Clinical and genetic predictors of dipeptidyl peptidase-4 inhibitor treatment response in Type 2 diabetes mellitus

**Aim:** To determine the clinical and genetic predictors of the dipeptidyl peptidase-4 (DPP-4) inhibitor treatment response in Type 2 diabetes mellitus (T2DM) patients.

**Patients & methods:** DPP4, WFS1 and KCNJ11 gene polymorphisms were genotyped in a cohort study of 662 T2DM patients treated with DPP-4 inhibitors sitagliptin, vildaglaptin or linagliptin. Genotyping was performed by Applied Biosystems TaqMan SNP genotyping assay. **Results:** Patients with triglyceride levels less than 1.7 mmol/l (odds ratio [OR]: 2.2; 95% CI: 1.031–4.723), diastolic blood pressure (DBP) less than 90 mmHg (OR: 1.7; 95% CI: 1.009–2.892) and KCNJ11 rs2285676 (genotype CC) (OR: 2.0; 95% CI: 1.025–3.767) were more likely to respond to DPP-4 inhibitor treatment compared with other patients, as measured by HbA1c levels. **Conclusion:** Triglycerides, DBP and KCNJ11 rs2285676 are predictors of the DPP-4 inhibitor treatment response in T2DM patients.

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**Keywords:** DPP4 • dipeptidyl peptidase-4 inhibitors • HbA1c • KCNJ11 • Type 2 diabetes mellitus • WFS1

Diabetes mellitus is an epidemic chronic disease with a global prevalence of approximately 366 million people, a figure expected to increase to 592 million by 2035 [1].

Type 2 diabetes mellitus (T2DM) results from a progressive insulin secretory defect with a background of insulin resistance and is characterized by increased lipolysis and free fatty acid production, increased hepatic glucose production and decreased skeletal muscle uptake of glucose [2].

Incretin-based therapies are a recent class of antidiabetic agents used to control blood glucose levels in T2DM by altering the patient’s incretin pathway [3,4]. Glucagon-like peptide-1 (GLP-1) and gastic inhibitory polypeptide (GIP) are the main incretin hormones released in response to ingestion of glucose or nutrients, thus stimulating insulin secretion from pancreatic β-cells into circulation [5–8]. DPP-4 enzyme rapidly deactivates GLP-1 (7–36) amide and GIP (1–42) amide into inactive forms of GLP-1 (9–36) amide and GIP (3–42) amide, respectively; which minimised the duration of action of both compounds resulting in reduced glucose-stimulated insulin secretions and decreased proliferation and survival of β cells [9,10].

Incretin-based therapies are classified into two groups, GLP-1R agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors [3]. DPP-4 inhibitors, including sitagliptin, vildaglaptin and linagliptin block the DPP-4 enzyme, thus preventing the degradation of active GLP-1 and GIP [11], thereby activating the expression of multiple genes resulting in insulin secretion, β-cell proliferation and survival. Insulin is secreted from β-cells into the circulation to control blood glucose levels [12].

Based on disease pathogenesis and the incretin pathway, we hypothesized that three
genes, DPP4, WFS1 and KCNJ11, play a role in the response to DPP-4 inhibitors. The genes may include the drug target, pathway genes and disease genes.

DPP4 gene, also known as CD26, encodes a membrane-bound enzyme that is identical to adenosine deaminase complexing protein-2 and T-cell activation antigen CD26 [13], involves mainly the endocrine systems, metabolism, immune systems, bone marrow mobilization, cancer growth, cell adhesion and nutrition [14]. DPP-4 primarily acts by cleaving the X-proline dipeptides from the N-terminal of dipeptides in sequence [13]. The DPP4 gene, with a cytogenetic location at 2q24.3 [15], plays an important role in glucose metabolism where it directly mediates the degradation of incretins, especially GLP-1 [16]. DPP-4 rapidly inactivates active GLP-1 to inactive GLP-1 to prevent it from binding to the GLP-1R receptor, thus leading to poor glycemic control resulting from insufficient insulin production by pancreatic β-cells [17]. Therefore, we concluded that the DPP4 gene plays a crucial role in the disruption of the ‘incretin effect’ of the pancreatic β-cell glycemic control mechanism.

The WFS1 gene, with a cytogenetic location at 4p16.1 [15], plays a significant role in the maintenance of homeostasis of the endoplasmic reticulum (ER) in pancreatic β-cells. ER homeostasis is important for insulin secretion as this organelle is specifically responsible for the maturation of the prohormone precursor proinsulin to insulin, and for its subsequent release into the pancreatic β-cell matrix [18]. The inactivation of β-cell WFS1 may disrupt ER homeostasis, resulting in β-cell dysfunction and contributing to T2DM [18]. The important role of WFS1 in the stimulus-secretion coupling of insulin was evidenced in subjects with both Wolfram syndrome and diabetes, in which the progressive loss of β-cells impaired insulin pathways in these cells [19]. For these reasons, we selected to study WFS1 gene as a candidate gene in determining the DPP-4 inhibitor treatment response in T2DM.

The KCNJ11 gene (cytogenetic location of 11q15.1) [15] regulates one of the pancreatic β-cell ATP-sensitive potassium (KATP) channel subunits that plays a significant role in controlling insulin secretion [20,21]. The influx of glucose causes ATP generation [20] and the efflux of ATP from pancreatic β-cells occurs through the KATP channels, thereby activating KCNJ11. This results in membrane depolarization of K+, causing an influx of Ca2+ and triggering the release of insulin from β-cells into the circulation [18]. Since KCNJ11 mediates insulin exocytosis from pancreatic β-cells into the circulation via KATP channels [20,21] to complete the incretin signaling pathway of insulin secretion, we selected this gene as our final candidate gene in determining the DPP-4 inhibitor treatment response.

Within the incretin pathway, intestinal L cells mediate the release of GLP-1 into the gastrointestinal circulation following ingestion of a meal [9]. Prevention of GLP-1 inactivation by inhibition of DPP-4 allows active GLP-1 to bind with its receptor (GLP-1R) on pancreatic β-cells [11,12] and activates the PI3K pathway [22]. The resulting signaling cascades lead to β-cell insulin secretion through a mechanism involving WFS1 gene expression in pancreatic ER [18,19]. The outcome of incretin pathway activation is the release of insulin into the circulation [23] through pancreatic β-cell ATP-sensitive potassium channels in a process mediated by the KCNJ11 gene [20,21].

