Pancreatic gene variants potentially associated with dipeptidyl peptidase-4 inhibitor treatment response in Type 2 diabetes

In the adult pancreas, the expression of the genes PAX4, KCNQ1, TCF7L2, KCNJ11, ABCC8, MTNR1B and WFS1 are mainly restricted to β cells to maintain glucose homeostasis. We have identified these genes as the main regulators of incretin-mediated actions, and therefore they may potentially influence the response of DPP-4 inhibitors. This review represents the first detailed exploration of pancreatic β-cell genes and their variant mechanisms, which could potentially affect the response of DPP-4 inhibitors in Type 2 diabetes. We have focused on the signaling pathways of these genes to understand their roles in gastrointestinal incretin-mediated effects; and finally, we sought to associate gene mechanisms with their Type 2 diabetes risk variants to predict the responses of DPP-4 inhibitors for this disease.

**KEYWORDS:** ABCB8, dipeptidyl peptidase-4 inhibitors, KCNJ11, KCNQ1, MTNR1B, PAX4, TCF7L2, WFS1

Type 2 diabetes (T2D) is attributed to genetic and environmental factors, and can be characterized by insulin resistance and dysfunctional pancreatic β cells. Treatments for diabetes have included physical exercise, diet modifications and drug therapy. This article will focus on the effects of incretins on pancreatic cells. The ‘incretin effect’ phenomenon is attributed to the gastrointestinal proteins, incretins, which are released in response to the ingestion of nutrients, thus stimulating glucose-induced production of insulin from pancreatic β cells. In other words, Tolhurst et al. describe the incretin effect as the phenomenon of more insulin release, triggered by oral ingestion of glucose compared with the same amount of glucose administered intravenously. In humans, incretin effects are mediated by two incretin hormones, GLP-1 and GIP. GLP-1 and GIP mediate their effects via GLP-1R and GIPR, respectively. GLP-1R is located on pancreatic α and β cells, as well as in the heart, CNS, kidney, lung and gastrointestinal tract. GIPR is mainly located on pancreatic β cells, in the CNS, adipose tissue and osteoblasts. GLP-1 is mainly produced by enteroendocrine L cells in the distal intestine. GLP-1 exists in two molecular forms, GLP-1 (7–37) and GLP-1 (7–36) amide. Although both forms are equipotent, only GLP-1 (7–36) amide circulates in high levels after food ingestion. GLP-1 potentiates glucose-dependent insulin secretion from pancreatic β cells in response to ingestion of carbohydrates and lipid-rich meals. Furthermore, GLP-1 exerts other effects: the suppression of glucagon secretion, stimulation of β cell neogenesis and insulin biosynthesis, inhibition of gastric emptying and acid secretion, reduction of food intake, and trophic effects on the pancreas. In addition, this hormone contributes to GLP-1 receptor-dependent (GLP-1R-dependent) regulation of glucose homeostasis via the modulation of gastric emptying and acquisition of neural circuits in the portal region and CNS. Two SNPs in GLP1R, rs6923761 and rs3765467 were found to be nominally associated with altered insulin secretory response to GLP-1 infusion, in the presence of hyperglycemia in nondiabetic subjects. To date, GLP1R gene variations have not been associated with T2D.

GIP is a 42-amino acid peptide predominantly secreted by proximal small intestinal K cells in response to ingestion of carbohydrates and lipids. GIP potentiates glucose-stimulated insulin release through G-protein-coupled receptors, mainly located on pancreatic β cells. mRNA expression was reduced in human islets in T2D, where the A allele of GIPR was associated with impaired glucose- and GIP-stimulated insulin secretion.

Incretin-based therapy is the most recent antidiabetic agent to control blood glucose levels. This control is achieved by altering the patient’s incretin pathway. Charbonnel and Cariou summarized 23 randomized controlled trials of DPP-4 inhibitor therapies, and demonstrated that DPP-4 inhibitors used as monotherapy or as combination therapy with one oral antidiabetic produce moderate glycated

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Dipeptidyl peptidase-4 (DPP-4) inhibitors are rapidly inactivated by the plasma half-life of 5–7 min, and circulating GLP-1 by the plasma half-life of 1–2 min\[10,17\]. The DPP-4 enzyme targets numerous substrates, including neuropeptides, cytokines and other gastrointestinal peptides\[10\]. Consequently, the enzyme is distributed in multiple tissues, including endothelial cells, lymphocytes, the CNS, kidney, lung and pancreas\[10\]. The distribution and rapid activity of the DPP-4 enzyme on both incretin hormones ultimately determines the outcome of glucose-stimulated insulin secretion, hence glycemic control.

