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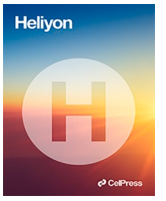

Research article

The effects of transcription factor 7-like 2 rs7903146 and paired box 4 rs2233580 variants associated with type 2 diabetes on the therapeutic efficacy of hypoglycemic agents

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ABSTRACT

Aim: This study aims to investigate the effects of the *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) variants associated with T2D on the therapeutic efficacies of various HAs in patients with T2D after follow-up for 3 years.
Methods: A total of 526 patients who were followed up at the Diabetic Clinic of Siriraj Hospital during 2016–2019 were enrolled. The variants *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) were genotyped using the RNase H2 enzyme-based amplification (rhAmp) technique and the associations between genotypes and glycemic control after treatments with different combinations HA were evaluated using Generalized Estimating Equations (GEE) analysis.
Results: Patients who carried *TCF7L2* rs7903146C/T + T/T genotypes when they were treated with biguanide alone had significantly lower fasting plasma glucose (FPG) than those of the patients who carried the C/C genotype ($p = 0.01$). Patients who carried the *PAX4* rs2233580 G/G genotype when they were treated with sulfonylurea alone had significantly lower FPG than those of the patients who carried G/A + A/A genotypes ($p = 0.04$).
Conclusion: Genotypes of *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) variants associated with T2D influence the therapeutic responses to biguanide and sulfonylurea. Different genotypes of these two variants might distinctively affect the therapeutic effects of HAs. This finding provides evidence of pharmacogenetics in the treatment of diabetes.

1. Introduction

The global prevalence of diabetes in 2019 was 9.3% (463 million people) and will increase to 10.2% (578 million people) by 2030, and 10.9% (700 million people) by 2045 [1]. Diabetes has significant impacts on health and health expenditure worldwide estimated

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Abbreviations

Bigua	Biguanide
BMI	Body mass index
FPG	Fasting plasma glucose
HAs	Hypoglycemic agents
HbA1c	Hemoglobin A1C
OGTT	Oral glucose tolerance test
PAX4	Paired box 4 gene
rhAmp	RNase H2 enzyme-based amplification
SU	Sulfonylurea
TCF7L2	Transcription factor 7-like 2 gene
T2D	Type 2 diabetes

at 760 billion USD and projected to reach 825 billion USD by 2030, and 845 billion USD by 2045 [2]. Type 2 diabetes (T2D) comprises 90% of diabetes cases worldwide.

Despite the various classes of antidiabetic drugs available, less than half of patients with T2D had HbA1c lower than 7% [3,4]. The treatment of type 2 diabetes consists of many classes of antidiabetic drugs including biguanides, sulfonylureas, thiazolidinediones, DPP4 inhibitors, SGLT2 inhibitors, and α-glucosidase inhibitors [5]. Several explanations have been proposed, such as poor medical adherence, suboptimal resources for diabetes self-management education (DSME), inaccessibility to proper medications and healthcare, and the discrepancy in outcome between randomized clinical trials and real-world studies [6]. A recent Thailand survey has also shown that less than 40% of T2D patients reached the HbA1c glycemic goal of less than 7% [7]. Furthermore, different drug responses and adverse effects among individuals have suggested the roles of pharmacogenetics in the outcome of medical treatment [5]. Previous studies had revealed that variants of certain genes influenced the therapeutic efficacies of hypoglycemic agents such as ATM serine/threonine kinase (*ATM*) [8] and solute carrier family 2 member 2 (*SLC2A2*) [9] loci with metformin. Transcription factor 7-like 2 (*TCF7L2*), ATP binding cassette subfamily C member 8 (*ABCC8*) [10], potassium voltage-gated channel subfamily J member 11 (*KCNJ11*) [10], and insulin receptor substrate 1 (*IRS1*) [11] loci with response to sulfonylurea and glinides; peroxisome proliferator activated receptor gamma (*PPARG*) locus with response to thiazolidinedione (*TZD*) [12]. Paired-box 4 (*PAX4*) locus with TZD and glinides response [13]. However, most research has been conducted in Caucasians, and to a lesser extent, in Asians such as Chinese and Korean [14].

