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► To cite this version:

Wenwen Gao, Ralf Jockers. Pharmacogenomics of GPCR genes in type 2 diabetes and obesity. *Current Opinion in Endocrine and Metabolic Research*, 2021, 16, pp.128 - 135. <10.1016/j.coemr.2020.11.008>. <hal-03493285>

HAL Id: hal-03493285

<https://hal.science/hal-03493285v1>

Submitted on 16 Dec 2022

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Pharmacogenomics of GPCR genes in type 2 diabetes and obesity

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Running title: GPCR genes in diabetes

Abstract:

G protein-coupled receptors (GPCRs) are important drug targets in type 2 diabetes (T2D). GPCR gene variants can modify the responsiveness to drugs and can be a risk factor for T2D development and associated traits. GPCR genes of interest in this respect are *GIPR*, *GLP1R*, *MC4R* and *MTNR1B*. Once the association of gene variants with T2D risk or altered drug response established, the consequences on receptor function have to be determined. Depending on the nature of the altered function, different types of compounds can be proposed to re-establishing normal receptor function and drug responsiveness providing eventually a genotype-based personalized therapy for carriers of GPCR gene variants.

Keywords: diabetes, obesity, genetic variants, personalized therapy, drug design

General introduction

G protein-coupled receptors (GPCR) are the largest family of transmembrane receptors with ~800 genes in humans. They detect a range of biological molecules to inform the cell about the *status-quo* and changes in the extracellular environment.

GPCRs are targeted by clinically relevant drugs representing 30-40% of all commercially available drugs [1, 2]. Single-cell RNA sequencing indicates that every cell co-expresses between 8 and 20 GPCRs simultaneously [3] providing several opportunities for therapeutic intervention by regulating cellular metabolism, proliferation, differentiation, insulin secretion, etc. GPCRs relay information to downstream effectors, at the first level to heterotrimeric G proteins and β -arrestins and then to other effectors of intracellular signaling pathways.

Genes are subject to variations over time (single nucleotide polymorphisms, insertions, deletions, etc.) resulting in sequence differences between individuals and ethnics [4-6]. The most abundant gene sequence present in a population is defined as the wild-type (WT) sequence and the others as either common (prevalence of >1%) or rare (prevalence of <1%) variants. Analyses revealed an average of 22 to 34 missense variants per 10,000 individuals in the coding region of 376 human GPCR genes, with every individual carrying statistically 1.2 non-synonymous variants in one of their GPCR genes [2, 7] (Figure 1). Gene variants may affect receptor function or may be neutral. Commonly observed alterations are receptor expression levels or export to the cell surface as well as diminished or exaggerated receptor activation and signaling (Figure 1). Of note, gene variants can not only affect the receptor response to the natural ligand but also to drugs, an aspect typically studied in the field of pharmacogenomics [2]. Gene variants can be associated with disease risk or traits and can be disease-causing in some rare cases [7] (Figure 1).

Here, we will focus on role of GPCR gene variants in type 2 diabetes (T2D). T2D is a major public health concern worldwide characterized by high blood glucose concentrations in the context of insulin resistance, diminished insulin sensitivity and secretion capacity and decreased β -cell mass [8]. The global diabetes prevalence in 2019 is estimated to be 9.3% (463 million people), rising to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 [8]. GPCRs are important drug targets in T2D treatment with several molecules already on the market or in clinical trials [9]. Furthermore, variants in GPCR genes participate in T2D risk. Variants in GPCR signaling genes associated with T2D risk have been also described for *ADCY5* encoding adenylate cyclase 5, *GPSM1* encoding G protein signaling modulator 1 (or AGS3) and *GRK5* encoding G protein-coupled receptor kinase 5 but will not be part of the current review (see [10] for review).