As illustrated in Figure 1, the DPP-4 inhibitor blocks the DPP-4 enzyme, and the degradation of active GLP-1 (7–36 amide) into inactive GLP-1 (9–36 amide) is thus prevented\[19\]. Active GLP-1 binds GLP-1R on the pancreatic β cell, thereby activating the expression of multiple genes resulting in insulin secretion, β-cell proliferation and survival. Insulin is secreted from the β cell into the circulation to control blood glucose levels\[20\]. DPP-4 inhibitors increase circulating GLP-1 to physiological levels\[21\]. The full efficacy of any selected DPP-4 inhibitor must be achieved at a dose that inhibits more than 80% of the DPP-4 enzyme for 24 h, because increasing the dose above its threshold will not raise the efficacy\[16\]. Since the DPP-4 inhibitors bind to the DPP-4 enzyme to enhance GLP-1 action, the efficacy of the DPP-4 inhibitors could be affected by the expression of DPP4 gene variants\[19,22\]. A study was conducted to associate DPP4 gene variants with vildagliptin efficacy using various SNPs: g-234A/C, rs13015258, IVS8-128A/G, rs17848920, IVS8+46C/T, rs109330040, IVS11-143A/G, rs2302873, G645G, rs17848910, IVS22+4C/T and rs2268891; however, the study could not find any significant associations because of a small sample size\[22\].

The DDP-4 inhibitors sitagliptin, vildagliptin and saxagliptin were the first three DPP-4 inhibitors (up until 2009) to be approved by the US FDA for their therapeutic use in T2D\[17,23\]. However, saxagliptin was only approved for use in Europe in 2011 for the treatment of T2D associated with moderate to severe renal insufficiency and mild hepatic insufficiency\[24\]. Alogliptin and linagliptin are the most recent DPP-4 inhibitors to be launched on the market\[24\]. Dutogliptin, teneligliptin and SYR472 are currently undergoing Phase III clinical trials\[24\], while other more recently developed DPP-4 inhibitors, such as KRP104, LC15-0444 and melogliptin are undergoing Phase II clinical trials\[24\].

Following the failure of metformin to reach HbA1c target levels after 3 months of therapy in T2D patients, the American Diabetes Association (ADA) recommended DPP-4 inhibitors as an add-on therapy, a third-line triple oral therapy selection (if the HbA1c target was unachievable after 3 months), an option if sulfonylurea therapy was contraindicated, or an option when the risk of hypoglycemia was a major issue\[3\]. According to ADA statements by Nathan et al., DPP-4 inhibitor monotherapy will not cause hypoglycemia, and is expected to decrease HbA1c by 0.5–0.8% with no effect on weight\[23\]. Although DPP-4 inhibitors exhibit fewer side effects, the long-term safety is still not established\[23\]. Furthermore, their high cost may limit their usage to higher income patients\[25\].

Given that DPP-4 inhibitors act on pancreatic β cells, which play a role in T2D, we examined the association of gene variants involved in the function of pancreatic β cells as putative candidates for DPP-4 inhibitor treatment in T2D.

Therefore, the aim of the present article, which focuses on T2D, was to review the genetic variants associated with oral incretin-based therapy, and response pathways mediated by DPP-4 inhibitors. In this review we have determined DPP-4 treatment responses based on the insulin outcome, in which good treatment responses signified gene-activated pancreatic β cell insulin secretion and vice versa. We also explored several genes in the PI3K and Wnt pathways, which are known to play vital roles in response to DPP-4 inhibitors. The genes investigated in this review include PAX4, KCNQ1, TCFL7L2, KCNJ11, ABCC8, WFS1 and MTNR1B.
Pancreatic β cell genes that may be associated with DPP-4 inhibitor treatment response

We identified seven genes (PAX4, KCNQ1, TCF7L2, KCNJ11, ABCC8, MTNR1B and WFS1) as having the potential of possible relevance to be associated with the response to DPP-4 inhibitors according to drug pathway, metabolism and disease pathogenesis. The selection of these genes was based on the understanding of the disease pathogenesis and the mechanism of drug action. Therefore, the gene selected may include the drug target, pathway genes, drug-metabolizing enzymes and also the disease genes.