TCF7L2 rs7903146 was one of several genetic variants that significantly contributed to the risk of developing T2D [15]. Moreover, it has been robustly replicated in several studies in multiple populations, including Thais [16–20]. In addition, insulin secretion was reduced in people with the risk allele [21]. The transcription factor that is a component of the Wnt signaling pathway in the β-cells is encoded by *TCF7L2* [22]. The insulin gene and the gene for the insulinotropic hormone glucagon-like peptide 1 (GLP-1) are all induced by the heterodimerization of the TCF7L2 protein with β-catenin.

Recent studies have shown that *PAX4* rs2233580 (R192H) is associated with T2D in Thais, Japanese, Han Chinese, Hong Kong, Korean, and younger onset T2D in Singaporean Chinese [23–27], but was not present in French populations [28]. *PAX4* rs2233580 (R192H) has been proposed to be an Asian-specific risk locus for T2D [26]. We have shown that this variant altered insulin and glucagon promoter repression activities and decreased transcript levels of genes required to maintain β-cell function, proliferation, and survival. The viability of β-cell was reduced under glucotoxic stress conditions for the cells overexpressing R192H [23]. Furthermore, another variant associated with diabetes of *PAX4* rs114202595 (R121W) identified in Japanese with T2D exhibited a low initial insulin response relative to glucose during the oral glucose tolerance test (OGTT) and significantly decreased the insulin/glucose ratio compared to that of non-diabetic subjects [25].

Taken together, *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) could alter the efficacy of available hypoglycemic agents due to the compromise of β-cell functions. However, no pharmacogenetic study has yet been carried out in Thai patients carrying these two variants. Therefore, the objective of this study is to determine the association between *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) polymorphisms and biochemical parameters that influence therapeutic responses to hypoglycemic agents in Thai patients with T2D. This study focused on four groups of hypoglycemic agents commonly used to treat T2D in Thai patients, including sulfonylurea, biguanide, thiazolidinediones, and insulin. All these medications are registered on the Thailand National List of Essential Medicines (NLEM).

2. Materials and methods

2.1. Study population

A total of 526 patients with T2D were recruited at Siriraj Diabetes Center and Diabetic Clinic, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. All patients have consistently attended the clinic from 2016 to 2019. The study protocol and informed consent were approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (COA no. Si491/2014). Written informed consent was obtained from all participants after a detailed

explanation of the purpose and protocol of the study. Diabetes was diagnosed based on the American Diabetes Association (ADA) [29]. T2D patients had to satisfy all of the following inclusion criteria: age at diagnosis >40 years, FPG ≥126 mg/dl (7.0 mmol/l), and HbA1c ≥ 6.5%. Demographic data including age, sex, age at diagnosis of T2D, duration of diabetes, body mass index (BMI), systolic and diastolic blood pressure were obtained. Biochemical parameters including fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), creatinine (Cr), eGFR, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were collected. Our study observed all biochemical parameters during T2D patients' follow-up at Siriraj Diabetes Center and Diabetic Clinic. Moreover, we examined the FPG and HbA1c levels during the patient's treatment with hypoglycemic agents. All studied participants have been treated with sulfonylurea, biguanide, thiazolidinediones, insulin, and combinations of these drugs.

2.2. Genotyping of TCF7L2 and PAX4

Genomic DNA was extracted from 2 ml of peripheral blood leukocytes using a commercial kit (Flexigene DNA kit, Hilden, Germany). *TCF7L2* rs7903146 was selected for analysis as this SNP was highly replicated as T2D risk loci in most ethnicities [30] and it has been proposed that *PAX4* rs2233580 is an Asian-specific T2D risk loci [26]. Genotyping was performed using RNase H2 enzyme-based amplification (rhAmp) technique (rhAmp SNP Assays, Redwood City, California, USA). The specific primers were identified using the rhAMP-genotyping design tool (IDT) (Supplementary material, Table 1). For this assay, PCR was carried out in 96-well plates with a total volume of 5 μl each, which consisted of 10 ng of DNA template, 2.65 μl of combined master mix and reporter mix, 0.25 μl of rhAmp SNP assay (20X), and 0.1 μl of nuclease-free water. The rhAmp genotyping assay was performed by using a LigthCycler® 480 system with LightCycler® 480 end-point genotyping software version 1.5 (Roche Diagnostic, Mannheim, Germany). The PCR condition consisted of an initial denaturation step at 95 °C for 10 min, followed by a 40-cycle program consisting of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, and elongation at 68 °C for 20 s, with a single acquisition mode for fluorescence signals (FAM or VIC). The quality of SNP genotyping was checked by the reproducibility of the genotypes of control DNA samples, which consisted of homozygous major allele and negative control. The genotyping success rate was greater than 98%.