Gene variants in GPCR genes and type 2 diabetes and obesity

Initially, exon sequencing of GPCRs was performed in targeted patient cohorts with the aim to identify rare, disease-causing variants in the coding region of candidate genes. Prominent examples of this candidate gene approach are *AVPR2* encoding the vasopressin 2 receptor (V2R) and *MC4R* encoding the melanocortin 4 receptor. *MC4R* variants are linked with severe obesity (see below). *AVPR2* variants are linked with nephrogenic diabetes insipidus (NDI), which is not related to diabetes, but it does share some of the same signs and symptoms such as extreme thirst (polydipsia) and excessive production of hypoosmotic urine (polyuria). More than 190 loss-of-function V2 mutants have been reported, of which most do not reach the cell surface because of retention and degradation in the endoplasmic reticulum (ER) [11, 12]. Another example of this approach is a variant located in the 3'UTR region of *ADRA2A*,

encoding the α 2A-adrenergic receptor, which associates with impaired insulin secretion [13].

More recently, genome-wide association studies (GWAS) identified common variants typically located outside of the coding region and with modest impact on disease risk. Typical examples associated with T2D risk through β -Cell function and/or insulin secretion are *MTNR1B* encoding the melatonin MT₂ receptor, *TSPAN8/LGR5* encoding the orphan leucine-rich repeat containing G protein-coupled receptor 5 (also called GPR49) and *GIPR* encoding the glucose-dependent insulintropic peptide receptor. Additional gene variants were found in *MC4R* also associated with T2D risk through insulin action (resistance/low sensitivity) and/or adiposity (see [10] for review). Genetic mutations in free fatty acid receptors FFAR1 (GPR40) and FFAR4 (GPR120), known to regulate insulin secretion have been also associated with impaired insulin secretion and increased fasting glucose levels, respectively [9, 14]. Below we will discuss in more detail the contribution of *MTNR1B*, *GIPR*, *MC4R* and *GLP1R* gene variants to T2D risk and their consequences on receptor function.

***MTNR1B* - melatonin MT₂ receptor**

In 1992, genome-wide association studies identified common variants in the *MTNR1B* (www.ncbi.nlm.nih.gov/gene/4544) gene that associate with type 2 diabetes risk [15-17]. The common rs10830963 variant showed the strongest association signal with T2D risk and was in addition associated with increased fasting plasma glucose levels and impaired insulin secretion, as well as increased risk of gestational diabetes mellitus Table 1 [18]. Cumulative evidence indicates that the effect of rs10830963 (intronic variant) probably starts early during the development of prediabetic fasting hyperglycemia by affecting insulin secretion. The mechanism of action of this variant

is not fully understood. The *MTNR1B* gene encodes the melatonin MT₂ receptor. Risk allele carriers of the intronic rs10830963 variant show increased *MTNR1B* mRNA expression, altered melatonin secretion and possibly further effects associated with the enhancer activity of the region surrounding this variant [19]. The increase in *MTNR1B* mRNA expression with T2D risk first suggested that MT₂ gain-of-function is associated with a higher risk of developing T2D possibly through an increased inhibitory effect on insulin secretion through the Gi-coupled MT₂ receptor as reported in pancreatic β -cells in rodents [20]. Later, more direct evidence for the contribution of the MT₂ receptor in T2D risk came from the identification and characterization of forty rare variants distributed all over the coding region of the *MTNR1B* gene of which those rather displaying a loss-of-function phenotype associated with T2D risk Table 1 [21]. The reason for this discrepancy is not fully understood at the moment [18]. A detailed in vitro analysis showed that defective melatonin-induced Gi and Gz protein activation, as well as spontaneous β -arrestin 2 recruitment associates with T2D risk [22]. The action of melatonin is special as this hormone can directly target metabolic tissues (liver, adipose tissue, skeletal muscle, pancreas) and indirectly regulated metabolic functions through its chronobiotic effect. Melatonin receptors are indeed expressed in the suprachiasmatic nucleus where they entrain the circadian rhythms of the biological master clock. Disruption of the master clock leads to metabolic diseases such as T2D [23]. As melatonin is a chronobiotic agent, its efficacy depends not only on the dose but also on the proper time of administration that still have to be properly sorted out accompanied with life-style recommendation such as meal times and regular sleep-wake cycles [18].

