusions of Phani N.M et al. [30], which may be related to individual differences in gene frequency distribution.

If racial genetic heterogeneity is excluded, the effect of gene–gene and gene–environment on the pharmacokinetic and pharmacodynamic efficacy of metformin may be an important factor in the difference. Previous studies have shown that MATE1 rs2252281-SLC22A2 rs316019 and SLC22A1 rs622342-SLC47A1 rs2289669 had significant effects on the pharmacokinetic and pharmacodynamic efficacy of metformin, respectively [38,39]. However, the interaction between SLC22A2 rs316019 and SLC47A1 rs2289669 in this study did not enter the model, which suggested that the interaction between SLC22A2 rs316019 and SLC47A1 rs2289669 did not significantly affect insulin resistance in patients with type 2 diabetic patients receiving metformin treatment. Our study found that the second-order interaction of dose30_g-SLC47A2 rs12943590 might have a significant effect on FINS in patients with type 2 diabetes taking metformin; however, other interaction models were not statistically significant (p > 0.05).

The results of this study showed that SLC47A2 rs12943590 predicted insulin resistance improvement in patients with type 2 diabetes mellitus treated with metformin, which may be helpful to guide the clinical use of metformin in the treatment of type 2 diabetes mellitus to some extent. Therefore, for type 2 diabetic patients with the SLC47A2 rs12943590 AG genotype, metformin may be an appropriate choice.

The present study had some limitations. First, compared with the previous study mentioned above [12], our study lasted for two months, which was not enough to observe the long-term effects of these four SNPs on the therapeutic efficacy of metformin. Our results could only show the preliminary effect, which remains to be verified by further study and longer follow-up time. Second, the participants in the present study were newly diagnosed, treatment-naive type 2 diabetic subjects. Therefore, whether our findings could be extended to patients with long-term T2DM needs further testing. Third, the patients in this study came from the Chaoshan region of China; hence, whether our findings were applicable to patients from other regions remains to be tested. Although there were limitations, metformin monotherapy and prospective design in previous studies could attenuate the influence of confounding factors, such as information bias and concomitant medication.

5. Conclusions

To sum up, we found that in a Chaoshan population with newly diagnosed T2DM, rs2289669 of the SLC47A1 gene and rs12943590 of the SLC47A2 gene polymorphism might affect the risk of T2DM by changing HOMA-IR parameters and other factors. Our data indicated that the AA or GG genotype of SLC47A1 rs2289669 and the AG genotype of SLC47A2 rs12943590 might affect HOMA-IR in patients with type 2 diabetes. No significant
The effects of both SLC22A1 rs628031 and SLC22A2 rs316019 against glycemic response, FINS, HOMA-IR, and HOMA-IS were detected in our study. In addition, dose30_g-SLC47A2 rs12943590 could be a risk factor for HOMA-IR in T2DM.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines10102546/s1. Table S1: Baseline characteristics of the participants in this study; Table S2: Comparison of baseline characteristics of different genotypes of SLC22A1 rs628031 and SLC22A2 rs316019; Table S3: Comparison of baseline characteristics of different genotypes of SLC47A1 rs2289669 and SLC47A2 rs12943590; Table S4: Genotypes of SLC22A1 rs628031 on the effect of metformin efficacy in subgroup analysis; Table S5: Genotypes of SLC22A2 rs316019 on the effect of metformin efficacy in subgroup analysis; Table S6: Genotypes of SLC47A2 rs12943590 on the effect of metformin efficacy in subgroup analysis.

Author Contributions: Conceptualization, S.C. (Shiyi Chen) and S.C. (Shenren Chen); methodology, P.C., Y.C., Y.G. and Z.L.; formal analysis Z.L.; writing—original draft preparation, P.C. and Y.C.; writing—review and editing, S.C. (Shiyi Chen) and Y.G.; supervision, S.C. (Shenren Chen). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Science and Technology Project of Guangdong Province, China (2012B031800269), and the Medical and Health Research of Meizhou city, Guangdong Province, China (2019-B-37, 2019-B-41).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Second Affiliated Hospital of Shantou University Medical College (date of approval: 9 March 2012; protocol code 2022-103 and date of approval: 28 September 2022).

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study.

Data Availability Statement: Data are available on request due to privacy or ethics.