2.3. Statistical analysis

SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Baseline clinical characteristics and laboratory parameters were compared between T2D patients carrying *TCF7L2* rs7903146 and *PAX4* rs2233580. For continuous data, descriptive statistics were used to show the mean with standard deviation (SD) or median with interquartile range, while categorical variables were reported as case numbers and percentages.

The correlation between the clinical characteristics of the patients and the combinations of genotypes of *TCF7L2* rs7903146/*PAX4* rs2233580 was analyzed using the Generalized Estimating Equations (GEE) model [31]. Since the results were repeatedly measured over three years of the follow-up period, GEE was used to take into account the correlation structure of the repeated results. The maximum number of studies was 16 combination drugs. The correlation structure of the continuous-time first-order autoregressive, AR (1) was then applied for repeated measurements. Independent variables were patient visits and genotype-drug group (32 categories). To compare the mean results of the same drug among different genotypes, no adjustment was made for multiple comparisons due to the exploratory analysis outcomes of this study. The number of observations (n) was 4633 for repeated measurement of the result in each genotype or genotype drug group (more than 2 observations were considered). All analyses were adjusted for age, sex, BMI, and drugs

Table 1
Baseline clinical characteristics and biochemical parameters of type 2 diabetic patients.

Variables	*TCF7L2* rs7903146		*p*-value	*PAX4* rs2233580		*p*-value
	C/C (n = 459)	C/T + T/T (n = 67)		G/G (n = 441)	G/A + A/A (n = 85)	
Gender (M:F)	135:324	22:45	-	131:310	26:59	-
Age (years)	64.56 ± 9.58	64.49 ± 11.23	0.96	64.52 ± 9.91	64.69 ± 9.22	0.88
Age at diagnosis (years)	54.98 ± 9.62	54.24 ± 9.45	0.56	54.99 ± 9.72	54.34 ± 8.97	0.57
Duration of diabetes (years)	9.20 ± 6.44	9.94 ± 7.05	0.38	9.16 ± 6.39	9.99 ± 7.15	0.28
BMI (kg/m^2)	25.77 ± 4.46	26.09 ± 4.37	0.59	25.79 ± 4.51	25.93 ± 4.14	0.78
Systolic blood pressure (mmHg)	133.52 ± 14.76	131.48 ± 14.37	0.29	133.40 ± 14.37	132.54 ± 16.48	0.62
Diastolic blood pressure (mmHg)	76.29 ± 10.11	76.403 ± 12.62	0.95	76.44 ± 10.45	75.61 ± 10.46	0.50
HbA1c (%)	6.97 ± 1.16	7.10 ± 1.27	0.41	6.97 ± 1.18	7.07 ± 1.14	0.49
Fasting plasma glucose (FPG) (mg/dl)	140.14 ± 38.07	140.62 ± 30.16	0.92	140.30 ± 38.77	139.67 ± 27.25	0.89
Creatinine (mg/dl)	0.96 ± 0.30	0.98 ± 0.33	0.65	0.96 ± 0.30	0.96 ± 0.29	0.99
eGFR ($mL/min/1.73m^2$)	73.91 ± 20.65	75.16 ± 23.34	0.74	74.01 ± 21.06	74.48 ± 21.08	0.89
TC (mg/dl)	183.05 ± 38.71	166.95 ± 33.05	0.10	180.32 ± 38.13	179.00 ± 38.98	0.89
TG (mg/dl)	150.93 ± 95.98	120.00 ± 45.74	0.15	149.69 ± 95.22	129.88 ± 64.42	0.33
Calculated LDL-C (mg/dl)	102.56 ± 30.41	87.94 ± 31.27	0.09	98.65 ± 28.51	101.72 ± 39.49	0.77
HDL-C (mg/dl)	53.33 ± 18.31	55.22 ± 13.29	0.68	52.64 ± 12.71	57.35 ± 27.96	0.47

Data was presented as mean ± SD.
Abbreviations: BMI: body mass index; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; eGFR: estimated glomerular filtration rate; TC: total cholesterol; TG: triglyceride; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.