***GIPR* - Glucose-dependent insulintropic peptide receptor (GIPR)**

In healthy individuals, the incretin effect of two hormones, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), count to up to 65% of the overall insulin secretory response to oral intake of glucose, amino acids, and free fatty acids. This effect is substantially diminished in individuals with various types of diabetes and hyperglycemia [24]. Some genetic studies indicate that variants of the *GIPR* (www.ncbi.nlm.nih.gov/gene/2696) gene may account for glycemic trait and T2D risk in both diabetic and non-diabetic subjects. GWAS showed that the common intronic rs10423928 variant in the *GIPR* gene associated with decreased insulin secretion and diminished incretin effect Table 1 [25]. *GIPR* mRNA levels were lower in human pancreatic islets of risk allele carriers compared to non-risk allele carriers and nondiabetic donors indicating a potential mechanism underlying the reduced β -cell function in risk carriers [26]. Intriguingly, a recent study reported that risk allele carriers show significantly higher insulin sensitivity, indicating an enhanced β -cell response [27]. Clearly, further studies are necessary to confirm the mechanism behind these contradictory results.

A meta-analysis of GWAS and exome-wide association studies demonstrated that the minor alleles of rs1800437 and rs2287019 in the *GIPR* locus are associated with lower fasting and lower 2-hour GIP concentrations Table 1. The rs1800437 variant corresponds to a missense mutation in the coding region of *GIPR* leading to the relatively well-characterized p.E354Q mutation, which displays reduced basal signaling [28]. However, an association with T2D risk was not observed in this earlier study [28]. Besides, carriers of the rs12453394 variant exhibited a small but significant risk of developing T2D [29]. Altogether, the extent to which altered *GIPR* signaling contribute to T2D risk remains uncertain and warrants further studies. Dual

agonists targeting GIPR and GLP-1R have been developed recently and might be also useful to recover defective mutants like p.E354Q (see below).

***GLP1R* - Glucagon-like peptide-1 receptor (GLP-1R)**

In the 1980s, since the discovery of the incretin hormone, GLP-1, the diverse and vital physiological role of GLP-1R signaling in the regulation of glucose homeostasis has been well determined. In the past two decades, even though people notice the importance of GLP-1R variants in the pharmacogenetic field, less progress has been made in associating these variants with disease risk or drug response. Since 1990s, several independent groups identified a limited number of nonsynonymous, typically common, genetic variants of the *GLP1R* gene (www.ncbi.nlm.nih.gov/gene/2740) that modified receptor function, but with limited impact on the related glycemic trait, such as insulin secretion or body mass index. Recently, the low-frequency missense variant rs10305492 in the coding region of *GLP1R* (p.A316T) showed for the first time an association with lower fasting glucose and lower T2D risk, as well as reduced early insulin secretion in human cohorts Table 1 [30]. An *in silico* analysis of 61,488 individuals with coronary artery disease and 163,728 controls displayed an additional association of the p.A316T mutation with protection against coronary artery disease [31]. The *in vitro* functional characterization of the p.A316T mutant revealed reduced expression levels of GLP-1R, no altered cAMP production nor ERK1/2 phosphorylation of this Gs-coupled receptor, and only a small alteration of the maximal effect of GLP-1 on Ca²⁺ mobilization [32]. In 2019, another variant in the coding region (rs3765467; p.R131Q) was identified in a GWAS and associated with increased T2D risk Table 1 [33]. *In vitro* functional characterization of p.R131Q did

not reveal major alterations in receptor responses in CHO cells [32]. An earlier pilot study indicated that *GLP1R* variants may alter insulin secretion in response to exogenous GLP-1 administration [34], an hypothesis that was subsequently confirmed for several GLP-1R variants which showed altered responses to exenatide and liraglutide drugs [35]. In summary, some *GLP1R* variants in the coding region may promote or protect against T2D development. It is also essential to verify the impact of genetic variation of the *GLP1R* gene in respect to drug responsiveness in clinical trials or at least in pharmacological studies. Athanathan and colleagues studied 88 healthy individuals' acute insulin response to GLP-1 administration over 2h and found that two variants (rs6923761(p.G168S) and rs3765467(p.R131Q)) were associated with modest alteration of β -cell responsiveness to GLP-1 infusion [36]. Another study with 90 patients with T2D and overweight revealed that carriers of Ser at position 168 show better anthropometric parameters with a greater decrease in body weight and fat mass after treatment with liraglutide [37].

