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Adhesion G-protein coupled receptors: Implications for metabolic function

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Adhesion G-protein coupled receptors: implications for metabolic function

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adipose, liver, muscle

ABSTRACT

Adhesion G-protein coupled receptors (aGPCRs) are emerging as important actors in energy homeostasis. Recent biochemical and functional studies using transgenic mice indicate that aGPCRs play important roles in endocrine and metabolic functions including β -cell differentiation, insulin secretion, adipogenesis and whole body fuel homeostasis. Most aGPCRs are orphans, for which endogenous ligands have not yet been identified, and many of the endogenous ligands of the already de-orphanised aGPCRs are components of the extracellular matrix (ECM). In this review we focus on aGPCR expression in metabolically active tissues, their activation by ECM proteins, and current knowledge of their potential roles in islet development, insulin secretion, adipogenesis and muscle function.

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Abbreviations: GPCRs - G-protein coupled receptors, aGPCRs - adhesion G-protein coupled receptors, T2D - type 2 diabetes, T1D - type 1 diabetes, ECM - extracellular matrix, GPS - GPCR proteolytic site, GAIN - GPCR autoproteolysis-inducing, WAT – white adipose tissue, BAT – brown adipose tissue, HGP – hepatic glucose production, GCGR – glucagon receptor

1. Introduction

Type 2 diabetes (T2D), a state of persistently elevated blood glucose that is associated with long term damage to multiple organs, is a chronic metabolic disorder that poses considerable economic and health challenges to individuals and society. The global rise in T2D prevalence is mainly driven by the increased incidence in obesity: in 2016 over 650 million adults were obese (WHO, 2016), approximately 425 million of whom have diabetes (IDF, 2017). The global incidence of T2D is predicted to continue to rise as a consequence of changing demographics and increases in the number of people adopting a sedentary lifestyle. It is therefore essential that effective pharmacotherapies are available to people with T2D to ensure appropriate blood glucose control and thus minimise the complications associated with uncontrolled hyperglycaemia.

1.1 Islet GPCRs as drug targets for type 2 diabetes

GPCRs are the largest class of plasma membrane proteins, and they control many physiological processes through transducing a diverse array of signals to the cell interior through coupling to a family of heterotrimeric GTP-binding proteins. GPCRs were initially classified, based on their sequence similarities, into six alphabetically-named classes: Class A (rhodopsin-like receptors), Class B (secretin receptors), Class C (metabotropic glutamate receptors), Class D (fungal receptors), Class E (cyclic AMP receptors) and Class F (frizzled receptors). Of these, only classes A, B, C and F are found in vertebrates (Kolakowski, 1994). However, this classification did not take into account the subtle differences between the GPCRs within the same group and an alternative system was proposed that related GPCR structures to their functions and ligand preference. This alternative classification, known as the GRAFS system, sub-divides the GPCR superfamily into five classes: **G**lutamate, **R**hodopsin, **A**dhesion, **F**rizzled and **S**ecretin (Fredriksson *et al.*, 2003; Alexander *et al.*, 2017). Although progress has been made in identifying cognate ligands for the GPCRs, 121 out of the ~350 non-sensory GPCRs are still orphans, for which no activating ligands have been identified (Alexander *et al.*, 2017). The rhodopsin family, which is the most studied, has 197 receptors with known ligands and about 87 of them are orphans. The frizzled family consists of 11 receptors, all of which have been de-orphanised. Of the 33 adhesion GPCRs, only 6 have been proposed to have an endogenous ligand (Davenport *et al.*, 2013; Alexander *et al.*, 2017).

Nearly 300 GPCRs, belonging to all classes of the GRAFS system, are expressed by human islets of Langerhans (Amisten *et al.*, 2013). The function of many of these receptors has not yet been established, but it is known that numerous islet GPCRs play important roles in regulating growth, differentiation, secretion, maturation and survival of islet cells (Ahrén, 2009;

Amisten *et al.*, 2013). As islets are crucial in maintaining glucose homeostasis through secretion of gluco-regulatory hormones, the importance of GPCR ligands in maintaining appropriate islet function has led to GPCRs being considered as attractive targets for novel T2D therapies. They have readily accessible druggable sites and are the targets of at least 30% of all prescription drugs in current use (Santos *et al.*, 2017; Sriram and Insel, 2018), which explains why pharmaceutical industries are investing heavily in them for new drug leads (Hauser *et al.*, 2017; Persaud, 2017).

GPCRs exert their effects via activation and release of the $G\alpha$ -subunit from the heterotrimeric G-protein complex, although G-protein-independent effects via β -arrestins have been described (Tuteja, 2009; Ravier *et al.*, 2014; Smith and Rajagopal, 2016). The coupling preference of GPCRs to the $G\alpha$ subtypes, $G\alpha_i$, $G\alpha_s$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$, determines their effects on insulin secretion. Generally, GPCRs that signal via $G\alpha_s$ or $G\alpha_{q/11}$ increase insulin secretion through the generation of cAMP and IP₃/DAG respectively, while signalling via $G\alpha_i$ -mediated reductions in cAMP inhibits insulin release. The roles of β -cell receptors coupled to $G\alpha_{12/13}$ are not fully understood, but actin cytoskeleton remodelling and activation of Rho-associated protein kinase downstream of these GPCRs is likely to be coupled to increased insulin secretion. Islet-expressed GPCRs that have received considerable attention for diabetes therapy include GLP-1R, GPR40, GPR119 and GPR120 (Persaud, 2017). Drugs that activate GLP-1R have been in use for the clinical management of T2D for the past 10-15 years, and those targeting GPR40, GPR119 and GPR120 are either currently undergoing clinical trials or have been discontinued due to lack of efficacy or for safety reasons. Recently, a novel GLP-1/GIP dual agonist, LY3298176, has shown very promising results in phase 2 trials in patients with T2D (Frias *et al.*, 2018).