We also hypothesized that four clinical factors, sCD26, HOMA2B, homeostatic model assessment of insulin resistance (HOMAIR) and HbA1c (glycated hemoglobin), would be associated with the response to DPP-4 inhibitors. The selection of these clinical factors was based on an understanding of the disease pathogenesis and the mechanism of drug action. Other general clinical parameters investigated were fasting plasma glucose (FPG), insulin levels, total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, diastolic blood pressure (DBP), systolic blood pressure (SBP), alanine transferase (ALT), aspartate aminotransferase (AST), BMI and waist circumference.

Given that DPP-4 inhibitors act on pancreatic β-cells, which play a role in T2DM, we evaluated genetic and clinical factors involved in pancreatic β-cell functioning in T2DM. Therefore, the aim of this study was to determine the genetic variants and clinical factors associated with the DPP-4 inhibitor treatment response and the effects on this response of genetic–clinical factor interactions in T2DM.

### Patients & methods

#### Subjects

The study was approved by the Medical Ethics Committee of the University Malaya Medical Centre (UMMC) (Institutional Review Board Reference Number: 944.59) and written consent was obtained from each subject after a full explanation of the research purpose. The study was compliant with the Declaration of Helsinki [24]. The number (N) of samples required for 80% power to detect a gene–gene interaction with magnitude $R_{gh}$ = 3.0, assuming $R_{g} = 1$, $R_{h} = 1$ and various genetic-susceptibility prevalence and dominance models, was 312 [25]. Ideally, 662 samples were collected from the case group (DPP-4 inhibitor treated) group (n = 331) and from the control group (n = 331).

Approximately 331 subjects aged 18–75 years, with a diagnosis of T2DM and receiving DPP-4
inhibitor treatment (sitagliptin, vildagliptin or linagliptin) for at least 3 months, were recruited from a diabetes clinic at the UMMC in Kuala Lumpur. Daily doses of DPP-4 inhibitor were 100 mg (sitagliptin) [26], 50–200 mg (vildagliptin) [27] or 5 mg (linagliptin) [28]. The DPP-4 inhibitors can either in dual therapy with metformin, or in triple therapy with metformin and sulphonylurea or thiazolidinedione or sodium-glucose co-transporter 2 inhibitor [29]. A control group of approximately 331 subjects, receiving antidiabetic treatment other than DPP-4 inhibitors at any recommended treatment dose for at least 3 months [30] was included; previous DPP-4 inhibitor treatment prior to the 3-month period was not permitted in control subjects [11,26–28]. Subjects with a history of pancreatice injury [26,27], not compliance to medications [31], using insulin [29], with HIV infection [32], or cancer [33] were excluded from participating in the study. We considered Malay, Chinese and Indian to be the main ethnicities in Malaysia [34]; other ethnicities were classified as ‘other’. With time, new populations are formed by the mixture of previously separate groups, which can lead to unusually high levels of linkage disequilibrium [35], and children between people of different ethnicities may affect allele frequencies within populations [36]. Although many studies have failed to find a correlation between ethnicities and health outcomes, ethnicities can cause a close biological similarity among groups [37] and has the potential to influence the results of our study.

Clinical & laboratory assessments
Fasting venous blood samples were taken from all subjects for the determination of HbA1c, FPG, lipids, SBP, DBP and liver enzymes; and all testing was performed at the diagnostic laboratory of the UMMC using standard clinical laboratory protocols [38]. HbA1c is a measure of glucose binding to β-chains of the hemoglobin molecule [2] and was used as the determinant of treatment response in our samples. HbA1c of less than or equal to 7.0% was considered as good treatment response and HbA1c of more than 7.0% was considered as poor treatment response [2]. Insulin levels were measured using an insulin (IRI) assay (ADVIA Centaur®, SIEMENS, IL, USA) [39]. HOMAIR and pancreatic β-cell function (HOMA2B) were calculated using the homeostatic model assessment (HOMA2) as (fasting serum insulin (µU/ml) [FPG (mmol/l)]/22.5) [40]. sCD26 levels were measured using enzyme-linked immunosorbent assay kits (Human sCD26 Platinum ELISA; Bender MedSystems GmbH, Vienna, Austria) [41]. Other data, such as duration of diabetes, weight, waist circumference and comorbidity, were also collected and reviewed.

Gene polymorphism selection & genotyping
The selection of polymorphisms for DPP4, WFS1 and KCNJ11 genes was based on HapMap Phase III studies in Asian populations [13] with minor allele frequency of more than 5% [42]. To the best of our knowledge, no study to date has associated DPP4 gene polymorphisms rs2970932 and rs2268889 with T2DM; we selected both polymorphisms for evaluation as DPP4 itself is associated with T2DM [43]. These polymorphisms were primarily genotyped in 11 populations including an Asian (Japanese) population [44] and were therefore considered relevant to our subject population. Schosser et al. investigated the DPP4 gene polymorphism rs1861975 as a diabetes risk factor associated with depression because of the potential role of DPP4 in depression through its incretin effects [45]. Therefore, the polymorphisms selected for the DPP4 gene were rs2970932, rs2268889 and rs1861975. Cheng et al. found that WFS1 gene polymorphisms rs734312 and rs10010131 had significant protective effects on the risk of developing T2DM [46], while Fawcett identified the rs1046320 polymorphism as highly correlated with a strong and comparable association with T2DM [47]. Based on these findings, we selected rs734312, rs10010131 and rs1046320 as WFS1 gene polymorphisms to be studied. For the KCNJ11 gene, rs2285676, rs5218 and rs5210 polymorphisms were selected as these were previously identified as common KCNJ11 polymorphisms associated with diabetes [48].

For genotyping, standard EDTA tubes were for the collection of approximately 2.5 ml blood samples. Genomic DNA was extracted from peripheral blood lymphocytes using the QIAamp DNA Mini Kit (Qia-gen, CA, USA) according to the manufacturer’s protocol. The concentration of isolated DNA was determined using a NanoDrop 2000c spectrophotometer (Thermo Scientific, DE, USA) at a wavelength of 260–280 nm. The minimum DNA sample concentration required for analysis was 5 ng/μl [49,50]. Real-Time PCR was performed using the StepOnePlus™ instrument (Applied Biosystems, CA, USA). Each of the DPP4 (rs2970932, rs2268889 and rs1861975), WFSI (rs1046320, rs734312 and rs10010131) and KCNJ11 (rs2285676, rs5218 and rs5210) polymorphisms were genotyped using predesigned TaqMan® SNP Genotyping Assays (Applied Biosystems, CA, USA) as described in the manufacturer’s protocol [49].