Acknowledgments: The authors thank the staff of the Department of Epidemiology and Biostatistics, School of Public Health, Peking University Health Science Center, for their support in the genotyping and statistics analysis of the study. We thank Dafang Chen and Xiaozhu Wang from Peking University Health Science Center, and Liuwei Zhang from Beijing Sport University for their expert technical assistance. We thank Qi Xu from the Second Affiliated Hospital of Shantou University Medical College for recruiting study participants.

Conflicts of Interest: There are no conflict of interest.

References
1. Chinese Diabetes Society. Chinese guidelines for the prevention and treatment of type 2 diabetes 2017. Chin. J. Diabetes Mellit. 2018, 10, 4–66.
2. American Diabetes Association. Standards of medical care in diabetes 2018. Diabetes Care 2018, 41, S1–S159. [CrossRef]
3. Garber, A.J.; Abrahamson, M.J.; Barzilay, J.I.; Blonde, L.; Bloomgarden, Z.T.; Bush, M.A.; Dagogo-Jack, S.; DeFronzo, R.A.; Einhorn, D.; Fonseca, V.A.; et al. Consensus statement by the American association of clinical endocrinologists and American college of endocrinology on the comprehensive type 2 diabetes management algorithm-2018 executive summary. Endocr. Pract. 2018, 24, 91–120. [CrossRef] [PubMed]
4. Ji, L.; Han, P.; Wang, X.; Liu, J.; Zheng, S.; Jou, Y.M.; O’Neill, E.A.; Golm, G.T.; Engel, S.S.; Kaufman, K.D.; et al. A randomized clinical trial of the safety and efficacy of sitagliptin and metformin co-administered to Chinese patients with type 2 diabetes mellitus. J. Diabetes Investig. 2016, 7, 727–736. [CrossRef] [PubMed]
5. Garber, A.J.; Duncan, T.G.; Goodman, A.M.; Mills, D.J.; Rohlf, J.L. Efficacy of metformin in type II diabetes: Results of a double-blind, placebo-controlled, dose-response trial. Am. J. Med. 1997, 103, 491–497. [CrossRef]
6. DeFronzo, R.A.; Goodman, A.M. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N. Engl. J. Med. 1995, 333, 541–549. [CrossRef] [PubMed]
7. Wu, K.; Li, X.; Xu, Y.; Zhang, X.; Guan, Z.; Zhang, S.; Li, Y. SLC22A1 rs622342 Polymorphism Predicts Insulin Resistance Improvement in Patients with Type 2 Diabetes Mellitus Treated with Metformin: A Cross-Sectional Study. Int. J. Endocrinol. 2020, 2020, 2975989. [CrossRef]
8. Becker, M.L.; Visser, L.E.; Van Schaik, R.H.; Hofman, A.; Uitterlinden, A.G.; Stricker, B.H.C. Genetic variation in the multidrug and toxin efflux transporter proteins influences the glucose-lowering effect of metformin in patients with diabetes: A preliminary study. *Diabetes* 2009, 58, 745-749. [CrossRef]

9. Umaraheswaran, G.; Praveen, R.G.; Damodaran, S.E.; Das, A.K.; Adithan, C. Influence of SLC22A1 rs622342 genetic polymorphism on metformin response in South Indian type 2 diabetes mellitus patients. *Clin. Exp. Med.* 2015, 15, 511–517. [CrossRef]

10. Christensen, M.M.H.; Hojlund, K.; Høth-Nielsen, O.; Stage, T.B.; Damkier, P.; Beck-Nielsen, H.; Brøsen, K. Steady-state pharmacokinetics of metformin is independent of the OCT1 genotype in healthy volunteers. *Eur. J. Clin. Pharmacol.* 2015, 71, 691–697. [CrossRef]

11. Jablonski, K.A.; McAteer, J.B.; de Bakker, P.I.; Franks, P.W.; Pollin, T.I.; Hanson, R.L.; Saxena, R.; Fowler, S.; Shuldiner, A.R.; Knowler, W.C.; et al. Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle interventions in the diabetes prevention program. *Diabetes* 2010, 59, 2672–2681. [CrossRef] [PubMed]

12. Yi, M.; Xu, W. Correlation analysis of C11orf65 and SLC47A1 gene polymorphisms with clinical efficacy of metformin. *Med. Innov. China* 2019, 16, 64–69.