2. Adhesion GPCRs

The adhesion GPCR family consists of 33 members, with nine distinct subclasses (Hamann *et al.*, 2015). A unified nomenclature was recently recommended for aGPCRs using the prefix ADGR, for adhesion G-protein coupled receptor, followed by a single letter and a number derived from their previous subfamily name and members within each subfamily, respectively (Table 1). In addition to the canonical seven transmembrane segments common to other GPCRs, aGPCRs have an extended N-terminal segment and they undergo self-cleavage during maturation at the GPCR proteolytic site (GPS) within the GPCR autoproteolysis-inducing (GAIN) domain (Figure 1). The GAIN domain is a unique evolutionarily conserved structure of aGPCRs that is required for self-cleavage (Prömel *et al.*, 2013). The N and C termini re-associate upon maturation and remain non-covalently attached to each other at the

GPS. The capacity of the N-terminal fragment of an aGPCR to cross-assemble with the C-terminus of a different aGPCR and vice versa, and the ability of a small proportion of the N-terminal segment, anchored independently to the cell membrane, to initiate downstream signalling upon ligand binding, increases the complexity of aGPCR heterodimer signalling considerably (Volynski *et al.*, 2004; Silva *et al.*, 2009; Huang *et al.*, 2018). The GPS is found immediately upstream of the first transmembrane loop of aGPCRs and it is rich in cysteine residues, alternating with tryptophan in a C-W-W-C-C conserved sequence (Araç *et al.*, 2012). The exceptions to this general aGPCR structure is GPR123, which does not have a GAIN domain, and GPR111, GPR114, GPR115 and CELSR1, which do not have cleavable GAIN domains (Araç *et al.*, 2012; Krishnan *et al.*, 2016; Wilde *et al.*, 2016). Other aGPCRs that are predicted to have a non-cleavable GAIN domain include GPR124, GPR125, EMR1 and CELSR3, but this atypical structural feature has not yet been demonstrated (Hamann *et al.*, 2015).

The extracellular N-termini of aGPCRs vary greatly in length and contain mucin-like motifs, which are thought to help them in their cell adhesion and cell-matrix interactions (Langenhan *et al.*, 2013). They also contain multiple functional domains such as hormone binding domains, thrombospondin repeats, epidermal growth factor-calcium binding domain, and cadherin repeats (Bjarnadóttir *et al.*, 2004; Hamann *et al.*, 2015). The CELSR family members (CELSR1, CELSR 2, CELSR 3), which are associated with planar cell polarity in epithelial cells, contain four functional domains including several cadherin repeats on the uppermost part of their N-terminal segments (Wang *et al.*, 2014), enabling them to participate in diverse physiological processes.

Most aGPCRs are widely expressed in tissues such as the reproductive tract, neurons, stomach, lungs and various tumour cells, where they play roles in angiogenesis, cell adhesion, immune recognition and neuronal migration (Simundza and Cowin, 2013). However, some aGPCRs have fairly restricted tissue expression. For example, GPR123, BAI1, BAI3 and VLGR1 have biased expression in the brain, GPR111, GPR112, GPR128 in the gastrointestinal tract and GPR110 in the kidney (Fagerberg *et al.*, 2014), suggesting that these receptors have particular functional capacities in those organs. Inactivating mutations in aGPCRs have been linked to several human diseases including bilateral frontoparietal polymicrogyria (Piao *et al.*, 2004), vibratory urticaria (Boyden *et al.*, 2016) and Usher syndrome type IIc (Weston *et al.*, 2004), further pointing to the importance of aGPCRs in physiological processes and disease.

2.1 Adhesion GPCR activation and signalling

As mentioned above, the N-terminal fragment of most mature adhesion GPCRs attaches non-covalently with the membrane bound C-terminal fragment at the GPS, and the cleavage of the N-terminus has been suggested to modulate receptor activity. Removal of the N-terminal domains in GPR56, CD97 and BAI1 mutants has been associated with increased receptor activities (Paavola *et al.*, 2011; Ward *et al.*, 2011; Stephenson *et al.*, 2013), which led to the proposal that the extracellular domain of aGPCRs acts as an inverse agonist of constitutive signalling. It is now established that removal of the N-terminus, either by ligand binding or mechanical detachment, is sufficient for receptor activation (Liebscher and Schöneberg, 2016). Another signalling mechanism has been proposed based on the observation that a part of the aGPCR extracellular domain called a stachel sequence, which is buried in the GAIN segment, acts as a tethered agonist and this is exposed to the seven transmembrane domain upon ligand binding. This scenario has been demonstrated in a number of aGPCRs including GPR56, GPR64, GPR114, GPR126 and GPR133 (Wilde *et al.*, 2016; Stoveken *et al.*, 2015; Liebscher *et al.*, 2014; Demberg *et al.*, 2015; Kishore *et al.*, 2016; Stoveken *et al.*, 2017). Due to high sequence conservation of the aGPCR stachel region, stachel-derived peptides for a given receptor may be able to activate other aGPCRs within and beyond the same sub-family (Demberg *et al.*, 2017). A further aGPCR activation mechanism has been identified through the observation that increased GPR126 signalling occurred when shaking forces were applied in the presence of the activating ligand laminin-211, presumably as a consequence of detachment of the N-terminal fragment and exposure of the stachel sequence (Petersen *et al.*, 2015). It is therefore likely that aGPCRs are activated in a tripartite manner *in vivo*, such that binding of the endogenous ligand coupled with mechanical force from cell movement exposes the tethered agonist to initiate receptor activation (Figure 2).