According to the incretin pathway, following ingestion of a meal, KCNQ1 in the intestinal L cells mediates the release of GLP-1 into the gastrointestinal circulation [7]. To prevent the GLP-1 from being inactivated, a DPP-4 inhibitor inhibits the DPP-4 enzyme resulting in active GLP-1 to bind with its receptor (GLP-1R) on the pancreatic β cell [19, 21]. This transmits a signal in the PI3K pathway [26], activating cascades of action that produce insulin, including the WFS1 gene in the endoplasmic reticulum of pancreatic cells, as WFS1 expression plays a significant role in insulin secretion in β cells [27, 28]. Next, following the incretin pathway, the expression of the MTNR1B gene may trigger the release of insulin into the circulation [29] through the pancreatic β cell KATP channels mediated by two genes, KCNJ11 and ABCC8 [30, 31]. The PAX4 and TCF7L2 genes are required for the maintainence of pancreatic β-cell proliferation and survival via the PI3K and Wnt pathways, respectively [32–34]. Altered expression of PAX4 and TCF7L2 genes may influence insulin production and secretion [32, 33–38] via the loss of pancreatic β-cell differentiation function, thus suggesting poor response to DPP-4 inhibitors [32, 39]. Through the identification of these seven genes according to the incretin pathway, we are hoping a set of pharmacogenomic markers may be provided in order to explore or determine response to existing DPP-4 inhibitor therapies.

Genes that play significant roles in insulin signaling by β cells can be mediated via the PI3K pathway. Kaneko et al., have shown glucose intolerance and reduced insulin secretion in response to glucose in mice lacking the Pik3r1 gene in β cells [26].

Class I PI3K enzymes control various cellular functions, including growth, differentiation, migration, survival and metabolism [14].

Upon insulin stimulation, PI3K generates PIP₃, which then activates Akt or PKB, and atypical forms of PKC λ/ζ through the activation of PDK1. Activated Akt suppresses gluconeogenesis and triggers glycogen synthesis through the phosphorylation of FoxO1 and GSK3β, respectively [40, 41].

Although DPP-4 inhibitors and GLP-1 receptor agonists work in the same incretin pathway in T2D, GLP-1 still requires the DPP-4 inhibitors to block the DPP-4 enzyme in order to prevent the inactivation of GLP-1. In an oral glucose tolerance test in nondiabetic subjects, KCNQ1 was associated with decreased GLP-1 concentrations while not affecting GLP-1 signaling [42]. This provides evidence that active GLP-1 concentration is crucial in the incretin pathway for producing insulin secretion, which is maintained by DPP-4 inhibitors.
We may conclude that the DPP-4 inhibitors response to increase or to prevent a reduction in insulin secretion could be more prominent than GLP-1, therefore the gene variants involved in the incretin pathway for insulin secretion are more related to DPP-4 inhibitor response.

**PAX4**

PAX4 encodes a family of transcription factors essential in pancreatic β-cell development and differentiation at the embryonic stage, thus promoting cellular proliferation, migration and survival [43–45]. PAX4 is important for the generation of islet cell progenitors, the maturation of both α and β cells during the embryonic stage, and the maturation of duodenal and jejunal endocrine cells [46–48]. PAX4 is located at the cytogenetic location of 7q32.1 [101].

Wang et al. provided more insight into the role of Pax4 in β-cell development by a comparative analysis of gene expression in the pancreas between Pax4-deficient versus wild-type mice newborns at various developmental stages. The study revealed a model of β-cell development, in which Ngn3 in β-cell precursors induced the expression of Pax4, Nkx2.2 and Nkx6.1, potentially leading to the expression of Islet1 and Pax6. Both Pax4 and Nkx2.2 play a role in β-cell differentiation. Therefore, increased levels of Pdx1 together with the induction of H69 expression, results in the synthesis of insulin. Pax4 function is required for the formation of insulin-producing cells. However, as the pancreas matures, Pax4 activity is only required for the proliferation and survival of β cells [32].