(hypoglycemic agents, lipid-lowering drugs, and blood pressure medications). $P < 0.05$ was considered statistical significance for all tests.

3. Results

3.1. Baseline clinical characteristics and biochemical parameters of patients with type 2 diabetes

The baseline clinical characteristics and biochemical parameters of patients with type 2 diabetes are shown in Table 1. The subjects were classified by *TCF7L2* rs7903146 and *PAX4* rs2233580 genotypes at baseline clinical characteristics. *TCF7L2* rs7903146 and *PAX4* rs2233580 genotypes frequencies are consistent with Hardy-Weinberg equilibrium (Supplementary material, Table 3). The subjects who carried the C/C and C/T + T/T genotypes *TCF7L2* rs7903146 had 459 and 67 participants, respectively. The average age, age at the time of diagnosis of diabetes, duration of diabetes, BMI, systolic and diastolic blood pressures, FPG, HbA1c, creatinine, eGFR, lipid profiles (TC, TG, calculated LDL-C and HDL-C) were not significantly different between the subjects who carried C/C and C/T + T/T genotypes *TCF7L2* rs7903146. The subjects who carried the G/G and G/A + A/A genotypes *PAX4* rs2233580 had 441 and 85 participants, respectively. The participants with G/G and G/A + A/A genotypes of *PAX4* rs2233580 did not differ substantially in terms of average age, age at the time of diagnosis of diabetes, duration of diabetes, BMI, systolic and diastolic blood pressures, FPG, HbA1c, creatinine, eGFR, lipid profiles (TC, TG, calculated LDL-C and HDL-C).

3.2. Relationships of clinical characteristics and laboratory parameters of type 2 diabetic patients and the TCF7L2 rs7903146 and PAX4 rs2233580 genotypes after 3 years of follow-up

Generalized estimating equations (GEE) analysis was performed to determine the independent association between clinical characteristics and laboratory parameters of patients carrying the *TCF7L2* rs7903146 and *PAX4* rs2233580 genotypes during 3 years of follow-up, as shown in Table 2. Serum triglyceride was significantly lower in patients who carried the *PAX4* rs2233580 G/A + A/A genotype compared to those of patients who carried the G/G genotype ($p = 0.04$). There was a trend for lower serum triglyceride in the patients with the *TCF7L2* rs7903146C/T + T/T genotype compared to patients with the C/C genotype ($p = 0.07$). Mean age, age at diagnosis, BMI, systolic blood pressure, diastolic blood pressure, FPG, HbA1c, Cr, eGFR, TC, calculated LDL-C and HDL-C were not significantly different between SNP genotypes. In addition, no association was identified between response to hypoglycemic agents and duration of diabetes (Supplementary material, Table 4).

3.3. TCF7L2 rs7903146 genotypes and therapeutic responses to sulfonylurea, biguanide, thiazolidinediones and insulin treatment after 3 years of follow-up

To determine the association of different genotypes of *TCF7L2* rs7903146 and *PAX4* rs2233580 and therapeutic responses to hypoglycemic agents, the cohort was categorized into 16 groups according to the combination of anti-diabetic drugs: biguanide (Bigua), sulfonylurea (SU), thiazolidinediones (TZD) and insulin (Ins) (Supplementary material, Table 2). We analyze the data using the dominant model because the numbers of patients homozygous for minor alleles of *TCF7L2* rs7903146 and *PAX4* rs2233580 were

Table 2
Relationships of changes of clinical characteristics and biochemical parameters of type 2 diabetic patients during the follow-up period and *TCF7L2* rs7903146 and *PAX4* rs2233580 genotypes. (n = 526 and observation = 4633).