The hybrid and enigmatic structural features of aGPCRs have impeded understanding of their signalling mechanisms, and progress has been further limited by more than two-thirds of aGPCRs being orphan receptors. The major endogenous ligands reported so far for the de-orphanised aGPCRs are components of the extracellular matrix (ECM), a meshwork of collagens, laminins and chondroitin sulphate proteoglycans that contains secreted proteins such as cytokines and growth factors trapped within it (Table 1). CELSR2 and 3 have no known ligands but are activated by N-terminal homophilic interactions (Shima *et al.*, 2007).

It has been postulated that aGPCRs will have multiple interacting ligands due to their unusually long N-terminal segment with adhesive motifs, and their broad expression in many tissues (Paavola and Hall, 2012). Four binding partners have been identified for the most studied aGPCR, GPR56: the ECM protein collagen III in the developing brain (Luo *et al.*, 2011), the

cross-linking enzyme transglutaminase 2 (TG2) in HEK293 cells (Xu *et al.*, 2006), the anti-coagulant heparin in A375 melanoma cells (Chiang *et al.*, 2016) and the stomach prohormone progastrin in colorectal cancer cells (Jin *et al.*, 2017). Whereas activation of GPR56 by collagen III or an antibody directed against its N-terminal has been shown to couple to RhoA through $G_{\alpha_{12/13}}$ (Iguchi *et al.*, 2008; Luo *et al.*, 2011, 2014), recruitment of downstream signalling has not been demonstrated so far for TG2, heparin or progastrin, suggesting that collagen III is the main endogenous agonist of GPR56. Complexes between GPR56 and the tetraspanins CD9 and CD81 that couple to G_{α_q} have also been reported (Little *et al.*, 2004), but there is currently no information on whether these complexes perform a physiological function.

Another mechanism that might regulate signalling downstream of GPR56 is the generation of splice variants in cells expressing this receptor. Full length GPR56 consists of 13 exons, yielding proteins of 693 and 697 amino acids in human and mouse, respectively, and human GPR56 is known to undergo alternative splicing at exons 2, 3 and 10 to produce four isoforms termed GPR56₆₈₇, GPR56₆₉₂, GPR56₅₂₃ and GPR56₅₁₈ (Kim *et al.*, 2010). Analysis of gene promoter activities in cells expressing GPR56 splice variants indicated that GPR56₆₈₇ and GPR56₆₉₂ increased serum response element (SRE) transcriptional activity, which may have been a consequence of deletion of six amino acids in the first intracellular loop contributing to enhanced $G_{\alpha_{12/13}}$ /RhoA signalling (Kim *et al.*, 2010). It has also been identified that the GPR56₅₁₈ variant, in which a frameshift deletion causes a new translation start site, has increased basal activity and it has been predicted that alternative splicing of GPR56 could diversify functionality (Salzman *et al.*, 2016). GPR56 is not the only aGPCR that undergoes alternative splicing: functional splice variants have been identified in all other aGPCRs except for GPR111, GPR115, GPR128, GPR144, BAI3, CELSR1, ELTD1 and VLGR1 (Bjarnadóttir *et al.*, 2007).

3. Function of adhesion GPCRs in metabolism

Quantitative trait loci analyses have revealed that the majority of human aGPCRs are associated with traits involved in metabolism such as body weight and body fat, and leptin, glucose and insulin levels (Kovacs and Schöneberg, 2016). Adhesion GPCRs have been detected in metabolically active tissues such as islets, liver, skeletal muscle and adipose tissue (Figure 3), and TG2, a putative GPR56 interacting partner, was identified as a candidate gene for maturity onset diabetes of the young (Porzio *et al.*, 2007).

3.1. Islets

Most aGPCRs are expressed at varying levels in islets, with GPR56 being the most highly expressed GPCR in both mouse and human islets, and ELTD1, CELSR and latrophilin family members showing moderate expression (Table 1). Both mouse and human islets express readily quantifiable levels of the latrophilins LPHN1 and LPHN2, but LPHN3 mRNA is of low abundance in mouse islets and undetectable in human islets (Amisten *et al.*, 2017). Therefore, if latrophilins have any translational value in regulating islet function focus should be limited to LPHN1 and LPHN2. Research to date on the role played by adhesion receptors in islets has largely focused on their interaction with the ECM, and their roles in β -cell development and insulin secretion.

3.1.1 The extracellular matrix and β -cell function

Islets of Langerhans are surrounded by a capsule of ECM, made up of the peri-insular basement membrane (BM) and a subjacent interstitial matrix, and this is among the first structures to be destroyed by auto-immune assault in type 1 diabetes (Winer *et al.*, 2003; Irving-Rodgers *et al.*, 2008; Korpos *et al.*, 2013). The functional significance of the ECM in normal islet function is well reported. Thus, ECM proteins are involved in a wide range of functions such as protecting β -cells from apoptosis, enhancing insulin secretion, β -cell migration and adhesion, and they play a role in pancreas development (Davis *et al.*, 2012; Kaido *et al.*, 2004; Huang *et al.*, 2011; Shih *et al.*, 2016; Arzouni *et al.*, 2017; Llacua *et al.*, 2018; Olaniru *et al.*, 2018a).

A substantial amount of ECM is also distributed throughout the islet interior as the perivascular basement membrane that is associated with, and largely generated by, the microvasculature endothelium (Otonkoski *et al.*, 2008). Unlike mouse islets, which have a single BM, the blood vessels of human islets have a duplex BM consisting of an outer parenchyma BM and an inner capillary BM (Virtanen *et al.*, 2008), and this is speculated to provide extra protection against autoimmune insulinitis (Otonkoski *et al.*, 2008). The majority of β -cells are in contact with the BM at the vasculature (Peiris *et al.*, 2014) and it is becoming apparent that insulin granule fusion is enriched at points where β -cells are in contact with the vascular BM (Low *et al.*, 2014; Gan *et al.*, 2018). In this way the ECM plays an important role in optimal insulin exocytosis by allowing targeted insulin secretion towards the vasculature.