Statistical analysis
All values were reported as percentages for categorical data and as mean ± standard deviation for continuous data. For normally distributed variables (age, duration of T2DM, waist circumference, FPG, HbA1c, insulin
level, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, SBP, DBP, ALT, AST, HOMA2B, HOMAIR and sCD26 levels), an independent t-test was performed to determine the association with DPP-4 inhibitor treatment response. For variables that are not normally distributed (sex, ethnicity, BMI, hypertension, peripheral neuropathy and dyslipidemia), a Pearson Chi-square test was performed.

TaqMan® Genotyper Software (Applied Biosystems) was used to analyze the genotypes, while Haploview 4.2 software was used to analyze the haplotypes and the Hardy–Weinberg equilibrium (HWE) was evaluated for all polymorphism groups using a goodness-of-fit X² test with one degree of freedom [51]; Bonferroni adjustment was used to obtain the pre-defined p-value for genetic associations [52], where the resulted p-value of less than 0.0056 were considered in agreement with HWE [51]. Predictor models for DPP-4 inhibitor treatment response were developed based on three main elements, demographic factors, clinical factors and genetic factors, using the binary logistic regression analysis (likelihood ratio method). Predictor variables were selected based on significance outcome during preliminary statistical analyses (Table 1 & 2). Hosmer and Lemeshow testing was used to evaluate the predictor models; p > 0.05 indicated that that test model data were adequate. Omnibus test values with p < 0.05 (significant) represented model efficiency. The Nagelkerke R square test, which estimates R² values in logistic regression models, was used to show percentage dependence variables predicted by the predictor variables. The predictor ranking of model was determined by the odds ratio (OR) and significance (p < 0.05) values.

Definitions
According to the American Diabetes Association, patients aged above 65 years are defined as older adults [2]. Therefore, subjects were categorized into two age categories: less than 65 years and 65 years or above. For the duration of T2DM, because the risk of cardiovascular disease or all-cause mortality increases after 10 years of a diabetes diagnosis, we classified subjects as less than 10 years and 10 or more years diagnosed with T2DM [54].

The target FPG level for diabetes patients is less than 7 mmol/l, according to the American Diabetes Association [2]. Therefore, FPG results were categorized into greater than or equal to 7 mmol/l and less than 7 mmol/l. FPI levels less than 174 pmol/l are recommended [55], therefore FPI levels were categorized into less than 174 pmol/l and greater than or equal to 174 pmol/l. Normal triglycerides levels are less than 1.7 mmol/l [2], so triglycerides data were categorized into greater than or equal to 1.7 mmol/l and less than 1.7 mmol/l. The target LDL cholesterol for diabetes patients is less than 2.59 mmol/l [2], so LDL cholesterol levels were categorized into less than 2.59 mmol/l and greater than or equal to 2.59 mmol/l. The DBP target in diabetes is less than 90 mmHg [2], so DBP data were categorized into less than 90 mmHg and greater than or equal to 90 mmHg. The normal AST range is 1–31 U/l [56], so these values were categorized into 1–31 U/l and greater than or equal to 31 U/l. HOMAIR values for subjects with normal glucose tolerance are between 1.7 and 2.5 [53,57]. However, insulin resistance is higher in diabetes patients [58]. Esteghamati et al. conducted a study in 3071 Iranian subjects to evaluate HOMAIR values in populations with or without diabetes and determined that 3.875 was the optimal cut-off point in T2DM patients [58]. We therefore categorized HOMAIR results into less than 3.875 (insulin sensitive) and greater than or equal to 3.875 (insulin resistance).

Results

Demographic & clinical characteristics
As shown in Table 1, age, duration of T2DM, FPG, HbA1c, insulin levels, triglycerides, LDL cholesterol, DBP, AST and HOMAIR were chosen as variables to potentially predict the DPP-4 inhibitor treatment response and were therefore used for further analyses.

Laboratory values
HbA1c values for the case group subjects were 1% lower than in the control group (7.4 [±1.4] and 8.4 [±2.3], respectively). Additionally, the case group had lower FPG values than the control group, with a mean difference of 0.7 mmol/l (Table 1). Blood lipid and liver enzyme values were similar between the groups. Mean blood pressure values in both groups were within the normal range for T2DM patients (Table 1).