Findings from Brun et al. showed that the PAX4 gene stimulates β-cell proliferation and survival through concomitant regulation of the onecogenes, C-MYC and BCL-XL. Mitogens such as β cellular bind to the EGF receptor, which activates the PAX4 gene via the PI3K pathway. However, the precise underlying mechanisms remain unknown [2,49]. According to Brun et al., activated PAX4 stimulates β-cell proliferation via induced gene expression of C-MYC and BCL-XL. C-MYC promotes ID2 gene expression, resulting in the activation of cell cycle replication. The antiapoptosis gene, BCL-XL, promotes cell survival by preventing mitochondria from initiating the apoptotic cascade. The increased expression of BCL-XL may reduce glucose-provoked insulin secretion, because of alterations of the caspase pathway on mitochondrial ATP function and calcium homeostasis [2]. According to a study carried out by Brun, glucose promotes PAX4 expression via insulin through the activation of PI3K, ERK1/2 and cAMP-PKA pathways [50]. PAX4 expression was found to have a dependency on incretin action, as total inhibition of the stimulatory GLP-1 effects or any obstruction in the cascade of these pathways may results in definite PAX4 inhibition [50,51]. It was found by Johnson, that exocytosed insulin protects the pancreas islets from apoptosis via PDX1 [52]. As such, the PAX4 gene might have the probability of providing the same effect on the incretin pathway via the P3K cascade. Thus, the PAX4 gene may have the ability to influence DPP-4 inhibitor treatment response.

Larsson et al. revealed that mutations of PAX4 in the stomach showed a significant reduction in the numbers of somatostatin and serotonin cells; however, gastrin cell numbers were not affected. These results provide evidence that PAX4 is required for the expression of all upper gastrointestinal hormones. Moreover, PAX4 mutations in the duodenum caused reductions in CCK- and GIP- and PYY-immunoreactive cells [48]. Shima-jiri et al. revealed that a missense mutation of the R121W variant (located at the PAX4 pair domain) affected PAX4 transcription activity, leading to the loss of β-cell differentiation function, thus its association with T2D [48].

**KCNQ1**

KCNQ1 is one of the risk-conferring genes susceptible to T2D in east Asian, Japanese and European populations [53–55]. Its cytogenetic location is at 11p15.5–p15.4 [101]. KCNQ1 is expressed in epithelial cells of the exocrine and endocrine glands of the pancreas, where it mediates the release of GIP from intestinal K cells and GLP-1 from the intestinal L cells in response to food (Figure 2) [56]. Although the involvement of KCNQ1 in incretin secretion has not been shown, KCNQ1 is reported to be involved in hormone and electrolyte transport processes in the gastrointestinal tract [57]. Therefore, due to the expression of KCNQ1 in the epithelial cells, the genetic variants of KCNQ1 could affect the efficacy of the incretin transport mechanism in the gastrointestinal tract [7].

The incretin hormones, GLP-1 and GIP, bind to the pancreatic β-cell receptors, GLP-1R and GIPR, respectively, thereby activating a cascade of actions via cAMP-mediated induction of exchange protein directly activated by cAMP2 (EPAC2) and PKA, resulting in an increase in intracellular calcium. High concentration of calcium mediates the release of insulin into the circulation [7]. An association between rs151290 and glucose-stimulated GLP-1 and GIP levels
was found, where the SNP produces the strongest effect on insulin secretion after an oral glucose load, indicating the potential link between KCNQ1 gene variants and altered pancreatic β-cell function [42]. This led to the conclusion that KCNQ1 gene variants are associated with changes in incretin secretion [42].

According to a Japanese population study by Yasuda et al., rs2237892 was found to be the most significant SNP in KCNQ1 associated with T2D. The findings demonstrated the lowest p-value of 6.7 × 10^{-13}, and the odds ratio of 1.49. Similar results were observed in Korean, Chinese, European and two independent Japanese populations, in which rs2237892 was also detected to have an association with T2D [54]. Unoki et al. conducted a genome-wide study using 207,097 SNP markers in Japanese individuals. They found that the KCNQ1 gene was a strong risk-conferring factor for T2D, with the variants of rs2283228, rs2237895 and rs2237897 detected to have consistent association with the disease [55].

Tan et al. found that KCNQ1 variants of rs2237897, rs2237892 and rs2283228, are associated with T2D, fasting glucose and β-cell function in three ethnic groups in Singapore [56]. Liu et al. confirmed that KCNQ1 gene variants are associated with the risk of developing T2D in the Chinese population. This study used approximately 1912 unrelated Type 2 diabetics and approximately 2041 controls within the same geographical region to investigate the risk of diabetes from SNPs of KCNQ1 genes rs2237895, rs2237897 and rs2237892 [1].