Receptors mediating cell-ECM interactions have been identified, but their functions and signalling mechanisms are not fully understood. It is known that signals arising from ECM proteins can be transmitted into β -cells via membrane-localised adhesion receptors such as integrins, cadherins and aGPCRs, with integrin-mediated signalling cascades being the most

studied (Olaniru and Persaud, 2018). Efforts to translate integrin- β -cell-ECM interactions to T2D therapies have made little progress due to the sheer complexity of integrin/ECM interactions and non-specificity of ligand binding. However, given that GPCR family members have been successful in drug development, aGPCRs may prove more tractable than integrins in translating our understanding of β -cell-ECM interaction into novel therapeutic targets, although much research needs to be carried out in this area.

3.1.2 Islet development

Islet development starts as early as embryonic day 9 (E9) in mouse and occurs in two phases. The first phase, which is also known as primary transition, takes place between E9 and E12.5 and at this stage most of the cells are multipotent progenitor cells (MPCs) (Shih *et al.*, 2013). As the nascent pancreas enlarges and buds the MPCs, marked by their expression of transcription factors such as Pdx1, Ptf1a, Nkx2.2 and Sox9, undergo extensive proliferation (Bastidas-Ponce *et al.*, 2017). Towards the end of the primary transition, the first wave of differentiation begins, with the majority of the differentiated cells being glucagon-expressing α -cells (Johansson *et al.*, 2007). After E12.5, substantial differentiation occurs, giving rise to all of the endocrine cell types, with appearance of β -cells, followed by δ -cells and then pancreatic polypeptide cells (Johansson *et al.*, 2007). This is known as phase II or secondary transition (Jensen, 2004). As the mouse matures, islet cells migrate from the epithelium to form clusters and they do not resemble the adult islet structure until E18.5 (Villasenor *et al.*, 2008). Some differences in human islet development have been reported, such as in the presence of a single wave of endocrine differentiation, delayed expression of key differentiation genes and the islet cytoarchitecture (Nair and Hebrok, 2015). Thus, at 30 days post-conception (dpc) in human pancreas the MPCs express key transcription factors such as Pdx1, Ptf1a, Sox9, Gata4 and Nkx6.1 but not Nkx2.2, which is only detectable after endocrine cell commitment at 10 weeks post-conception (wpc) (Jennings *et al.*, 2013). Also, in humans insulin-positive β -cells are the first endocrine cells to appear (at 8wpc), rather than the α -cells that are observed first in developing mouse pancreas (Jeon *et al.*, 2009; Salisbury *et al.*, 2014). In addition, approximately 30% of the early human pancreas endocrine cells express more than one hormone, as the MPCs differentiate to other endocrine cell types, whereas the appearance of polyhormonal cells in mouse islets is scarce (Herrera, 2000; Riedel *et al.*, 2012). Despite these species-specific differences in endocrine pancreas specification the transcription factors regulating pancreas development are common to mice and humans and the overall islet structure is similar.

There is increasing evidence implicating ECM proteins and aGPCRs in pancreas development. It is now thought that ECM components provide cues for cells to migrate during organ development (Langenhan *et al.*, 2016), govern cell fate decisions of bipotent pancreatic progenitors to follow either duct or endocrine lineages (Mamidi *et al.*, 2018), and they regulate branching morphogenesis during pancreas development (Shih *et al.*, 2016). GPR56 is highly expressed in NGN3⁺ endocrine progenitors in the developing pancreas (Gu *et al.*, 2004), but its function in islet development is not yet known. Studies in neuronal and seminiferous tubule development in mice have indicated that GPR56 is critical for proper formation of the cerebellum, as it allows the developing neurons to adhere to the ECM of the pial basement membrane (Li *et al.*, 2008; Luo *et al.*, 2011), and its absence during embryogenesis leads to reduced male fertility (Chen *et al.*, 2010). Considering the high expression of GPR56 in the developing pancreas it may play a role in islet formation, and our preliminary studies showing fewer β -cells and more α -cells in islets from neonatal GPR56 null mice support this (Olaniru *et al.*, 2018b). In addition, it has been established that CELSR3 is critical for islet development, as mice with pancreatic progenitor Pdx1-specific deletion of CELSR3 are glucose intolerant in adult life, as a result of severe islet β -cell differentiation deficiency (Cortijo *et al.*, 2012).

3.1.3 Insulin secretion

Insulin release from islet β -cells is a tightly controlled process that is mainly regulated by nutrient availability and feedback communication between heterogenous islet cells and insulin-sensitive tissues. Although the contribution of classical GPCRs, such as GLP-1R, M3 muscarinic receptors and β_2 -adrenergic receptors (β_2 -AR), to glucose-induced insulin secretion is well established (Ahrén, 2009; Kong *et al.*, 2010; Santulli *et al.*, 2012), the involvement of aGPCRs is only just starting to be appreciated. In our screen of all GPCRs expressed by human islets, we detected 22 of the 33 known aGPCRs, including GPR56, GPR116, latrophilins (LPHNs), BAI3, ELTD1 and CELSR family members (Amisten *et al.*, 2013), and recent studies are beginning to reveal the effects of individual aGPCRs on islet secretory function.