Variables	*TCF7L2* rs7903146		*p*-value	*PAX4* rs2233580		*p*-value
	C/C (n = 459)	C/T + T/T (n = 67)		G/G (n = 441)	G/A + A/A (n = 85)	
Age first visit (years)	64.6 ± 9.6	64.5 ± 11.2	[a]0.96	64.5 ± 9.9	64.7 ± 9.2	[a]0.88
Age onset (years)	55.0 ± 9.6	54.2 ± 9.4	[a]0.56	55.0 ± 9.7	54.3 ± 9.0	[a]0.57
Duration of diabetes (years)	10.6 ± 2.5	11.82 ± 3.0	0.23	10.7 ± 2.6	11.3 ± 2.5	0.43
BMI (kg/m^2)	25.9 ± 1.0	25.9 ± 1.3	0.76	25.9 ± 1.1	25.9 ± 1.0	0.88
Systolic blood pressure (mmHg)	133.7 ± 5.0	132.7 ± 5.1	0.22	133.4 ± 4.9	134.5 ± 5.3	0.36
Diastolic blood pressure (mmHg)	74.7 ± 4.6	75.8 ± 5.0	0.25	74.9 ± 4.7	74.5 ± 4.2	0.63
HbA1c (%)	7.07 ± 0.42	7.08 ± 0.37	0.77	7.07 ± 0.42	7.05 ± 0.39	0.76
Fasting plasma glucose (FPG) (mg/dl)	140.4 ± 8.6	139.4 ± 7.2	0.81	140.4 ± 8.6	140.0 ± 7.8	0.61
Creatinine (mg/dl)	1.03 ± 0.36	1.40 ± 0.36	0.40	1.10 ± 0.37	0.94 ± 0.36	0.08
eGFR ($mL/min/1.73m^2$)	76.3 ± 12.3	75.0 ± 15.2	0.98	75.9 ± 12.7	77.5 ± 12.5	0.30
TC (mg/dl)	167.4 ± 9.1	167.0 ± 9.6	0.98	168.2 ± 9.1	162.8 ± 9.3	0.15
TG (mg/dl)	137.3 ± 13.2	124.78 ± 14.50	0.07	137.9 ± 13.4	124.7 ± 13.4	***0.04***
Calculated LDL-C (mg/dl)	87.6 ± 9.0	85.1 ± 9.8	0.65	88.1 ± 9.2	82.8 ± 9.4	0.13
HDL-C (mg/dl)	54.4 ± 4.6	55.6 ± 5.1	0.42	54.4 ± 4.6	55.6 ± 4.9	0.47

Data was presented as mean ± SD.
Means were adjusted by age, BMI, sex, and duration of diabetes, hypoglycemic agents, lipid-lowering drugs, and blood pressure medications using GEE.
P value < 0.05 indicates statistical significance.
[a] Means were analyzed using Independent Samples T-Test.

Table 3
Changes of fasting plasma glucose (FPG) and HbA1c after 3 years of treatment with hypoglycemic agents versus *TCF7L2* rs7903146 genotypes.

Variables	Types	*TCF7L2* (rs7903146) genotypes				*p-value*
		C/C	n	C/T and T/T	n	
FPG (mg/dl)	SU	136.4 ± 4.5	256	139.7 ± 2.8	57	0.25
	SU + Combinations	145.5 ± 6.1	2441	144.3 ± 5.6	326	0.93
	Bigua	130.7 ± 4.2	973	127.2 ± 4.5	144	***0.01***
	Bigua + Combinations	145.4 ± 6.7	2488	143.8 ± 4.5	321	0.64
	TZD	146.0 ± 6.3	12	–	–	–
	TZD + Combinations	144.4 ± 7.7	1048	144.8 ± 7.3	142	0.83
	Ins	154.5 ± 5.7	34	182.4 ± 1.3	7	0.26
	Ins + Combinations	154.2 ± 13.3	195	144.8 ± 6.3	12	0.73
HbA1C (%)	SU	6.74 ± 0.25	256	6.67 ± 0.16	57	0.54
	SU + Combinations	7.25 ± 0.35	2441	7.31 ± 0.30	326	0.51
	Bigua	6.70 ± 0.24	973	6.65 ± 0.25	144	0.13
	Bigua + Combinations	7.26 ± 0.37	2488	7.34 ± 0.29	321	0.58
	TZD	7.53 ± 0.38	12	–	–	–
	TZD + Combinations	7.32 ± 0.37	1048	7.59 ± 0.39	142	0.11
	Ins	7.31 ± 0.38	34	7.91 ± 0.07	7	0.52
	Ins + Combinations	8.01 ± 0.35	195	8.61 ± 0.05	12	0.16

Data as presented mean ± SD. P value < 0.05 indicates statistical significance.
Means were adjusted by age, BMI, sex, and duration of diabetes, hypoglycemic agents, lipid-lowering drugs, and blood pressure medications using GEE.
SU: sulfonylureas; Bigua: biguanides; TZD: thiazolidinediones; Ins: insulin.
n = Number of observations.
Type of drug combinations (Supplementary material, Table 2).