Genotyping results
DPP4, WFS1 and KCNJ11 polymorphisms were genotyped and results are presented in Table 2. For DPP4 gene, the frequencies of the CC, CT and TT genotypes of the DPP4 rs2970932 polymorphism were 73%, 25% and 2% in subjects with good DPP-4 inhibitor treatment response, and 77%, 21.3%, 1.6% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele C is protective against DPP-4 inhibitor treatment response (C vs T, OR: 0.8 [95% CI: 0.626–1.537]; p = 0.297). Meanwhile, the frequencies of the AA, AG and GG genotypes of the DPP4 rs2268889 polymorphism were 14.9%, 48.9%, 36.5% in subjects with good DPP-4 inhibitor treatment response, and 18.6%, 44.3%, 37.2% in subjects with poor DPP-4 inhibitor treatment response, respectively.
### Table 1. Demographics and clinical characteristics of T2DM patients receiving DPP-4 inhibitor treatment and comparison with control patients.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n = 331)</th>
<th>Control (n = 331)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>– Male</td>
<td>168 (50.8%)</td>
<td>163 (49.2%)</td>
<td>0.687†</td>
</tr>
<tr>
<td>– Female</td>
<td>163 (49.2%)</td>
<td>168 (50.8%)</td>
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</tr>
<tr>
<td>Ethnicity:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>– Malay</td>
<td>150 (45.3%)</td>
<td>169 (51.1%)</td>
<td>0.462†</td>
</tr>
<tr>
<td>– Chinese</td>
<td>99 (29.9%)</td>
<td>89 (26.9%)</td>
<td></td>
</tr>
<tr>
<td>– Indian</td>
<td>63 (19.0%)</td>
<td>57 (17.2%)</td>
<td></td>
</tr>
<tr>
<td>– Others</td>
<td>19 (5.7%)</td>
<td>16 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>56.3 (±6.8)</td>
<td>58.0 (±7.3)</td>
<td>0.036‡,§</td>
</tr>
<tr>
<td>Duration of T2DM (year)</td>
<td>9.1 (±4.5)</td>
<td>8.8 (±4.2)</td>
<td>0.043‡,§</td>
</tr>
<tr>
<td>BMI (kg/m²):</td>
<td></td>
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</tr>
<tr>
<td>– Underweight (&lt;18.50)</td>
<td>16 (4.8%)</td>
<td>3 (0.9%)</td>
<td>0.449†</td>
</tr>
<tr>
<td>– Normal weight (18.50–24.99)</td>
<td>112 (33.8%)</td>
<td>94 (28.4%)</td>
<td></td>
</tr>
<tr>
<td>– Overweight (25.00–29.99)</td>
<td>165 (49.8%)</td>
<td>174 (52.6%)</td>
<td></td>
</tr>
<tr>
<td>– Obesity (&gt;30.00)</td>
<td>38 (11.6%)</td>
<td>60 (18.1%)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
<td>103.0 (±12.4)</td>
<td>106.4 (±13.3)</td>
<td>0.284‡</td>
</tr>
<tr>
<td>Laboratory values:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– FPG (mmol/L)</td>
<td>8.8 (±1.6)</td>
<td>9.5 (±2.4)</td>
<td>0.008‡,§</td>
</tr>
<tr>
<td>– A1c (%)</td>
<td>7.4 (±1.4)</td>
<td>8.4 (±2.3)</td>
<td>&lt;0.001‡,§</td>
</tr>
<tr>
<td>– Insulin level (pmol/L)</td>
<td>123.7 (±77.7)</td>
<td>141.8 (±79.0)</td>
<td>0.047‡,§</td>
</tr>
<tr>
<td>– Total cholesterol (mmol/L)</td>
<td>4.9 (±1.1)</td>
<td>5.0 (±1.2)</td>
<td>0.654†</td>
</tr>
<tr>
<td>– Triglyceride (mmol/L)</td>
<td>2.0 (±1.0)</td>
<td>2.2 (±0.9)</td>
<td>0.029‡,§</td>
</tr>
<tr>
<td>– HDL cholesterol (mmol/L)</td>
<td>1.1 (±0.3)</td>
<td>1.4 (±0.5)</td>
<td>0.392‡</td>
</tr>
<tr>
<td>– LDL cholesterol (mmol/L)</td>
<td>2.5 (±0.7)</td>
<td>2.6 (±0.9)</td>
<td>0.016‡,§</td>
</tr>
<tr>
<td>– DBP (mmHg)</td>
<td>77.4 (±9.1)</td>
<td>81.2 (±8.8)</td>
<td>0.009‡,§</td>
</tr>
<tr>
<td>– SBP (mmHg)</td>
<td>129.5 (±12.7)</td>
<td>133.4 (±15.6)</td>
<td>0.317‡</td>
</tr>
<tr>
<td>– ALT (IU/L)</td>
<td>25.8 (±7.0)</td>
<td>27.5 (±6.7)</td>
<td>0.559‡</td>
</tr>
<tr>
<td>– AST (IU/L)</td>
<td>20.9 (±5.6)</td>
<td>19.4 (±5.3)</td>
<td>0.038‡,§</td>
</tr>
<tr>
<td>– HOMA2B (%)</td>
<td>60.6 (±29.7)</td>
<td>61.5 (±30.8)</td>
<td>0.571‡</td>
</tr>
<tr>
<td>– HOMAIR (%)</td>
<td>2.5 (±1.6)</td>
<td>3.1 (±1.7)</td>
<td>0.013‡,§</td>
</tr>
<tr>
<td>– sCD26 level (ng/ml)</td>
<td>485.3 (±77.8)</td>
<td>608.6 (±97.3)</td>
<td>0.441‡</td>
</tr>
<tr>
<td>Comorbidities:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Hypertension</td>
<td>Yes</td>
<td>Yes</td>
<td>0.666‡</td>
</tr>
<tr>
<td></td>
<td>308 (93.1%)</td>
<td>318 (96.1%)</td>
<td></td>
</tr>
<tr>
<td>– No</td>
<td>308 (93.1%)</td>
<td>318 (96.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23 (6.9%)</td>
<td>13 (3.9%)</td>
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</tr>
</tbody>
</table>

Results expressed as n (%) or mean values (standard deviation). Chi-square test or t-test was used to determine p-values where appropriate. P-values <0.05 were considered statistically significant.

†Pearson Chi-square.
‡Independent t-test.
§Statistically significant.

DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; T2DM: Type 2 diabetes mellitus.
with poor DPP-4 inhibitor treatment response, respectively. It was found that allele A is protective against DPP-4 inhibitor treatment response (A vs G, OR: 0.6 [95% CI: 0.709–1.327]; p = 0.691). The frequencies of the AA, AC and CC genotypes of the DPP4 rs1861975 polymorphism were 16.9%, 41.9% and 41.2% in subjects with good DPP-4 inhibitor treatment response and 13.1%, 44.8%, 42.1% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele C is associated with DPP-4 inhibitor treatment response (C vs A, OR: 1.1 [95% CI: 0.733–1.384], p = 0.538).

For WFS1 gene, the frequencies of the AA, AG and GG genotypes of the WFS1 rs1046320 polymorphism were 64.9%, 19.6%, 15.5% in subjects with good DPP-4 inhibitor treatment response, and 61.7%, 26.2%, 12% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele A is associated with DPP-4 inhibitor treatment response (A vs G, OR: 1.0 [95% CI: 0.733–1.384], p = 0.538).

For KCNJ11 gene, the frequencies of the CC, CT and TT genotypes of the KCNJ11 rs2285676 polymorphism were 37.8%, 42.6%, 19.6% in subjects with good DPP-4 inhibitor treatment response, and 25.1%, 53.6%, 21.3% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele T is associated with DPP-4 inhibitor treatment response (T vs C: OR: 1.3 [95% CI: 0.749–1.390]; p = 0.128). Meanwhile, the frequencies of the AA, AG and GG genotypes of the KCNJ11 rs5218 polymorphism were 11.5%, 52.7%, 35.8% in subjects with good DPP-4 inhibitor treatment response, and 14.8%, 41.5%, 43.7% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele G is associated with DPP-4 inhibitor treatment response (G vs A, OR: 1.1 [95% CI: 0.733–1.384]; p = 0.538). Finally, as for the KCNJ11 rs5210 polymorphism, the frequencies of the AA, AG and GG genotypes were 16.2%, 56.8%, 27% in subjects with good DPP-4 inhibitor treatment response, and 20.8%, 51.9%, 27.3% in subjects with poor DPP-4 inhibitor treatment response, respectively. It was found that allele A is protective against DPP-4 inhibitor treatment response (A vs G, OR: 0.9 [95% CI: 0.731–1.352]; p = 0.585).