13. Xiao, D.; Guo, Y.; Li, X.; Yin, J.Y.; Zheng, W.; Qiu, X.W.; Xiao, L.; Liu, R.R.; Wang, S.Y.; Gong, W.J.; et al. The Impacts of SLC22A1/OCT1 (rs628031) Polymorphism and Its Interactions with Dietary Micronutrients in Type 2 Diabetes. *Diabetes Metab.* 2021, 37, 691–697. [CrossRef] [PubMed]

14. Donnelly, L.A.; Doney, A.S.; Hattersley, A.T.; Morris, A.D.; Pearson, E.R. The effect of obesity on glycaemic response to metformin in Han Chinese patients with type-2 diabetes mellitus. *Clin. Pharmacol. Ther.* 2011, 90, 674–684. [CrossRef] [PubMed]

15. Harries, L.W.; Hattersley, A.T.; Doney, A.S.; Colhoun, H.; Morris, A.D.; Sutherland, C.; Hardie, D.G.; Peltonen, L.; McCarthy, M.I.; Holman, R.R. Common variants near ATM are associated with glycemic response to metformin in type 2 diabetes. *Nat. Genet.* 2011, 43, 117–120.

16. Van Leeuwen, N.; Nijpels, G.; Becker, M.L.; Deshmukh, H.; Zhou, K.; Stricker, B.H.C.; Uitterlinden, A.G.; Hofman, A.; van’t Riet, E.; Palmer, C.N.A.; et al. A gene variant near ATM is significantly associated with metformin treatment response in type 2 diabetes: A replication and meta-analysis of five cohorts. *Diabetologia* 2012, 55, 1971–1977. [CrossRef]

17. Zhang, S.; Tang, Y.; Cai, D.; Li, Y.; Guo, X.; Su, Q.; Guo, L.; Zhao, D.; Li, Q.; Li, H.; et al. A common 5′-UTR variant in MATE2-K is associated with poor response to metformin. *Clin. Pharmacol. Ther.* 2011, 90, 674–684. [CrossRef] [PubMed]

18. Zhou, Y.; Ye, W.; Wang, Y.; Jiang, Z.; Meng, X.; Xiao, Q.; Zhao, Q.; Yan, J. Genetic variants of OCT1 influence glycaemic response to metformin in Han Chinese patients with type-2 diabetes mellitus in Shanghai. *Int. Clin. Exp. Pathol.* 2015, 8, 9533–9542.

19. Reséndiz-Abarca, C.A.; Flores-Alfaro, E.; Suárez-Sánchez, F.; Cruz, M.; Valladares-Salgado, A.; del Carmen Alarcón-Romero, L.; Vázquez-Moreno, M.A.; Wacher-Rodarte, N.A.; Gómez-Zamudio, J.H. Altered Glycemic Control Associated With Polymorphisms in the SLC22A1 (OCT1) Gene in a Mexican Population With Type 2 Diabetes Mellitus Treated With Metformin: A Cohort Study. *J. Clin. Pharmacol.* 2019, 59, 1384–1390. [CrossRef]

20. Shokri, F.; Ghaedi, H.; Fard, S.G.; Movafagh, A.; Abediankenari, S.; Kashi, Z.; Omrani, M.D. Impact of ATM and ATM rs11212617 polymorphisms with metformin efficacy in patients with type 2 diabetes. *Pharm. Genom.* 2015, 5, 1–7. [CrossRef] [PubMed]

21. Ding, J.; He, W.; Gong, Y. Association between SLC47A1 gene polymorphism and therapeutic efficacy of metformin for type 2 diabetes mellitus. *Cent. South Pharm.* 2018, 9, 1336–1338+1342.

22. Ito, H.; Ishida, H.; Takeuchi, Y.; Antoku, S.; Abe, M.; Mifune, M.; Togane, M. Long-term effect of metformin on blood glucose control in non-obese patients with type 2 diabetes mellitus. *Nutr. Metab.* 2010, 7, 83. [CrossRef] [PubMed]

23. Donnelly, L.A.; Doney, A.S.F.; Hattersley, A.T.; Morris, A.D.; Pearson, E.R. The effect of obesity on glycaemic response to metformin or sulphonylureas in type 2 diabetes. *Diabet. Med.* 2006, 23, 128–133. [CrossRef] [PubMed]

24. Ong, C.R.; Molyneaux, L.M.; Constantino, M.I.; Twigg, S.M.; Yue, D.K. Long-term efficacy of metformin therapy in nonobese individuals with type 2 diabetes. *Diabetes Care* 2006, 29, 2361–2364. [CrossRef] [PubMed]

25. Alberti, K.G.; Zimmet, P.Z. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet. Med.* 1998, 15, 539–553. [CrossRef]