Table 4
Changes of fasting plasma glucose (FPG) and HbA1c after 3 years of treatment with hypoglycemic agents versus *PAX4* (rs2233580) genotypes.

Variables	Type	*PAX4 (rs2233580)* genotypes				*p-value*
		G/G	n	G/A and A/A	n	
FPG (mg/dl)	SU	135.5 ± 4.1	252	142.9 ± 5.0	61	***0.04***
	SU + Combinations	145.7 ± 6.2	2303	143.8 ± 5.1	464	0.43
	Bigua	130.5 ± 4.2	958	129.0 ± 3.9	159	0.43
	Bigua + Combinations	145.6 ± 6.5	2332	143.0 ± 5.7	477	0.31
	TZD	149.3 ± 6.4	10	139.6 ± 3.8	2	0.26
	TZD + Combinations	144.7 ± 7.6	1032	143.8 ± 7.9	158	0.94
	Ins	154.8 ± 5.6	35	187.4 ± 1.4	6	0.15
	Ins + Combinations	156.1 ± 13.8	186	130.4 ± 16.8	21	***0.004***
HbA1c (%)	SU	6.71 ± 0.23	252	6.79 ± 0.30	61	0.55
	SU + Combinations	7.26 ± 0.35	2303	7.20 ± 0.31	464	0.58
	Bigua	6.70 ± 0.24	958	6.68 ± 0.27	159	0.62
	Bigua + Combinations	7.29 ± 0.37	2332	7.21 ± 0.32	477	0.58
	TZD	7.18 ± 0.41	10	8.19 ± 0.20	2	0.25
	TZD + Combinations	7.34 ± 0.38	1032	7.39 ± 0.36	158	0.54
	Ins	7.27 ± 0.38	35	8.31 ± 0.05	6	0.09
	Ins + Combinations	8.05 ± 0.36	186	7.99 ± 0.34	21	0.83

Data as presented mean ± SD. P value < 0.05 indicates statistical significance.
Means were adjusted by age, BMI, sex, and duration of diabetes, hypoglycemic agents, lipid-lowering drugs, and blood pressure medications using GEE.
SU: sulfonylureas; Bigua: biguanides; TZD: thiazolidinediones; Ins: insulin.
n = Number of observations.
Type of drug combinations (Supplementary material, Table 2).

too small for analysis with the additive model.

After three years of Bigua treatment, patients carrying the *TCF7L2* rs7903146C/T + T/T genotypes had significantly lower FPG compared to patients carrying the C/C genotype (127.2 ± 4.5 mg/dl vs 130.7 ± 4.2 mg/dl, p = 0.01) (Table 3 and Fig. 1). Other drug combinations did not show any significant impact on FPG and HbA1c.

3.4. PAX4 rs2233580 genotypes and therapeutic response to sulfonylurea, biguanide, thiazolidinediones, and insulin treatment after 3 years of follow-up

Sulfonylurea (SU) monotherapy was associated with higher FPG in patients who carried the *PAX4* rs2233580 G/A + A/A genotype compared to those of patients who carried the G/G genotype (142.9 ± 5.0 mg/dl vs. 135.5 ± 4.1 mg/dl, p = 0.04) (Table 4 and Fig. 1).