Genotypes selected for association modeling

The genotypes of WFS1 rs734312 (X² = 5.479; p = 0.0192; HWE: 0.7759), KCNJ11 rs2285676 (X² = 4.559; p = 0.0327; HWE: 0.9596) were chosen for further association studies. Although the p-values

### Table 1. Demographics and clinical characteristics of T2DM patients receiving DPP-4 inhibitor treatment and comparison with control patients (cont.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case (n = 331)</th>
<th>Control (n = 331)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comorbidities (cont.):</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>– Peripheral Neuropathy</td>
<td>Yes</td>
<td>Yes</td>
<td>0.687†</td>
</tr>
<tr>
<td></td>
<td>55 (16.6%)</td>
<td>41 (12.4%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>276 (83.4%)</td>
<td>290 (87.6%)</td>
<td></td>
</tr>
<tr>
<td>– Dyslipidemia</td>
<td>Yes</td>
<td>Yes</td>
<td>0.276†</td>
</tr>
<tr>
<td></td>
<td>236 (71.3%)</td>
<td>270 (81.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>No</td>
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<tr>
<td></td>
<td>95 (28.7%)</td>
<td>61 (18.4%)</td>
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</tbody>
</table>

Results expressed as n (%) or mean values (standard deviation). Chi-square test or t-test was used to determine p-values where appropriate. P-values <0.05 were considered statistically significant.

†Pearson Chi-square.
‡Independent t-test.
§Statistically significant.

DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; SBP: Systolic blood pressure; T2DM: Type 2 diabetes mellitus.
Table 2. Genotype and haplotype analysis of the DPP4, WFS1 and KCNJ11 genes polymorphisms. Hardy–Weinberg Equilibrium value of more than 0.05 is considered in agreement with Hardy–Weinberg Equilibrium [53].

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype, n (%)</th>
<th>Assoc Allele</th>
<th>Alleles, n (%)</th>
<th>OR</th>
<th>OR (95% CI)</th>
<th>X²</th>
<th>p-value</th>
<th>MAF</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C/C</td>
<td>T/T</td>
<td>C/C</td>
<td>T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPP4</td>
<td>rs2970932</td>
<td>a 108 (73.0)</td>
<td>37 (25.0)</td>
<td>3 (2.0)</td>
<td>253 (85.5)</td>
<td>43 (14.5)</td>
<td>0.825</td>
<td>0.626–1.537</td>
<td>1.088</td>
<td>0.2969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 141 (77.0)</td>
<td>39 (21.3)</td>
<td>3 (1.6)</td>
<td>321 (87.7)</td>
<td>45 (12.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 249 (75.2)</td>
<td>76 (23.0)</td>
<td>6 (1.8)</td>
<td>574 (86.7)</td>
<td>88 (13.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs2268889</td>
<td>a 22 (14.9)</td>
<td>72 (48.9)</td>
<td>54 (36.5)</td>
<td>116 (39.2)</td>
<td>180 (60.8)</td>
<td>0.644</td>
<td>0.709–1.327</td>
<td>0.158</td>
<td>0.6912</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 34 (18.6)</td>
<td>81 (44.3)</td>
<td>68 (37.2)</td>
<td>149 (40.7)</td>
<td>217 (59.3)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 56 (16.9)</td>
<td>153 (46.2)</td>
<td>122 (36.9)</td>
<td>265 (40.0)</td>
<td>397 (60.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs1861975</td>
<td>a 25 (16.9)</td>
<td>62 (41.9)</td>
<td>61 (41.2)</td>
<td>112 (37.8)</td>
<td>184 (62.2)</td>
<td>1.105</td>
<td>0.733–1.384</td>
<td>0.379</td>
<td>0.5379</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 24 (13.1)</td>
<td>82 (44.8)</td>
<td>77 (42.1)</td>
<td>130 (35.5)</td>
<td>236 (64.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 49 (14.8)</td>
<td>144 (43.5)</td>
<td>138 (41.7)</td>
<td>242 (36.6)</td>
<td>420 (63.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WFS1</td>
<td>rs1046320</td>
<td>a 96 (64.9)</td>
<td>29 (19.6)</td>
<td>23 (15.5)</td>
<td>221 (74.7)</td>
<td>75 (25.3)</td>
<td>0.989</td>
<td>0.702–1.422</td>
<td>0.004</td>
<td>0.9527</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 113 (61.7)</td>
<td>48 (26.2)</td>
<td>22 (12.0)</td>
<td>274 (74.9)</td>
<td>92 (25.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 209 (63.1)</td>
<td>77 (23.3)</td>
<td>45 (13.6)</td>
<td>495 (74.8)</td>
<td>167 (25.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs734312</td>
<td>a 2 (1.4)</td>
<td>47 (31.8)</td>
<td>99 (66.9)</td>
<td>51 (17.2)</td>
<td>245 (82.8)</td>
<td>1.697</td>
<td>0.675–1.746</td>
<td>5.479</td>
<td>0.0192</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 3 (1.6)</td>
<td>34 (18.6)</td>
<td>146 (79.8)</td>
<td>40 (10.9)</td>
<td>326 (89.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 5 (1.5)</td>
<td>81 (24.5)</td>
<td>245 (74.0)</td>
<td>91 (13.7)</td>
<td>571 (86.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs10010131</td>
<td>a 78 (52.7)</td>
<td>57 (38.5)</td>
<td>13 (8.8)</td>
<td>213 (72.0)</td>
<td>83 (28.0)</td>
<td>1.103</td>
<td>0.718–1.413</td>
<td>0.321</td>
<td>0.5708</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 87 (47.5)</td>
<td>82 (44.8)</td>
<td>14 (7.7)</td>
<td>256 (69.9)</td>
<td>110 (30.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 165 (49.8)</td>
<td>139 (42.0)</td>
<td>27 (8.2)</td>
<td>469 (70.8)</td>
<td>193 (29.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNJ11</td>
<td>rs2285676</td>
<td>a 60 (40.5)</td>
<td>62 (41.9)</td>
<td>26 (17.6)</td>
<td>182 (59.1)</td>
<td>114 (40.9)</td>
<td>1.479</td>
<td>0.753–1.403</td>
<td>4.559</td>
<td>0.0327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b 46 (25.1)</td>
<td>98 (53.6)</td>
<td>39 (21.3)</td>
<td>190 (51.9)</td>
<td>176 (48.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total 106 (32.0)</td>
<td>160 (48.3)</td>
<td>65 (19.6)</td>
<td>372 (56.2)</td>
<td>290 (43.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a: Good DPP-4 inhibitor treatment response (HbA1c <7%); A: Adenine; b: Poor DPP-4 inhibitor treatment response (HbA1c ≥7%); C: Cytosine; G: Guanine; HWE: Hardy–Weinburg equilibrium; MAF: Minor allele frequency; OR: Odds ratio; T: Thymine; X²: Chi-square test.
of both polymorphisms were not achieved within the Bonferroni adjustment (p < 0.0056), both polymorphisms were nominally significant (p < 0.05) and commonly associated with T2DM [46,48].