Our observation that GPR56 is the most abundant GPCR in human and mouse islets (Amisten *et al.*, 2013, 2017) is consistent with its previously reported high expression in mouse pancreas (Haitina *et al.*, 2008). Collagen III, the endogenous ligand of GPR56 (Luo *et al.*, 2011), activates the receptor in a $G\alpha_{12/13}$ /RhoA dependent manner (Iguchi *et al.*, 2008; Luo *et al.*, 2014). In the islets, collagen III is not expressed by the β -cells but by the vascular endothelial cells within the islets and the basement membrane (Van Deijnen *et al.*, 1994; Olaniru *et al.*, 2018a). Exposure of islets and β -cells to exogenous collagen III potentiates glucose-mediated

insulin secretion and protects them from apoptosis, in a GPR56-dependent manner (Dunér *et al.*, 2016; Olaniru, *et al.*, 2018a), demonstrating that islets use β -cell-expressed GPR56 to sense signals from ECM collagen III to improve β -cell mass and secretory function. Consistent with this, islets in which GPR56 expression was acutely downregulated with siRNA had less insulin content and reduced insulin secretory capacity (Dunér *et al.*, 2016). However, GPR56 knockout mice are normal and are able to regulate blood glucose levels appropriately, and islets isolated from them respond appropriately to glucose (Olaniru *et al.*, 2018a). In contrast, deletion of TG2, which is thought to be a GPR56 binding partner, resulted in glucose intolerance in mice and islets isolated from them showed reduced glucose-induced insulin secretion, although signalling via GPR56 was not explored as TG2 had not yet been identified as a GPR56 activating ligand (Bernassola *et al.*, 2002). This role for TG2 in regulating blood glucose levels via stimulation of insulin release could not be replicated in a separate study where deletion or constitutive activation of TG2 did not affect islet insulin secretory responses to glucose or whole body glucose homeostasis (Iismaa *et al.*, 2013).

The black widow spider neurotoxin, α -latrotoxin, was shown to induce exocytotic release of insulin from clonal β -cells that expressed latrophilin, and transient overexpression of latrophilin in a β -cell line with low endogenous latrophilin expression conferred insulin secretory responsiveness to α -latrotoxin in those cells (Lang *et al.*, 1998). It was proposed that this stimulatory effect of α -latrotoxin may have been a consequence of it mimicking a natural ligand of the latrophilin receptor, but it is not yet known whether specific LPHN activation in islets is coupled to increased insulin secretion since LPHNs are still classified as orphans for which endogenous ligands have not yet been defined. Fibronectin leucine rich transmembrane proteins (FLRT) (O'Sullivan *et al.*, 2012) and teneurins (Silva *et al.*, 2011; Boucard *et al.*, 2014) have been proposed as endogenous binding partners for LPHNs, but nothing is known about the effects of these proteins on islet function.

Less is known about aGPCR signal transduction mechanisms than for the other GPCR families, most likely because most of them are orphans. However, there is evidence of aGPCR signalling via both stimulatory $G\alpha_s$ and inhibitory $G\alpha_i$ coupling (Hamann *et al.*, 2015), so it might be expected that they could have inhibitory as well as stimulatory effects on insulin secretion. In this context, it has recently been reported that the secreted protein, complement 1q like-3 (C1ql3), which has been identified as an activating ligand for BAI3 (Bolliger *et al.*, 2011; Hamoud *et al.*, 2018), decreased glucose-induced insulin secretion from mouse and human islets in a BAI3-dependent manner by inhibiting cAMP production (Gupta *et al.*, 2018). These data were generated using C1ql3 present in conditioned medium from HEK293 cells

transfected with a C1ql3 plasmid, so it would be useful to identify whether similar results are also obtained with exposure of islets to recombinant C1ql3. A related C1q domain-containing protein, C1ql4, has recently been identified as an inhibitor of Bal3 while stabilin-2 is an activator. It is thought that the relative expression of these two Bal3 binding partners is important in the regulation of myoblast fusion (Hamoud *et al.*, 2018), but there is currently no information on Bal3 regulation by C1ql4 or stabilin-2 in islets.

3.2 Adipose tissue

The traditional function of adipose tissue is to store energy and release it in the form of fatty acids when required, but it is now accepted it plays an important role as an endocrine organ that secretes a range of adipokines that are critical for maintaining whole body energy homeostasis. The major adipokine secreted by WAT is leptin, which is involved in metabolic regulation through its anorexigenic effects to inhibit food intake (Friedman, 2016). There is an imbalance in adipokine expression in obesity, with adipocyte hypertrophy leading to up-regulation of pro-inflammatory adipokines (e.g. IL-6, leptin, resistin and TNF α) and concomitant reductions in anti-inflammatory adipokines (e.g. adiponectin, adipolin and omentin-1) (Ohashi *et al.*, 2014; Nakamura *et al.*, 2014). This chronic, low-grade inflammatory environment in obesity contributes to cardiovascular and metabolic disorders (Nakamura *et al.*, 2014).

Adipose tissue is generally sub-classified as white adipose tissue (WAT) and brown adipose tissue (BAT). In addition, beige or brite adipocytes with brown-like properties have been found in WAT depots (Wu *et al.*, 2012), and it is now generally accepted that these arise from browning of WAT rather than being a distinct type of adipocyte. WAT is the primary energy storage organ that expands and contracts under conditions of energy excess and deficit, respectively. BAT is mainly found in neonates and infants where it has an essential function to generate non-shivering heat during cold exposure. Adipocytes in BAT have many mitochondria with high expression of uncoupling protein 1 (UCP-1), allowing them to efficiently convert lipids into heat, and BAT deficiency in mice is associated with obesity, insulin resistance and reduced thermogenic ability (Lowell *et al.*, 1993; Hamann *et al.*, 1998). It has been calculated that 40-50g of maximally activated BAT in adult humans could account for 20% of total energy expended (Rothwell and Stock, 1983), and the importance of BAT in energy metabolism has heightened the search for BAT activators for use as obesity and T2D therapies (Scheideler *et al.*, 2017).