Because KCNQ1 plays a significant role in activating the incretin pathway in pancreatic β-cells, it is essential to understand its role in the development of T2D.

![KCNQ1 pathway in insulin production by pancreatic β cells](source_image_url)

**Figure 2. KCNQ1 pathway in insulin production by pancreatic β cells.**

ER: Endoplasmic reticulum. Adapted with permission from [7].
### Table 2. Data representing KCNQ1 SNPs associated with Type 2 diabetes in human populations worldwide.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Population</th>
<th>HGN C approved gene symbol†</th>
<th>Full gene titles</th>
<th>RefSNP alleles</th>
<th>Gene type</th>
<th>Function class</th>
<th>OMIM number†</th>
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<th>SNP associated with T2D</th>
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</tbody>
</table>

†The Online Mendelian Inheritance in Man (OMIM) number and the cytogenetic locations are obtained from OMIM [101]. HGNC: HUGO Gene Nomenclature Committee; T2D: Type 2 diabetes.
β-cell insulin release, the response by DPP-4 inhibitors may be predicted by the presence of the at-risk KCNQ1 gene variants. However, caution should be taken with KCNQ1 variants (discussed in Table 2) when initiating DPP-4 inhibitor therapy in case of low insulin release, and thus a poor response to treatment.

**WFS1**
The WFS1 gene, with the cytogenetic location at 4p16.1 (Table 3), 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 because this organelle is responsible for the maturation of the prohormone precursor, proinsulin, to insulin, and for its subsequent release into the pancreatic β-cell matrix [27]. High intracellular calcium levels resulting from the activation of the KCNQ1 gene triggers the release of insulin from the pancreatic β cell into the circulation (Figure 2). Therefore, WFS1 gene expression plays a major role in the secretion of insulin in the human pancreas [7]. Inactivation of β cell WFS1 disrupts ER homeostasis, resulting in β-cell dysfunction, and thus contributing to T2D [27].

WFS1 plays an important role in stimulus secretion of insulin [28]. This role was evidenced in subjects with both Wolfram syndrome and diabetes, in which the progressive loss of β cells impaired stimulus secretion of insulin by these cells [28]. The findings were also supported by Fonseca et al., who showed that WFS1 gene expression in mouse islets was increased with 16.7 mM glucose and 30 mM KCl, and thereby concluded that WFS1 upregulation is crucial for insulin secretion [27]. WFS1 rs10010131 was found to specifically impair incretin-induced insulin secretion independently of insulin sensitivity [58]. A study by Heni et al. also concluded that diabetes risk gene variants of WFS1 are associated with impaired incretin signaling [59]. Since the results of both studies confirmed the association of WFS1 gene variants with incretin-induced insulin secretion, the response of oral incretin therapy such as DPP-4 inhibitors may be predicted by the presence of these gene variants based on the incretin signaling pathway.

Studies of the SNPs of WFS1 genes associated with T2D revealed that among the 31 tagged SNPs, the strongest association was with rs1046320 (Table 3) [60]. This strong association may thus lead to low levels of insulin secretion, despite treatment with antidiabetics, including DPP-4 inhibitors.

**TCF7L2**
The genetic variant of the TCF7L2 gene (cytogenetic location 10q25.3 [101]), rs7903146, is strongly associated with T2D. In vitro and in vivo studies have shown that altering the expression of TCF7L2 in pancreatic islets influences the secretion of insulin (Table 4) [33,35–38,61]. Furthermore, the risk T allele of the SNP of this gene was found to affect the enteroinsular axis and the relationship between its target hormones: glucagon, insulin and GIP [62]. TCF7L2 promotes cellular proliferation and survival in pancreatic β cells via the Wnt pathway [33,34]. The Wnt pathway consists of Wnt signaling molecule proteins that are ubiquitously secreted in order to regulate the cellular morphology, proliferation, motility and cell fate [62]. Binding of Wnt ligands to the cell surface receptors, FZD6 and LRPS or 6, inhibits APC–GSK3β activity, resulting in the accumulation of cytosolic β-catenin (Figure 3) [39,62]. Nuclear translocation of β-catenin and subsequent binding to LEF/TCF7L2 to form a β-catenin–T cell factor–DNA complex stimulates gene transcription of Cyclin D, PTX2 and PPARα, leading to cellular proliferation [62,63]. Without Wnt ligand binding, cytoplasmic β-catenin is rapidly phosphorylated by GSK3β, subsequently undergoing ubiquitination and degradation by proteasomes within the APC–GSK3β–axin protein complex [39]. This degradation thus reduces cellular proliferation [39].