Antidiabetic treatment response

T2DM patients receiving DPP-4 inhibitor treatment showed better treatment responses than T2DM patients receiving other antidiabetic regimens (Table 3). The number of case group subjects with HbA1c levels of 7.0% or greater was lower (n = 183) compared with control group subjects (n = 236) (Table 3).

Predictive models

Predictive model of DPP-4 inhibitor treatment response with genetic variables

Significant genetic variables were WFS1 rs734312 and KCNJ11 rs2285676, rs5218 and rs5210 (Table 2). For binary logistic regression analysis, these variables were categorized into two groups each.

Subjects with KCNJ11 rs2285676 (genotype CC) were 2-times more likely to respond to DPP-4 inhibitor treatment than other subjects (OR: 2.1; 95% CI: 1.094–3.923). This indicated that KCNJ11 rs2285676 is a genetic predictor of DPP-4 inhibitor treatment response.

Predictive model of DPP-4 inhibitor treatment response with demographic, clinical & genetic variables

All demographic (Table 1), clinical (Table 1) and genetic variables were included in overall model predictors of DPP-4 inhibitor treatment response. Subjects with triglycerides less than 1.7 mmol/l were 2.2-times more likely to respond to DPP-4 inhibitor treatment compared with other subjects (OR: 2.2; 95% CI: 1.031–4.723). Subjects with DBP less than 90 mmHg were 1.7 more likely to respond to DPP-4 inhibitor treatment compared with other subjects (OR: 1.7; 95% CI: 1.009–2.892). Subjects with KCNJ11 rs2285676 (genotype CC) were 2-times more likely to respond to DPP-4 inhibitor treatment than other subjects (OR: 2.0; 95% CI: 1.025–3.767). Triglycerides, DBP and KCNJ11 rs2285676 were thus considered predictors of DPP-4 inhibitor treatment response.

Discussion

A meta-analysis by Kim et al. found that BMI was significantly correlated with HbA1c-lowering effects of DPP-4 inhibitors in Asian T2DM patients [59]. However, BMI was not found to be statistically significant in our study during the preliminary analysis (Table 1), thus not included in our model of predict-
ing the DPP-4 inhibitor treatment response. A study by Monami et al. found that HbA1c was significantly reduced by DPP-4 inhibitors at 24 weeks by 0.6 (0.5–0.7%), as compared with placebo [60]. The study also found no difference in HbA1c in comparison with α-glucosidase inhibitors and thiazolidinediones, whereas metformin and sulfonylureas generated a greater HbA1c lowering effects as compared to DPP-4 inhibitors [60]. Monami et al. concluded that DPP-4 inhibitors are more effective in older patients with mild to moderate fasting hyperglycemia [60]. In 2015, Esposito et al. observed a decreased of HbA1c from baseline (by -0.77% [95% CI: -0.82 to -0.72%]) from 98 RCTs with 100 arms consisting of 26 arms with vildagliptin, 37 with sitagliptin, 13 with saxagliptin, 13 with linagliptin and 11 with alogliptin [61]. Age, duration of treatment and previous diabetes drugs provided low predictive power (less than 1%) to the DPP-4 inhibitor treatment response [61]. Tanaka et al. investigated the characteristics of patients in whom receiving full HbA1c lowering effect of vildagliptin, had found that age, gender and BMI showed no significant differences among categories, and also no significant difference in HbA1c lowering effect of concomitant oral antidiabetics with vildagliptin, nor antidiabetics replaced to vildagliptin [62]. Similarly in our current study, we found that age and duration of T2DM showed no significant difference to the DPP-4 inhibitors treatment response. However, triglyceride, DBP and KCNJ11 rs2285676 (genotype CC) showed to be the significant predictors of DPP-4 inhibitors treatment response.

DPP-4 inhibitors affect postprandial lipid levels; a study by Matikainen et al. showed that vildagliptin treatment for 4 weeks in T2DM patients improved postprandial plasma triglycerides and apolipoprotein B-48-containing triglyceride-rich lipoprotein particle metabolism following consumption of a lipid-rich meal [63]. DPP4 itself is highly expressed in human adipocytes, and further increased in subcutaneous and visceral adipose tissue of obese individuals [64,65]. Elevated DPP4 expression in the adipose tissue of obese patients correlates with various metabolic syndrome markers such as plasma triglycerides, BMI, waist circumference, HOMAIR, fat cell volume and adipokine leptin [64,65]. DPP4 release also increased significantly during in vitro adipocyte differentiation [64,65], a finding supported by Rosmaninho-Salgadoa et al. who showed that DPP4 stimulates lipid accumulation, PPAR-γ expression and neuropeptide Y cleavage, suggesting that it might stimulate adipocyte differentiation [66]. Turcot et al. suggest that increased DPP4 gene expression in visceral adipose tissue may serve as a marker of visceral adipose tissue inflammation, known to be associated with metabolic disturbances; the study also found that DPP4 mRNA abundance correlated positively with the plasma total-/HDL-cholesterol ratio, suggesting that DPP4 gene expression is potentially associated with lipid profiles in severely obese individuals [67]. Shirakawa et al. investigated the effect of DPP-4 inhibition on adipose tissue and found that the DPP-4 inhibitor des-fluoro-sitagliptin improved linoleic acid-induced adipose tissue hypertrophy in β-cell-specific glucokinase haploinsufficient mice, a nonobese model of T2DM [68]. Shimasaki et al. reported that des-fluoro-sitagliptin reduced body adiposity without affecting food intake in C57BL/6 mice with diet-induced obesity [69]. Similar findings were obtained by Fukuda-Iisuru et al. using teneligliptin as the DPP-4 inhibitor [70]. Based on these studies, we conclude that DPP-4 inhibitors improve lipid profiles, including triglycerides [64,65,67,68], in line with our findings that T2DM patients with normal triglycerides are more likely to have a good response to DPP-4 inhibitor treatment. Possible mechanisms supporting our findings are the DPP-4 inhibitors suppressed postprandial elevation of triglycerides due to largely mediated by the inhibition of intestinal lipid absorption [71] and partly by the delayed gastric emptying [72].