Human subcutaneous WAT and mouse mature BAT express over 150 and 230 GPCRs, respectively (Amisten *et al.*, 2015; Klepac *et al.*, 2016). In terms of aGPCRs, GPR56, GPR116,

GPR124 and ELTD1 are highly expressed in human subcutaneous adipose tissue (Amisten *et al.*, 2015; Figure 3) and profiling of mouse brown adipose tissue has indicated relatively high expression of GPR124, GPR125, GPR133, LPHN1 and LPHN2 (Klepac *et al.*, 2016). Research on the role of GPCRs in adipose tissue has largely focused on β -adrenergic receptor signalling, with observations that selective activation of β_3 receptors in BAT and WAT was associated with increased energy expenditure and reduced food intake in mice (Grujic *et al.*, 1997). However, the enthusiasm for a β_3 adrenergic receptor-directed therapy was diminished in human studies due to lack of agonist efficacy and undesirable side effects (Poekes *et al.*, 2015).

Studies have been performed to identify the role of GPR116 in white adipocyte differentiation and whole body glucose homeostasis. It was found that depletion of GPR116 in pre-adipocytes in vitro impaired their capacity to differentiate to mature adipocytes (Nie *et al.*, 2012). In addition, adipose tissue-specific deletion of GPR116 produced mice that were glucose intolerant and insulin resistant, which was exacerbated by high fat feeding (Nie *et al.*, 2012), suggesting that if ligands are identified that selectively activate GPR116 they could be useful therapeutically in normalising glucose homeostasis.

The recent profiling of the BAT GPCRome (Klepac *et al.*, 2016) has led to renewed interest in the possibility of targeting BAT GPCRs to combat obesity. In particular, in that study it was identified that antagonising $G\alpha_q$ signalling could provide a mechanism for increasing brown and beige adipocyte number, and this is a potential strategy for increasing energy expenditure in obesity. In addition, since aGPCRs are the second most abundant family of GPCRs (after the large rhodopsin receptor family) in BAT, and there are altered expression profiles of aGPCRs upon differentiation of pre-adipocytes to mature brown adipocytes (Klepac *et al.*, 2016) there is strong rationale for research exploring aGPCR function in BAT. CELSR2 is down-regulated in mature brown adipocytes (Klepac *et al.*, 2016) and the potential importance of this aGPCR in metabolic control is indicated by the correlation of single nucleotide polymorphisms in CELSR2 with altered low-density lipoprotein levels in humans (Kathiresan *et al.*, 2008). The roles of aGPCRs that show altered expression during brown adipocyte maturation will provide important information on whether they may be targeted therapeutically, but as with identifying aGPCR function in other metabolically active tissues progress is limited by the lack of suitable ligands for most of the receptors.

3.3 Skeletal muscle

Skeletal muscle cells take up glucose following food intake through insulin-dependent translocation of GLUT4 transporters to the myocyte plasma membrane, and glucose that is not required for muscle activity is stored as glycogen (DeFronzo and Tripathy, 2009, Bradley *et al.*, 2015). Glucose transport into myocytes can also occur in an insulin-independent manner through exercise-induced muscle contractility (Sinacore and Gulve, 1993), thus ensuring that myocytes obtain an appropriate fuel supply in the post-absorptive state. The insulin resistance that commonly occurs in obesity, T2D, and other cardiometabolic disorders impairs insulin-mediated glucose uptake via GLUT4 and it is now apparent that β_2 adrenergic receptor activation can promote glucose uptake into skeletal muscle (Sato *et al.*, 2014). This increase in glucose clearance is dependent on GLUT4 translocation, but occurs independently of the insulin-mediated signal transduction cascade, offering the potential of circumventing myocyte insulin resistance.

The possibility of targeting aGPCRs to treat muscle disease through promoting skeletal muscle cell growth has been raised recently (White, 2016), and there is an increasing body of evidence indicating that aGPCRs are crucial for myoblast differentiation and muscle mass regulation. The majority of aGPCR family members that have been detected in human skeletal muscle are expressed at low levels (GPR56, GPR64, GPR113, CD97, BAI3, CELSR2, and ELTD1), and GPR116 and GPR125 are reported to have moderate expression (Jean-Baptiste *et al.*, 2005; Duff *et al.*, 2015).

Cell-cell fusion of myoblasts is a crucial and tightly regulated process in muscle formation and repair (Rochlin *et al.*, 2010). By interacting with the conserved ELMO-DOCK1-Rac pathway, BAI3 is obligatory for myoblast fusion in myogenesis and in adult muscle regeneration (Hamoud *et al.*, 2014). BAI3 can either inhibit or promote myoblast fusion by binding to C1q-like proteins or stabilin-2, respectively (Hamoud *et al.*, 2018). In chicken embryos, a loss of function mutation in BAI3 blocked myoblast fusion, leading to reduced myofibre length (Hamoud *et al.*, 2014), while BAI3 knockout mice have smaller muscle fibres that are less efficient in recovering from injury (Hamoud *et al.*, 2018). It has been identified that GPR56 expression is upregulated at the early stage of myoblast differentiation, and its deletion resulted in reduced myoblast fusion (Wu *et al.*, 2013). However, no difference in muscle phenotype was observed in GPR56 knockout mice (Wu *et al.*, 2013), suggesting that GPR56 is not required for skeletal muscle development in vivo. A role for GPR56 in regulating muscle mass is supported by observations that it is up-regulated under conditions of excess load on skeletal muscle, that its overexpression in vitro leads to myocyte hypertrophy and that it regulates PGC-14 α -mediated muscle hypertrophy in vivo (White *et al.*, 2014). Additionally,

GPR56 increases muscle hypertrophy via $G_{\alpha_{12/13}}$ signalling, with subsequent downstream activation of mTOR and IGF-1 leading to increased protein synthesis (White *et al.*, 2014). The sarcoplasmic reticulum of myofibres from CD97 null mice have altered morphology, but this does not translate to any changes in muscle function and the authors concluded that its role is most likely associated with skeletal muscle differentiation (Zyryanova *et al.*, 2014). Overall, aGPCRs are important players in muscle physiology and further research is required to identify whether they also play a role in contraction-induced glucose transport or other metabolic parameters in skeletal muscle. In this context it is of interest that CD97 mRNA upregulation has been implicated in the development of skeletal muscle insulin resistance (Poelkens *et al.*, 2013), but further work is required to determine if this is a causative relationship and whether myocyte aGPCRs could be targeted therapeutically to improve glucose homeostasis.