MC4R - Melanocortin-4 receptor (MC4R)

MC4R is essential for the maintenance of weight homeostasis in humans and MC4R (www.ncbi.nlm.nih.gov/gene/4160) mutations may be a substantial monogenic cause of obesity with a reported prevalence of 0.5% to 6%, in severely obese adults [38, 39]. MC4R mutations show typically loss-of-function phenotypes with altered receptor expression levels, reduced ligand binding and signaling properties and, in most of the cases with defective cell surface expression due to intracellular retention Table 1 [40]. In order to target the different types of MC4R mutants, several therapeutic strategies have been developed, including aminoglycosides (Geneticin) to

improve receptor biosynthesis and chemical and pharmacological chaperones (i.e. ML00253764) to improve receptor trafficking [41]. In addition to loss-of-function of MC4R mutants, a recent study demonstrated that gain-of-function mutants in the MC4R associate with protection against obesity, T2D and coronary artery disease Table 1 [42]. In this study, the authors examined the functional profile of 61 mutants comprising nonsense/frameshift and missense variants in terms of recruitment of β -arrestin and canonical Gas-mediated cAMP production [42]. Surprisingly, the gain-of-function mutants exhibited signaling selectivity by preferentially increasing the recruitment of β -arrestin rather than Gs coupling and cAMP production. The functional consequences of increased β -arrestin recruitment remain to be determined but do not seem to be translated into increased internalization, as would have been expected. The understanding of the function of MC4R variants and its impact on clinical phenotypes facilitate MC4R drug design targeting this pathway to treat common obesity and its complications in individuals carrying these mutations. It has been reported that patients with a mutation in the gene encoding POMC can be treated with an MC4R agonist setmelanotide [43].

Therapeutic perspectives

Future T2D treatments are likely to rely on genotype-base therapies implying the treatment of subpopulation of T2D patients in a personalized manner [44]. To reach this goal the genetic predisposition for disease risk and altered drug response has to be defined [2]. The sequencing of an increasing number of human genomes to detect GPCR gene variants will help to reach this goal. To translate this knowledge into tailored treatments, the consequences of these variants on GPCR function in a physiologically relevant context have to be determined (Figure 2). In some cases, this

knowledge already exists as for the rs553668 variant in the *ADRA2A* gene that is associated with T2D risk as a consequence of receptor hyperactivation. Consequently, treatment of risk allele carriers with an α 2A-adrenergic receptor antagonist normalized the otherwise diminished insulin response of these T2D patients [45]. In the majority of cases the therapeutic goal will be rather to recover an impaired receptor activity either by improving receptor expression, trafficking to the cell surface or signaling [41]. Chemical chaperones, i.e. low molecular weight molecules that non-selectively promote protein folding have been successfully applied to V2 and MC4R mutants in vitro (Figure 2a). Before further in vivo application, efficacy and safety issues have to be addressed. Pharmacological chaperons are cell-permeant GPCR antagonists or agonists that facilitate folding and export of the receptor to the plasma membrane by stabilizing the receptor by constraining and stabilizing its conformational flexibility (Figure 2a). Such compounds have been mostly developed for V2 and MC4R but applications in humans are still rare because of weak efficacy and persistence of chaperon binding to the receptor once at the cell surface [12, 41], evidencing the need of improvement of this strategy.

Many GPCR mutants reach the cell surface successfully but exhibit impaired ligand binding, receptor activation or signal transmission. Several pharmacological tools have been developed over the years with the potential to overcome these problems and to become future drug candidates. The most advanced strategy in terms of progress in clinical studies consists in designing sequence-intermixed single molecule hybrids targeting two GPCR with the goal to synergistically combine their therapeutic benefits (Figure 2d) [46]. Such dual peptidic agonists were first designed as GLP-1R/GIPR co-agonists followed by GLP-1R/glucagon receptor co-agonists and GLP-1R, GIPR, and glucagon receptor triple agonists. Several showed greater effect on

body weight loss in rodents compared to the single agonists (see [46] for review).