According to studies on different racial populations, rs7903146, rs12255372, rs7901695, rs11196205 and rs10885390 were determined as TCF7L2 variants associated with T2D (Table 4). Furthermore, T2D risk by TCF7L2 variants were found to be associated with impaired β-cell function but not with insulin resistance [61]. Therefore, because impaired β-cell function may disrupt the secretion of insulin, response by DPP-4 inhibitor treatment is predicted to be low in T2D patients with such risk variants. TCF7L2 variants with decreased protein is reported to be associated with reduced islet GLP1R and GIPR expression, thus resulting in reduced glucose-stimulated insulin secretion by GLP-1 and GIP [64]. In conclusion, the presence of at-risk polymorphisms could be the marker for detecting the differences in incretin-based treatment response [65]. This would further suggest that the TCF7L2 variants could influence the dose of DPP-4 inhibitors used.

In a different study, the TCF7L2 variants rs7903146 and rs12255372, were associated
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Table 4. Data representing TCF7L2 SNPs associated with Type 2 diabetes in the worldwide population.

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†The Online Mendelian Inheritance in Man (OMIM) number and the cytogenetic locations are obtained from OMIM [10].

HGNC: HUGO Gene Nomenclature Committee; T2D: Type 2 diabetes.
with impaired incretin-induced insulin secretion. Although the pathogenic mechanism remains unclear, a defect in the incretin-induced stimulus of insulin secretion coupling in the incretin signaling pathway may influence a reduction of insulin secretion in TCF7L2 variant carriers [66].

**KCNJ11 & ABCC8**

Pancreatic β cell KATP channels are made of two subunits, the potassium channel, Kir6.2, and the sulphonylurea receptor, SUR1. Both subunits play a significant role in controlling the secretion of insulin [30,31]. The genes KCNJ11 and ABCC8 (cytogenetic location of 11q15.1 for both subunits [101]) encode Kir6.2 and SUR1, respectively. Generation of ATP occurs via the influx of glucose (Figure 4) [30]. Efflux of ATP from pancreatic β cells occurs through the KATP channels, thereby activating KCNJ11 and ABCC8. Activation of these genes results in membrane depolarization of potassium (K⁺), causing the influx of calcium (Ca²⁺) and triggers the release of insulin from the β cell into the circulation [30]. Both KCNJ11 and ABCC8 mediate insulin exocytosis from the pancreatic β cells into the circulation via the KATP channels [30,31], which completes the incretin signaling pathway for insulin secretion. KATP channel expression could be reduced by mutations in the KATP channel subunits, resulting in ‘overactive’ channels that may decrease the pancreatic β-cell membrane excitability and, thus, insulin secretion will be reduced [67–69]. Based on these findings, we may conclude that mutations in the KATP channel, comprising KCNJ11 and ABCC8 variants, could impair the stimulus to the incretin pathway that may influence a reduction in insulin secretion.

Investigation of the association of common polymorphisms of KCNJ11 and ABCC8 with T2D revealed that the KCNJ11 rs5219 polymorphism was associated with diabetes in a case–control group, despite no evidence of a familial association with diabetes in this group (Table 5) [31]. However, the ABCC8 polymorphisms, exon 16–3 T/C and exon 18 T/C, were found to not be associated with diabetes [31].

DPP-4 inhibitor treatment with functioning KCNJ11 KATP channels of the pancreatic β cells is thus hypothesized to provide adequate release of insulin into the circulation to control blood glucose levels, despite other findings [31]. Regardless, KCNJ11 and ABCC8 are expressed together in the KATP channel of pancreatic β cells, and therefore responses by DPP-4 inhibitors can be predicted via the regulatory levels of both genes.

**MTNR1B**

Melatonin (MLT) is a hormone produced by the pineal gland, and regulates cardiovascular, visual, circadian and neuroendocrine systems [70]. The effects of MLT are mediated by MT1 and MT2, which are encoded by the MTNRIA and MTNR1B genes, respectively. Both genes
suggests pancreatic β-cell dysfunction \cite{74,75}. However, this hypothesis needs to be tested in a pharmacogenomic setting.