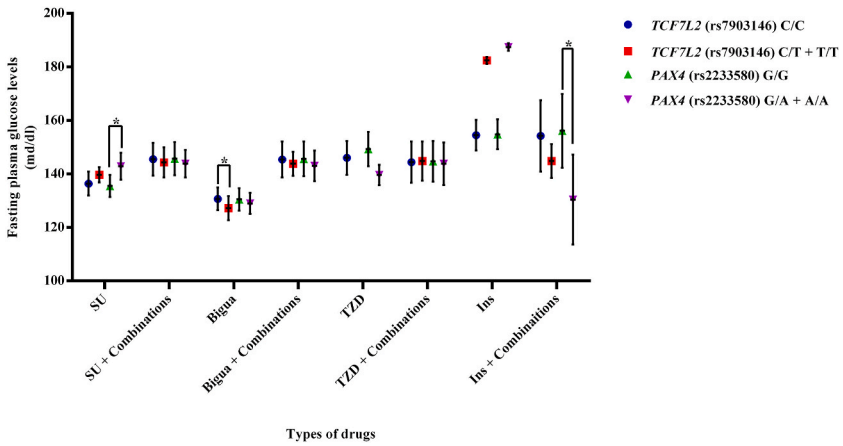

Fig. 1. Fasting plasma glucose (FPG) changes in comparison to *TCF7L2* rs7903146 and *PAX4* rs2233580 (R192H) genotypes following three years of hypoglycemic medications therapy.

However, treatment with a combination of insulin and oral hypoglycemic agents resulted in a lower FPG in patients who carried the G/A + A/A genotype (130.4 ± 16.8 mg/dl vs 156.1 ± 13.8, p = 0.004) (Table 4 and Fig. 1). Other drug combinations did not show any significant impact on both glycemic parameters.

4. Discussion

Our study provides supporting evidence that the genotypes of diabetes-associated variants of *TCF7L2* rs7903146 and *PAX4* rs2233580 influence the therapeutic efficacy of HAs after adjusting for confounding factors. Treatment with Bigua alone had a better outcome, as demonstrated by a lower FPG after three years of follow-up in patients with T2D who carried the T allele of *TCF7L2* rs7903146. Our results were supported by data from Dujic et al. which showed that the T allele of this SNP was associated with lower FPG after six months of metformin treatment in newly diagnosed patients with T2D [32], although the magnitude of the difference in the FPG level was smaller in our study than that reported by Dujic et al. (3.5% vs 6.7%). Our patients had a much longer duration of diabetes (almost 10 years) and β-cell function should have been substantially reduced. Therefore, it is possible that the therapeutic response to HAs in our patients might be lower than that of the patients previously reported. Interestingly, this T allele of *TCF7L2* rs7903146 was also associated with a lower FPG after metformin administration in non-diabetic individuals in the SUGAR-MGH study [33]. Since the Diabetes Prevention Program (DPP) study demonstrated that metformin treatment in prediabetes could delay the development of T2D [34] and the T allele of *TCF7L2* rs7903146 was associated with an increased risk of T2D [22]. Thus, metformin treatment is an attractive strategy to prevent diabetes in subjects with this allele. However, the mechanism by which this T allele affects metformin efficacy is unknown and merits further investigation. Our result was in contrast to the result of the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) study [35], which revealed that this T allele did not have an effect on the therapeutic response of metformin. This discrepancy might be attributable to differences in genetic background, characteristics of the studied subjects, or study designs. The GoDARTS study and other studies in European cohorts [36–38] have demonstrated a less favorable response to SU treatment. For example, smaller reduction in FPG and/or HbA1c, and a higher chance of secondary SU failure in patients who carried this T allele. It is possible that our study did not show a significant change in glycemic parameters between different genotypes of *TCF7L2* rs7903146 after SU treatment, perhaps due to a relatively small number of observations.

Until now, knowledge of the role of *PAX4* variants and the therapeutic efficacy of anti-diabetic medications has been limited. We showed that patients who carried *PAX4* rs2233580 G/A + A/A genotypes and had been treated with SU alone for 3 years had higher FPG than patients who carried the G/G genotype. To our knowledge, there are no studies that examined the relationship between *PAX4* rs2233580 (R192H), the Asian-specific variant of T2D risk, and the therapeutic response to hypoglycemic agents. A study by Gong et al. showed an attenuated effect of repaglinide on lowering postprandial plasma glucose in patients with the A allele of a different type of diabetes-associated *PAX4* rs114202595 (R121W), compared to patients with the G allele [39]. However, very few patients with T2D were treated with repaglinide in our cohort; therefore, we did not include this drug treatment in our analysis. Since SU and repaglinide belong to a similar class of HA or insulin secretagogue. Interestingly, treatment with a combination of insulin and oral hypoglycemic agents produced a better outcome, as demonstrated by a lower FPG in patients who carried the *PAX4* rs2233580 allele A (R192H) (Table 4). It is possible that this allele influenced the efficacy of the anti-diabetic drug that stimulates insulin secretion. Thus, a therapeutic regimen that includes exogenous insulin is needed to achieve better FPG. Confirmation of this finding is required in a future study with a larger number of patients.