Clinical trials are on the way. Combining the stimulation of the residual activity of a mutant GPCR with the simultaneous stimulation of another, full functional, GPCR, might become a feasible way to recover a defective response, like insulin secretion, in an integrative way in GPCR variant carriers.

Another potential strategy to recover the function of mutant GPCRs comprises the use of biased ligands, which activate only a subset of signaling pathways as compared to the endogenous reference ligand (Figure 2c). Such ligands present a considerable gain in specificity and safety as they enhance specifically the defective pathway without interfering with other pathways. This can be of particular importance if one pathway has been identified as beneficial and another as detrimental for a particular response as indicated for the GLP-1R for which the Gs/cAMP pathway promotes and the β -arrestin pathway limits insulin secretion due to receptor internalization [47]. G protein-biased ligands such as ex4-phe1 show indeed improved insulin secretion upon long-term stimulation [47]. Dual-agonists for GLP-1R and GIPR may be also of interest as they have biased properties [48, 49].

Incretin receptors like GLP-1R and GIPR bind their peptidic ligands with large ligand-receptor interfaces that vary depending on the type of ligand. Similarly, the impact of mutations in the binding site may depend on the type of ligand and other orthosteric ligands may still bind with high affinity to recover the defective response of mutant receptors (Figure 2b). This is exemplified by the impaired exendin-4-mediated cAMP production of the p.T149M GLP-1R mutant, an effect that was much less affected for GLP-1 [32].

Another class of ligands that are likely to gain importance in the future are compounds binding to allosteric sites (Figure 2b,c). Such compounds can have two

distinct actions, either the potentiation of the residual receptor activity towards the endogenous ligand (positive allosteric modulator (PAM) effect) or to activate the mutated receptor on its own by stabilizing active conformational states of mutant receptors that are not or less stabilized by the endogenous ligand. The latter effect has been already described for the p.T149M GLP-1R mutant which shows severely diminished binding for orthosteric ligands but was responsive to the allosteric Compound 2 [32]. The recently described GLP-1R PAM (LSN3160440) represents another interesting case as it binds at the interface of the receptor and the peptide ligand [50]. The stabilization of the defective ligand-receptor interaction in mutant receptors can be easily envisioned with this type of allosteric ligand.

In conclusion, several new compounds targeting GPCRs relevant for the treatment of T2D are currently in clinical trials (dual agonist compounds, etc.), others are at the pre-clinical stage (biased, allosteric compounds). These compounds are developed to improve T2D treatment in general but might also turn out to be particularly useful for carriers of GPCR mutants. Recent solved structures of GPCRs relevant for T2D treatment will greatly contribute to rational drug design in the field.

Declaration of interest

The authors declare no competing interests.

Acknowledgements

We thank Drs. Julie Dam, Erika Cecon and Lei Liu (Institut Cochin, Paris) for careful reading of the manuscript. This work was supported by the Fondation de la Recherche Médicale (Equipe FRM DEQ20130326503), Agence Nationale de la Recherche (ANR-2011-BSV1-012-01 “MLT2D” and ANR-2011-META “MELA-BETES”),

Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS); W.G. was supported by a doctoral fellowship from the Chinese Scholarship Council (China).

Figure legends

Figure 1: Workflow of genotype-based treatment. GPCR genes comprise ~ 400 non-odorant genes. Large-scale sequencing projects revealed an average of 22 to 34 missense variants (MV) per 10,000 individuals in the coding region of human GPCR genes. The functional characterization of GPCR mutants helps to define GoF, neutral, and LoF mutants and to determine the drug response of mutants. In parallel, genetic association studies focus on the contribution of variants to disease risk to further instruct the phenotype and treatment strategy of diseases. By combining pharmacological and genetic information, tailored therapeutic solutions can be elaborated. MVs, missense variants; GoF, gain-of-function; LoF, loss-of-function; *p refers to statistical significance.