Among all the variants (studied in Indian Asians and European–Caucasians) associated with MTNRIB, rs2166706 was the most closely linked with T2D \textit{(Table 6)} \cite{76}. Both variants, rs1387153 and rs10830963, were found to be associated with glucose in European–Caucasians \cite{76}. However, Indian–Asians and European–Caucasians have very similar risk allele frequencies for rs2166706 \cite{76}.

MTNRIB rs10830963 is associated with fasting glucose, HbA1c and homeostatic model assessment of β-cell function (HOMA-B) \cite{73} \textit{(Table 6)}. In addition, the effect size of rs10830963 on fasting glucose was comparable among different Asian populations \cite{73}. Since rs10830963 is identified as a T2D risk factor, through a series of isolated impaired fasting glycemia tests, and a reduction of oral and intravenous glucose-stimulated insulin release, it may be related to a defect in incretin stimulatory effects, which suggests pancreatic β-cell dysfunction \cite{74,75}. However, this hypothesis needs to be tested in a pharmacogenomic setting.

Among all the variants (studied in Indian Asians and European–Caucasians) associated with MTNRIB, rs2166706 was the most closely linked with T2D \textit{(Table 6)} \cite{76}. Both variants, rs1387153 and rs10830963, were found to be associated with glucose in European–Caucasians \cite{76}. However, Indian–Asians and European–Caucasians have very similar risk allele frequencies for rs2166706 \cite{76}.

The MTNRIB variants rs10830963 and rs2166706 are associated with T2D \cite{73,77}. Therefore, these T2D risk variants may serve as DPP-4 inhibitor response predictors, based on MTNRIB-mediated circulation of insulin levels from pancreatic β cells \cite{73}.

**Conclusion**

To date, there is no current study proving that a patient with a specific variant in a specific gene shows a better response to a particular DPP-4
inhibitor. However, this review has provided new insights into the regulation of DPP-4 inhibitors, by providing evidence of the effects of gene variants and their mechanistic pathways that could contribute to the understanding of how DPP-4 inhibitor responses are controlled by gene expression. Characterization of gene variants, various transcription factors and glucose and hormonal cues would allow for gene-expression levels to be targeted, thus increasing the ability to determine treatment outcomes of DPP-4 inhibitors. In the long term, the mechanism of expression of these genes may represent an exciting new molecular target pathway for the development of novel therapeutic strategies for T2D patients with pancreatic β cell gene dysfunction.

**Future perspective**

The article provides new insights into the regulation of DPP-4 inhibitors, strengthened by providing evidence of the effects of gene variants and their mechanistic pathways that could contribute to the understanding of how DPP-4 inhibitor responses are controlled by gene expression. This article provides new perspectives into increasing the ability to determine treatment outcomes of DPP-4 inhibitors according to the gene expression point of view.

In the future, the information in this article may be useful for the development and improvement of better DPP-4 inhibitor drug formulations with new specific genomic targets for better treatment outcome and of high clinical utility.

In 5–10 years time, the mechanism of expression of the genes explored in this article may represent a new molecular target pathway for the development of novel therapeutic strategies for T2D patients with pancreatic β-cell gene dysfunction.

**Financial & competing interests disclosure**

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No writing assistance was utilized in the production of this manuscript.
**Executive summary**

**DPP-4 inhibitor therapy**
- DPP-4 inhibitor therapy produces moderate HbA1c reductions, does not induce hypoglycemia and is neutral on weight.
- It can also be used as monotherapy or combination therapy with one oral antidiabetic.

**Expression of genes: PAX4, KCNQ1, TCF7L2, KCNJ11, ABCC8, MTNR1B & WFS1**
- PAX4, KCNQ1, TCF7L2, KCNJ11, ABCC8, MTNR1B and WFS1 have been identified as the main regulators of incretin-mediated actions.
- These genes may potentially influence the response of DPP-4 inhibitors.

**PAX4**
- PAX4 encodes a family of transcription factors essential in pancreatic β-cell development and differentiation at the embryonic stage, thus promoting cellular proliferation, migration and survival.
- A missense mutation of the R121W variant (located at the PAX4 pair domain) affected PAX4 transcription activity, leading to the loss of β-cell differentiation function, thus its association with Type 2 diabetes (T2D).