3.4 Other metabolic tissues with potential for aGPCR function

aGPCRs are expressed by other metabolic tissues such as liver, gastrointestinal tract and hypothalamus, but much less is known about their functions in these tissues than in islets, adipose tissue and skeletal muscle.

3.4.1 Liver

The liver plays a pivotal role in regulating glucose homeostasis by acting as a storage organ in the absorptive state and by maintaining glucose levels through glycogenolysis and gluconeogenesis during the post-absorptive state and fasting. Hepatocytes express many GPCRs, which are predicted to play important roles in liver function (Regard *et al.*, 2008). The glucagon receptor (GCGR), a G_{α_s} -coupled GPCR, is one of the most studied hepatocyte receptors since it directly controls hepatic glucose production: when blood glucose levels are low glucagon is secreted from islet α -cells and it increases liver glycogenolysis and gluconeogenesis through GCGR activation and generation of cAMP. This is an important physiological process, but it can be disrupted in T2D where reduced anabolic input from insulin and increased hepatic GCGR signalling can lead to excessive hepatic glucose production (HGP) (Lin and Accili, 2011). As G_{α_i} -coupled GPCRs inhibit cAMP production it has been proposed that they could be targeted therapeutically to combat the increased HGP that occurs in T2D. This has recently been investigated by a chemogenetic approach where mice expressing a G_{α_i} -coupled GPCR in their hepatocytes were exposed to a ligand that specifically activated that receptor, and the effects on glucose regulation in vivo and in vitro were evaluated (Rossi *et al.*, 2018). Selective activation of G_{α_i} in mouse hepatocytes paradoxically led to increased HGP and impaired glucose homeostasis, indicating that this is not an

appropriate strategy for reducing glucose levels in T2D, most likely because antagonism of hepatocyte cAMP generation will have an impact on multiple processes in addition to blockade of HGP. The authors concluded that rather than activating hepatocyte $G\alpha_i$ -coupled signalling it would be more appropriate to antagonise it for treating T2D, but it seems likely that a more nuanced approach will be necessary.

The involvement of aGPCRs in hepatocyte function has not yet been described, but global transcriptomic analysis has revealed that multiple aGPCRs are expressed by human liver, with GPR125, GPR126, LPHN2 and CD97 showing high levels of expression, while GPR116, GPR124, GPR128 and ELTD1 were detected at moderate levels (Fagerberg *et al.*, 2014). Our analysis of mouse liver has indicated a similar high expression pattern for GPR125 and LPHN2, and moderate expression of GPR116 and ELTD1 (Atanes and Persaud, unpublished). In contrast, while CD97 mRNA was highly expressed in human liver our quantifications indicated that it was expressed at low levels in mouse liver, as were GPR124 and GPR126, while we could not detect GPR128 in mouse liver by qPCR. Human hepatocytes are available for research, but as rodent samples are often used as surrogates it is important that research is focused on those rodent aGPCRs that are also expressed by human hepatocytes so that data have high translation value. Further work is required to define the function of liver aGPCRs and whether they present an opportunity for therapeutic intervention to combat liver dysfunction that occurs in fatty liver disease and diabetes.

3.4.2 Gastrointestinal tract

The gastrointestinal (GI) tract is the largest endocrine organ in mammals. It is involved in various metabolic processes such as appetite regulation, digestion, nutrient absorption and potentiation of glucose-induced insulin secretion through the release of the incretins GLP-1 and GIP from specialised enteroendocrine L cells. GLP-1 secretion is stimulated by activation of GPR40 and GPR119 by triacylglycerol metabolites and the bile acid GPCR, TGR5 (Hauge *et al.*, 2017), and elevations in GLP-1 and PYY, another L cell-derived GPCR ligand, have been implicated in improved metabolic outcomes following bariatric surgery (Pucci and Batterham, 2018). The production of GPR119 agonists by human gastrointestinal bacteria has recently been associated with increased GLP-1 secretion and improved glucose tolerance in mice (Cohen *et al.*, 2017). These observations indicate that there are additional physiological mechanisms regulating GLP-1 release, and open up the possibility that bacteria engineered to produce GPR119 ligands could have therapeutic potential to treat T2D. There has been interest from pharmaceutical companies to enhance the incretin response and ligands targeting a number of enteroendocrine cell-expressed GPCRs, including GLP-1R, GPR40, GPR119 and GPR120, are undergoing investigation as potential therapies for T2D (Mace *et*

al., 2015; Hoque *et al.*, 2018). It has been reported that aGPCRs expressed in the GI tract include GPR56, GPR126, GPR128, CD97, LPHN2 and CELSR1 (Fagerberg *et al.*, 2013; Hamman *et al.*, 2015). However, the role, if any, that GI tract aGPCRs play in regulating fuel homeostasis is currently unknown and most studies to date have focused on their contribution to colorectal carcinoma (Hilbig *et al.*, 2018; Ji *et al.*, 2018).