Our study revealed that the *PAX4* rs2233580 (R192H) variant had an effect on lipid level, as shown in Table 2, that serum triglyceride level was significantly lower in patients with an A allele after adjusting for lipid-lowering medications and three years of follow-up. The patients who carried a T allele of *TCF7L2* rs7903146 were not associated with lipid levels after adjusting for lipid-lowering medications and three years of follow-up. This is in contrast to a meta-analysis by Wang et al. who showed that the T

allele of *TCF7L2* rs7903146 was associated with a lower serum triglyceride level in diabetes [40]. However, most of the studies included in this meta-analysis were conducted in non-Asian populations. It is possible that a difference in genetic background could lead to different results.

We have investigated the impact of *TCF7L2* and *PAX4* polymorphisms and their combination on the efficacy of commonly used hypoglycemic agents in Thailand. The patients enrolled in this study were typical patients with T2D who visited our diabetic clinic. We selected *TCF7L2* rs7903146 because they were the most replicated T2D-associated loci in several ethnic groups and *PAX4* rs2233580 (R192H) was an Asian-specific T2D risk locus. Furthermore, the relatively long period of follow-up (3 years) was responsible for 4633 observations for repeated measurement of outcomes in each genotype or genotype drug group. We had adjusted for potential factors that might influence the therapeutic response of anti-diabetic medications and still genotypes of both genes had an impact on the glycemic control of various drugs. This is in contrast with previous studies that generally considered one gene-one drug at a time. Furthermore, the follow-up period was relatively shorter resulting in a smaller number of observations.

The limitations of our study are that a relatively small number of patients were enrolled, and it was not a randomized clinical trial. We also could not exclude the effect of dosage adjustment by attending physicians during the subsequent period on glycemic control. For example, agent switching due to hypoglycemia due to the use of insulin secretagogues (SU) or secondary failure of SU treatment were not included in the study design. In addition, information about lifestyle changes such as dietary control, physical exercise, and medication compliance was not available. We did not consider genes involved in the metabolism of certain drugs such as SU and thiazolidinedione. Variations of these genes may also have an impact on the effectiveness of hypoglycemic agents. Although the methodology qualities of the recruited participants were generally good, it should be noted that we followed up the baseline and monitored the characteristics of study subjects according to their therapies. Therefore, our results were derived from unadjusted estimations, and failure to conduct further adjusted analyses for baseline characteristics of participants such as age, gender, and co-morbidity conditions may influence the veracity of our findings and the number of each subgroup. Moreover, significant heterogeneities were detected in certain subgroup comparisons, which indicated that the inconsistent results of included studies could not be fully explained by differences in environmental background, and other unmeasured characteristics of participants may also be partially attributed to between-study heterogeneities. Taking these limitations into consideration, the results of the current study should be interpreted with caution.

5. Conclusion

Our study is the first pharmacogenetic study of anti-diabetic medications in Thai patients with T2D. We have shown that the diabetes-associated variants of *TCF7L2* and *PAX4* had an impact on the therapeutic efficacy of hypoglycemic agents commonly used in Thailand after three years of treatment. Additionally, these variants influenced serum triglyceride levels. Prospective randomized clinical trials that include information on diet and physical activities of the patients are needed to confirm the results of our study. In addition, analysis of variations of genes involved in sulfonylurea, metformin, thiazolidinedione, and insulin metabolism should also be taken into account in future studies.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Nipaporn Teerawattanapong: Data curation, Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. **Lanraphat Srisawat:** Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Tassanee Narkdontri:** Data curation, Formal analysis, Investigation, Methodology. **Pa-thai Yenchitsomanus:** Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing. **Watip Tangjittipokin:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Nattachet Plengvidhya:** Conceptualization, Funding acquisition, Resources, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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