**Table 3. The antidiabetic treatment responses in case and control groups.**

<table>
<thead>
<tr>
<th>Treatment response</th>
<th>Case (n = 331)</th>
<th>Control (n = 331)</th>
<th>Total (n = 662)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good response (HbA1c &lt;7.0%)</td>
<td>148 (45.1%)</td>
<td>95 (39.1%)</td>
<td>243 (36.7%)</td>
</tr>
<tr>
<td>Poor response (HbA1c ≥7.0%)</td>
<td>183 (43.7%)</td>
<td>236 (56.3%)</td>
<td>419 (63.3%)</td>
</tr>
</tbody>
</table>

**Table 4. Genetic variables categorized by genotype.**

<table>
<thead>
<tr>
<th>Genetic variables</th>
<th>Case (n = 331)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFS1 rs734312</td>
<td></td>
</tr>
<tr>
<td>Genotype AA†</td>
<td>5 (1.5%)</td>
</tr>
<tr>
<td>Genotype AG</td>
<td>81 (24.5%)</td>
</tr>
<tr>
<td>Genotype GG</td>
<td>245 (74.0%)</td>
</tr>
<tr>
<td>KCNJ11 rs2285676</td>
<td></td>
</tr>
<tr>
<td>Genotype CC</td>
<td>102 (30.8%)</td>
</tr>
<tr>
<td>Genotype CT</td>
<td>161 (48.6%)</td>
</tr>
<tr>
<td>Genotype TT†</td>
<td>68 (20.5%)</td>
</tr>
</tbody>
</table>

†Designated the reference category as the element of variability present was of lower risk.

A: Adenine; C: Cytosine; G: Guanine; T: Thymine.
may therefore serve as a predictor of DPP-4 inhibitor treatment response.

A study by Sufiun et al. found that vildagliptin significantly increased urine sodium excretion and normalized blood pressure in Dahl salt-sensitive rats, suggesting that vildagliptin may have antihypertensive effects [71]. Mason et al. also found that saxagliptin treatment (10 mg/kg/day) for 8 weeks reduced mean arterial pressure by 12 mmHg (p < 0.001) in hypertensive rats, suggesting that DPP-4 inhibition reduces blood pressure [72]. Meanwhile, Mistry et al. showed that sitagliptin significantly reduced SBP and DBP (2–3 mmHg and 1.6–1.8 mmHg, respectively) in a 24-h period in nondiabetic individuals with mild-to-moderate hypertension [73]. Conversely, Jackson et al. found that the inhibition of DPP-4 increased arterial blood pressure via Y(1) receptors when elevated blood pressure was reduced with antihypertensive drugs, provided that the sympathetic nervous system was functional [74]. Marney et al. showed that sitagliptin reduced blood pressure during low-dose ACE inhibition (enalapril, 5 mg) but that the reverse was observed during high-dose ACE inhibition (enalapril, 10 mg), suggesting that the combination of a DPP-4 inhibitor with a high-dose ACE inhibitor can cause the activation of sympathetic tone, thus impairing blood pressure reduction [75]. Our findings conclude that T2DM patients with DBP less than 90 mmHg will show a good treatment response to DPP-4 inhibitor therapy. Based on the previous studies, however, blood pressure reduction by DPP-4 inhibitors might only occur if patients are not receiving antihypertensive agents or low-dose ACE inhibitors. Nevertheless, this observation requires further investigation in a longer prospective study. Pacheco et al. suggested possible mechanism of blood pressure reduction by DPP-4 inhibitor was due to the decreasing of the expression of sodium/hydrogen exchanger isoform 3 in the microvilli membranes of the proximal renal tubule, resulting to increase in the urinary sodium excretion as well as the urinary volume leading to reducing blood pressure [76]. However, to the best of our knowledge, there is no exact mechanism explaining on how DPP-4 inhibitor reduces diastolic blood pressure alone.

Our findings supported our theory that KCNJ11 genes are associated with DPP-4 inhibitor treatment response as they control the secretion of insulin from pancreatic β-cells into the circulation via KATP channels [4]. Hart et al. investigated the chymotrypsinase in the regulation of the incretin pathway had found that the rs7202877 near CTRB1/2 gene was significantly associated with a lower HbA1c (0.51 ± 0.16% [5.6 ± 1.7 mmol/mol]; p = 0.0015) response to DPP-4 inhibitor treatment in T2DM patients with G-allele [77]. Chymotrypsinogens are highly expressed in the exocrine pancreas as well as in pancreatic β-cells [78]. Meanwhile, Zimdahl et al. produced a recent study on the HbA1c lowering effect of TCF7L2 on patients prescribed with linagliptin to treat T2DM [79]. According to the study, linagliptin significantly reduced HbA1c in T2DM patients with TCF7L2 rs7903146 (genotype CC [n = 356]: -0.82% [-9.0 mmol/mol]; p < 0.0001; genotype CT [n = 264]: -0.77% [-8.4 mmol/mol]; p < 0.0001; genotype TT [n = 73]: -0.57% [-6.2 mmol/mol]; p < 0.0006) [79]. Although the study concluded that linagliptin significantly improved hyperglycemia in T2DM patients both with or without the TCF7L2 gene diabetes risk alleles, it was found that linagliptin treatment response was better in patients with TT genotype compared to CC genotype (∼2.26% [-2.8 mmol/mol]; p = 0.0182), thus suggesting that diabetes susceptibility genes may influence the inter-individual variability of response to antidiabetic treatments [79].

Relating to our study of KCNJ11 gene, previous studies showed the HbA1c lowering effects of KCNJ11 in T2DM patients receiving oral antidiabetics [80,81,82]. Javorsky et al. found that KCNJ11 K-allele produced a significant HbA1c lowering effect (by 0.16% [95% CI: 0.01–0.32; p = 0.038]) in patients treated with glimepiride after metformin monotherapy failure [80]. Meanwhile, a study by He et al. examined the association between KCNJ11 E23K and therapeutic response to repaglinide in Chinese population, found that HbA1c lowering effect was greater in patients

<table>
<thead>
<tr>
<th>Predictor variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCNJ11 rs2285676 (genotype CC)</td>
<td>2.1</td>
<td>1.094</td>
<td>3.923</td>
</tr>
<tr>
<td>KCNJ11 rs2285676 (genotype CT)</td>
<td>0.9</td>
<td>0.522</td>
<td>1.727</td>
</tr>
</tbody>
</table>

*Statistically significant.