**KCNQ1**
- KCNQ1 is expressed in epithelial cells of the exocrine and endocrine glands of the pancreas. Here it mediates the release of GIP from intestinal K cells and GLP-1 from intestinal L cells in response to food intake.
- Although involvement of KCNQ1 in incretin secretion has not been demonstrated, KCNQ1 is reported to be involved in hormone and electrolyte transport processes in the gastrointestinal tract, suggesting that, due to the expression of KCNQ1 in the epithelial cells, the genetic variants of KCNQ1 could affect the efficacy of the incretin transport mechanism in the gastrointestinal tract.
- Because KCNQ1 plays a significant role in activating the incretin pathway in pancreatic β-cell insulin release, response by DPP-4 inhibitors may be at similar levels to those of the normal KCNQ1 gene itself. However, caution should be taken with KCNQ1 variants when initiating DPP-4 inhibitor therapy in case of low insulin release, and thus a poor response to treatment.

**WFS1**
- WFS1 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.
- Inactivation of β-cell WFS1 disrupts ER homeostasis, resulting in β-cell dysfunction, and thus contributing to T2D.
- Studies of the 31 SNPs of WFS1 revealed that rs1046320 polymorphism has the strongest T2D association thus may lead to low levels of insulin secretion, despite treatment with antidiabetics, including DPP-4 inhibitors.

**TCF7L2**
- TCF7L2 promotes cellular proliferation and survival in pancreatic β cells via the Wnt pathway.
- rs7903146, rs12255372, rs7901695, rs11196205 and rs10885390 were determined as TCF7L2 variants associated with T2D.
- T2D risk by TCF7L2 variants were found to be associated with impaired β-cell function but not with insulin resistance.
- Because impaired β-cell function may disrupt the secretion of insulin, responses by DPP-4 inhibitor treatment is predicted to be low in T2D patients with such risk variants.

**KCNJ11 & ABCC8**
- Both genes have a significant role in controlling the secretion of insulin from β cells into the circulation via the pancreatic β-cell ATP-sensitive KATP channels.
- DPP-4 inhibitor responses can be predicted via the regulatory levels of both genes.

**MTNR1B**
- MTNR1B encodes MT2.
- MT2 is suggested to be involved in the secretion of insulin and T2D, because of its expression in β cells and its upregulation in pancreatic islets of T2D patients.
- T2D risk variants may serve as DPP-4 inhibitor response predictors, based on MTNR1B-mediated circulation of insulin levels from pancreatic β cells.

**Conclusion**
- This review has provided new insights into the regulation of DPP-4 inhibitors by providing evidence of the effects of gene variants and their mechanistic pathways that could contribute to the understanding of how DPP-4 inhibitor responses are controlled by gene expression.
- Characterization of gene variants, various transcription factors, glucose and hormonal cues would allow for gene-expression levels to be targeted, thus increasing the ability to determine the treatment outcomes of DPP-4 inhibitors.

**References**

Papers of special note have been highlighted as:
- of interest
- of considerable interest

Pancreatic gene variants & dipeptidyl peptidase-4 inhibitor treatment response in Type 2 diabetes

**Explains the mechanism of pancreatic β-cell proliferation and survival via PAX4 expression.**


4 Gautier JF, Ferita S, Sobngwi E, Martin CS. Biological actions of the incretins GIP and GLP-1 and therapeutic perspectives in patients with Type 2 diabetes. Diabetes Metab. 31(3 Pt 1), 233–242 (2005).


8 Explains the mechanism of pancreatic β-cell insulin secretion, involving *KCNQ1, TCF7L2* and *WFS1* genes. Also briefly explains the incretin system, including GLP-1 and GIP.


25 Explains the mechanism of the DPP-4 inhibitors that inhibit the DPP-4 enzyme, in order to maintain the active level of GLP-1, to control the blood glucose level via insulin production.


33 Damcott CM, Pollin TI, Reinhart LJ et al. Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with Type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55, 2654–2659 (2006).


**Strengths the evidence of a PAX4 relationship with insulin secretion. The manuscript proved that glucose promotes PAX4 expression via insulin through the activation of the PI3K, ERK1/2 and cAMP–PKA pathways.**


**Concluded that the diabetes risk gene variants of WFS1 are associated with impaired incretin signaling, suggesting the association of WFS1 gene variants with incretin-induced insulin secretion.**


Pancreatic gene variants & dipeptidyl peptidase-4 inhibitor treatment response in Type 2 diabetes


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