26. Chen, P.; Cao, Y.; Guo, Y.; Xu, Q.; Wang, X.; Zhang, L.; Liu, Z.; Chen, D.; Chen, S.; Chen, S. Association of SLC22A1 rs622342 and ATM rs11212617 polymorphisms with metformin efficacy in patients with type 2 diabetes. *Pharm. Genom.* 2022, 32, 67–71. [CrossRef] [PubMed]

27. Tanigawa, Y.; Masuda, S.; Sato, T.; Katsura, T.; Ogawa, O.; Inui, K.I. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H+-organic cation antiporters. *Biochem. Pharmacol.* 2007, 74, 359–371. [CrossRef]

28. Zolk, O. Disposition of metformin: Variability due to polymorphisms of organic cation transporters. *Ann. Med.* 2012, 44, 119–129. [CrossRef]

29. Zepeda-Carrillo, E.A.; Ramos-Lopez, O.; Martinez-Lopez, E.; Barrón-Cabrera, E.; Bernal-Pérez, J.A.; Velasco-González, L.E.; Rangel-Rios, E.; Bustamante Martínez, J.F.; Torres-Valadez, R. Effect of Metformin on Glycemic Control Regarding Carriers of the SLC22A1/OCT1 (rs594709 and SLC47A1 rs2289669 Polymorphisms on Metformin Therapeutic Efficacy in Chinese Type 2 Diabetes Patients. *Int. J.* 2016, 43, 1120–1124. [CrossRef] [PubMed]
30. Phani, N.M.; Vohra, M.; Kakar, A.; Adhikari, P.; Nagri, S.K.; D'Souza, S.C.; Umakanth, S.; Satyamoorthy, K.; Rai, P.S. Implication of critical pharmacokinetic gene variants on therapeutic response to metformin in Type 2 diabetes. *Pharmacogenomics* 2018, 19, 908–911. [CrossRef]

31. Siddiqui, K.; Musambil, M.; Usmani, A.M. Established type 2 diabetes susceptibility genetic variants in Saudi ethnicity: A minisystematic review. *JBC Genet.* 2018, 1, 57–65.

32. Birnbaum, M.J.; Shaw, R.J. Genomics: Drugs, diabetes and cancer. *Nature* 2011, 470, 338–339. [CrossRef] [PubMed]

33. Zhou, B.F.; Cooperative Meta-Analysis Group of the Working Group on Obesity in China. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults–study on optimal cut-off points of body mass index and waist circumference in Chinese adults. *Biomed. Environ. Sci.* 2002, 15, 83–96. [PubMed]

34. Obesity: Preventing and Managing the Global Epidemic. In *Report of a WHO Consultation*; World Health Organization Technical Report Series; WHO: Geneva, Switzerland, 2000; Volume 894, pp. 1–253.

35. Lickteig, A.J.; Cheng, X.; Augustine, L.M.; Klaassen, C.D.; Cherrington, N.J. Tissue distribution, ontogeny and induction of the transporters multidrug and toxin extrusion (MATE) 1 and MATE2 mRNA expression levels in mice. *Life Sci.* 2008, 83, 59–64. [CrossRef]

36. Nies, A.T.; Damme, K.; Kruck, S.; Schaeffeler, E.; Schwab, M. Structure and function of multidrug and toxin extrusion proteins (MATEs) and their relevance to drug therapy and personalized medicine. *Arch. Toxicol.* 2016, 90, 1555–1584. [CrossRef] [PubMed]

37. Marta, M.; Sánchez-Pozos, K.; Jaime-Santoyo, J.; Monroy-Escutia, J.; Rivera-Santiago, C.; de los Ángeles Granados-Silvestre, M.; Ortiz-López, M.G. Pharmacogenetic Evaluation of Metformin and Sulphonylurea Response in Mexican Mestizos with Type 2 Diabetes. *Curr. Drug Metab.* 2020, 21, 291–300. [CrossRef] [PubMed]

38. Becker, M.L.; Visser, L.E.; van Schaik, R.H.; Hofman, A.; Uitterlinden, A.G.; Stricker, B.H.C. Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response. *Pharm. Genom.* 2010, 20, 38–44. [CrossRef] [PubMed]

39. Christensen, M.M.; Pedersen, R.S.; Stage, T.B.; Brasch-Andersen, C.; Nielsen, F.; Damkier, P.; Beck-Nielsen, H.; Brøsen, K. A gene-gene interaction between polymorphisms in the OCT2 and MATE1 genes influences the renal clearance of metformin. *Pharm. Genom.* 2013, 23, 526–534. [CrossRef]