Omnibus test of model coefficients: $\chi^2 = 17.681$, p = 0.001.
Nagelkerke $R^2$: 0.070.
Hosmer and Lemeshow Test: $\chi^2 = 2.692$, p = 0.442.
OR: Odds ratio.
with EK and KK genotypes than in EE genotypes (EE: 1.52 ± 1.03%, EK: 2.33 ± 1.53%, and KK: 2.65 ± 1.73%, p = 0.022) [81]. Sesti et al. found that KCNJ11 E23K variant may influence the response to sulphonylureas treatment in T2DM patients where K allele showed no differences in glucose-stimulated insulin secretion and more prone to reduced response upon glibenclamide stimulation (p = 0.09) [82]. E23K variant showed worse response to glibenclamide after 24 h exposure to high (16.7 mmol/L) glucose concentration where insulin release was significantly reduced (p = 0.01) [82]. As our findings, we suggest that KCNJ11 rs2285676 is a genetic predictor of DPP-4 inhibitor treatment response. Sakamoto et al. found that the KCNJ11 rs2285676 polymorphism was associated with T2DM in a Japanese population [83], Koo et al. reported its association with T2DM in a Korean population [84], and Liu et al. identified KCNJ11 rs2285676 as a T2DM susceptibility gene in a Chinese Han population [85]. Although the preliminary analyses found that DPP4 rs2970932, WFS1 rs734312 was also associated with DPP-4 inhibitor treatment response, logistic regression analyses failed to reach statistical significance; investigation was designed as a case–control study aimed at evaluating the associations between genetic variations and the DPP-4 inhibitor treatment response. Therefore, a larger population size is required to obtain greater statistical significance in future studies.

An overall model of DPP-4 inhibitor treatment response was developed using demographic, clinical and genetic variables to determine the optimal DPP-4 inhibitor treatment response in T2DM patients. From this model, the T2DM patients most likely to benefit from DPP-4 inhibitor treatment are those with a KCNJ11 rs2285676 (genotype CC) polymorphism and triglycerides and DBP values within normal ranges. We also propose that DPP-4 inhibitors are most suitable for the treatment of early-stage T2DM.

**Conclusion**

Our study has provided insights into DPP-4 inhibitor therapy as triglycerides, DBP and KCNJ11 rs2285676 were found to be predictors of DPP-4 inhibitor treatment response. Our predictive models may facilitate future genetic and clinical investigations, leading to a better understanding of the underlying disease, improvements in targeted therapy and better prescribing.

**Study limitations**

To determine the predictors of drug response, a large, well-designed clinical study population is the optimum requirement. However, this limitation was addressed here by collecting more samples than our minimal required sample size.

**Future perspective**

Our study may provide a better understanding of the exact relationship between DPP-4 inhibitor response and genes polymorphisms in the incretin pathway. Here, our findings may be used to increase the efficacy of the DPP-4 inhibitor treatment by the identification of the demographic, clinical and genetic factors involved in drug response.

In 5–10 years, we hope that our findings may be used to facilitate the development of better drug formulations for T2DM-targeted therapy, and we also hope that our findings will successfully assisting into strategizing the standard operating procedure of a diabetes clinic settings, as well as into the clinical practice guidelines for the treatment of diabetes; as in the term of selection of suitable candidate for starting the DPP-4 inhibitor treatment.
Executive summary

DPP-4 inhibitors
- Oral antidiabetic agents used to control blood glucose levels in Type 2 diabetes mellitus (T2DM) by altering the patient’s incretin pathway.
- Block the dipeptidyl peptidase-4 (DPP-4) enzyme, thus promoting active GLP-1 binds GLP-1R on pancreatic β-cells, thereby activating the expression of multiple genes resulting in insulin secretion from β-cells into the circulation to control blood glucose levels.

Selection of demographic predictors variables
- Generally, our demographic parameters take the form of age, gender, race and duration of T2DM.

Selection of clinical predictors variables
- Based on an understanding of the disease pathogenesis and the mechanism of drug action, we hypothesized that four clinical factors, sCD26, HOMA2B, HOMAIR and HbA1c (glycated hemoglobin), would be associated with the response to DPP-4 inhibitors.
- Serum sCD26 levels correlated strongly with circulating DPP-4 activity in humans and increased sCD26 may be associated with reduced sitagliptin efficacy. Studies found that DPP-4 inhibitors improved insulin resistance and HbA1c in T2DM.
- Other general clinical parameters investigated are fasting plasma glucose, insulin level, total cholesterol, triglyceride, HDL and LDL cholesterol, diastolic blood pressure (DBP), systolic blood pressure, alanine transferase, body mass index and waist circumference.

Selection of genetic predictors variables
- Based on disease pathogenesis and the incretin pathway, we hypothesized that three genes, DPP4, WFS1 and KCNJ11, play a role in the response to DPP-4 inhibitors.
- DPP4 encodes DPP4 enzyme that rapidly inactivates active GLP-1 to inactive GLP-1 to prevent it from binding to the GLP-1R receptor, thus leading to poor glycemic control resulting from insufficient insulin production by pancreatic β-cells.
- WFS1 plays a significant role in the maintenance of homeostasis of the endoplasmic reticulum which is important for subsequent insulin release into the pancreatic β-cell matrix. Inactivation of β-cell WFS1 may disrupt endoplasmic reticulum homeostasis, resulting in β-cell dysfunction and contributing to T2DM.
- KCNJ11 regulates one of the pancreatic β-cell ATP-sensitive potassium (KATP) channel subunits, where it mediates insulin exocytosis from pancreatic β-cells into the circulation via KATP channels to complete the incretin signaling pathway of insulin secretion.

Genetic predictor model
- KCNJ11 rs2285676 was found to be associated with DPP-4 inhibitor treatment response.
- Subjects with KCNJ11 rs2285676 (genotype CC) were 2.1-times more likely to response to DPP-4 inhibitor treatment.

Combination of demographic, clinical & genetic predictors model
- We combined demographic, clinical and genetic factors that were found to be associated with DPP-4 inhibitor treatment response to built this predictors model.
- Subjects with triglycerides less than 1.7 mmol/l were 2.2-times more likely to response to DPP-4 inhibitor treatment.
- Subjects with DBP less than 90 mmHg were 1.7 more likely to response to DPP-4 inhibitor treatment.
- Subjects with KCNJ11 rs2285676 (genotype CC) were 2-times more likely to response to DPP-4 inhibitor treatment.

Conclusion
- Triglycerides, DBP and KCNJ11 rs2285676 were found to be predictors of DPP-4 inhibitor treatment response.
Explained the mechanism of pancreatic β-cell insulin secretion, involving dipetidyl peptidase-4, WFS1 and KCNJ11 genes in the incretin pathway.


Explained the level of HbA1c 7% as the glycemic control target for Type 2 diabetes mellitus (T2DM), the definition of age and the levels of fasting plasma glucose, TG, LDL and diastolic blood pressure in diabetics.


Our theory that KCNJ11 genes are associated with dipetidyl peptidase-4 (DPP-4) inhibitor treatment response as they control the secretion of insulin from pancreatic β-cells into the circulation via KATP channels.

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