Figure 2: Functional defects of natural GPCR mutant and therapeutic agents to rescue their activity. **(a)** GPCR mutants with folding and trafficking problems can be rescued by chemical and pharmacological chaperons. **(b)** GPCR mutants with defective ligand binding can be rescued by orthosteric ligands relying on partially different molecular determinant or by allosteric ligands activating the receptor on its own by binding to allosteric binding sites. **(c)** GPCR mutants with defective signaling properties can be rescued by biased ligands or by allosteric ligands either on their own or through their PAM effect. **(d)** Binding or signaling defective GPCRs can be rescued by dual agonist binding to the mutated receptor and another receptor that synergistically merges towards the same functional outcome, i.e. insulin secretion.

Table 1: Characteristics of GPCR gene variants associated with T2D and obesity risk.

Gene	Genetic Variant(s)	Molecular mechanism	Disease risk	Metabolic trait	Reference
<i>MTNR1B</i>	rs10830963 (intronic)	Increase in <i>MTNR1B</i> mRNA expression	higher T2D risk	Increased FG levels, impaired insulin secretion, increased risk of GDM	[18]
	Forty rare variants (point mutations in coding region)	Defective MLT-induced Gi and Gz protein activation, and spontaneous β -arrestin 2 recruitment	higher T2D risk	more irregular sleep, higher behavioral circadian misalignment, more frequent caloric intake compared to T2D controls	[22]
<i>GIPR</i>	rs10423928 (intronic)	Decreased mRNA levels	higher T2D risk	decreased insulin secretion and diminished incretin effect	[25, 26]
			n.d.	significantly higher insulin sensitivity	[27]
	rs1800437 (E354Q mutation)	Reduced basal signaling	n.d.	lower fasting and lower 2-hour GIP concentrations	[28, 51]
	rs2287019 (intronic)		n.d.		
	rs12453394 (intronic)		higher T2D risk	n.d.	[29]
<i>GLP1R</i>	rs10305492 (A316T mutation)	Reduced expression levels	lower T2D risk	Lower FG, reduced early insulin secretion	[31, 32]
	rs3765467 (R131Q mutation)		higher T2D risk	n.d.	[33]
<i>MC4R</i>	Missense mutations, inframe deletion mutations, and nonsense	Reduced receptor expression levels, ligand binding and signaling	higher obesity risk	Deficient in maintaining energy homeostasis	[40]

	mutations, as well as dozens of frameshift mutations	properties			
		The gain-of-function in β -arrestin	lower obesity, T2D and coronary artery disease risk	Lower body mass index, lower resting heart rate, lower systolic blood pressure, lower diastolic blood pressure	[42]

T2D, Type 2 diabetes; GDM, Gestational diabetes mellitus, FG, fasting plasma glucose. n.d., not determined

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GPCR Coding region



ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly

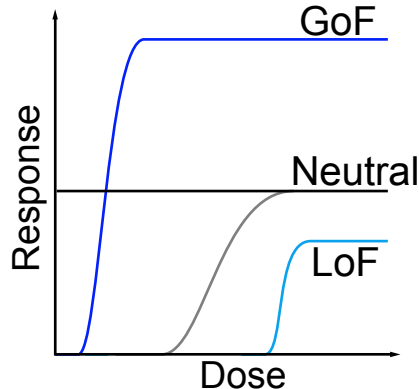


ATG	GAC	GCA	CGT
Met	Asp	Ala	Gly

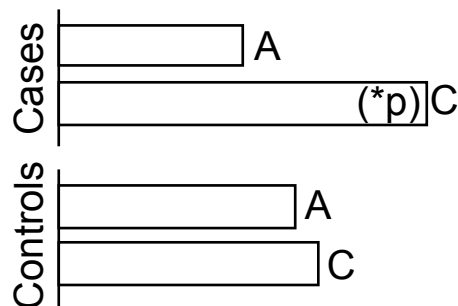


Gene variants

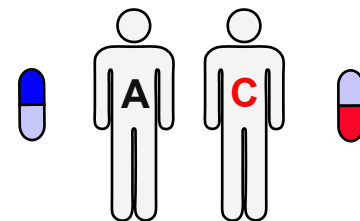
22-34 MVs per 10,000 individuals per gene



Functional characterization of GPCR mutants (Altered drug response)



Genetic association studies (Disease risk)



Tailored therapeutic solution