3.4.3 Hypothalamus

The importance of the hypothalamus in many metabolic processes such as feeding and energy homeostasis is well known, and many of these functions are mediated via hypothalamic GPCRs for ligands such as neuropeptide Y, opioids, serotonin and γ -amino butyric acid (Qiu *et al.*, 2006, 2007; Fu and van den Pol, 2010). The aGPCRs GPR56, GPR64, and VLGR1 are highly expressed in the hypothalamus (Liu *et al.*, 1999; Haitina *et al.*, 2008) and at least 23 aGPCRs have been detected in the arcuate nucleus, which is the metabolic centre of the hypothalamus (Rønnekleiv *et al.*, 2014). Of the aGPCRs expressed in the arcuate nucleus, GPR56 and BAI1 are the most abundant (Rønnekleiv *et al.*, 2014), but there is still no data available on whether these receptors or other arcuate nucleus aGPCRs are present on the NPY/AgRP or POMC/CART neurons that regulate food intake, or whether they may represent novel targets for appetite regulation in obesity.

4 Conclusion and future directions

Research defining the importance of aGPCRs in regulating appropriate energy homeostasis is still at an early stage, and considerable further research is required to determine whether members of this family of receptors may be suitable candidates for treating metabolic disorders such as obesity and T2D. Nonetheless, the expression of a wide range of aGPCRs by tissues important in ensuring that blood glucose levels are regulated within a tight range and the close association between aGPCRs and metabolic traits supports continued exploration of their utility as druggable targets.

Figure 4 summarises some of the key information relating to aGPCR function in islet β -cells, adipocytes and myocytes, and as aGPCR metabolic research progresses we will be able to build up a clearer picture of the key candidates that may have therapeutic potential. Nonetheless, there are still barriers to rapid progress in this field and future research will be greatly facilitated by de-orphanisation of the remaining aGPCRs and identification of specific small molecule agonists and antagonists. In addition, more studies are required to fully understand the signalling mechanisms downstream of aGPCR activation, and this too will be assisted by availability of highly selective ligands. Given their broad expression, it will also be

interesting to identify aGPCR splice variants that may be specifically targeted in metabolically active tissues without causing undesirable side effects.

Conflict of Interest statement

The authors declare that they have no conflicting interests.

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References

- Ahrén B (2009) Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nat Rev Drug Discov* 8:369–385.
- Alexander SP, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD, Pawson AJ, Sharman JL, Southan C, Davies JA, and CGTP Collaborators (2017) The concise guide to pharmacology 2017/18: G protein-coupled receptors. *Br J Pharmacol* 174 Suppl 1:S17–S129.
- Amisten S, Atanes P, Hawkes R, Ruz-Maldonado I, Liu B, Parandeh F, Zhao M, Huang GC, Salehi A, and Persaud SJ (2017) A comparative analysis of human and mouse islet G-protein coupled receptor expression. *Sci Rep* 7:46600.
- Amisten S, Neville M, Hawkes R, Persaud SJ, Karpe F, and Salehi A (2015) An atlas of G-protein coupled receptor expression and function in human subcutaneous adipose tissue. *Pharmacol Ther* 146:61–93.
- Amisten S, Salehi A, Rorsman P, Jones PM, and Persaud SJ (2013) An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. *Pharmacol Ther* 139:359–391.
- Araç D, Boucard AA, Bolliger MF, Nguyen J, Soltis SM, Südhof TC, and Brunger AT (2012) A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO J* 31:1364–1378.
- Arzouni AA, Vargas-Seymour A, Rackham CL, Dhadda P, Huang G-C, Choudhary P, Nardi N, King AJF, and Jones PM (2017) Mesenchymal stromal cells improve human islet function through released products and extracellular matrix. *Clin Sci* 131:2835–2845.
- Bastidas-Ponce A, Scheibner K, Lickert H, and Bakhti M (2017) Cellular and molecular mechanisms coordinating pancreas development. *Development* 144:2873–2888.
- Bernassola F, Federici M, Corazzari M, Terrinoni A, Hribal ML, De Laurenzi V, Ranalli M, Massa O, Sesti G, McLean WHI, Citro G, Barbetti F, and Melino G (2002) Role of transglutaminase 2 in glucose tolerance: knockout mice studies and a putative mutation in a MODY patient. *FASEB J* 16:1371–1378.
- Bjarnadóttir TK, Fredriksson R, Höglund PJ, Gloriam DE, Lagerström MC, and Schiöth HB (2004) The human and mouse repertoire of the adhesion family of G-protein-coupled receptors. *Genomics* 84:23–33.
- Bjarnadóttir TK, Geirardsdóttir K, Ingemansson M, Mirza MAI, Fredriksson R, and Schiöth HB (2007) Identification of novel splice variants of Adhesion G protein-coupled receptors. *Gene* 387:38–48.
- Bolliger MF, Martinelli DC, and Südhof TC (2011) The cell-adhesion G protein-coupled receptor BAI3 is a high-affinity receptor for C1q-like proteins. *Proc Natl Acad Sci USA* 108:2534–2539.
- Boucard AA, Maxeiner S, and Südhof TC (2014) Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. *J Biol Chem* 289:387–402.
- Boyden SE, Desai A, Cruse G, Young ML, Bolan HC, Scott LM, Eisch AR, Long RD, Lee C-CR, Satorius CL, Pakstis AJ, Olivera A, Mullikin JC, Chouery E, Mégarbané A, Medlej-Hashim M, Kidd KK, Kastner DL, Metcalfe DD, and Komarow HD (2016) Vibratory Urticaria Associated with a Missense Variant in ADGRE2. *N Engl J Med* 374:656–663.

- Bradley H, Shaw CS, Bendtsen C, Worthington PL, Wilson OJ, Strauss JA, Wallis GA, Turner AM, and Wagenmakers AJM (2015) Visualization and quantitation of GLUT4 translocation in human skeletal muscle following glucose ingestion and exercise. *Physiol Rep* 3:1–11.
- Chen G, Yang L, Begum S, and Xu L (2010) GPR56 is essential for testis development and male fertility in mice. *Dev Dyn* 239:3358–3367.
- Chiang N-Y, Chang G-W, Huang Y-S, Peng Y-M, Hsiao C-C, Kuo M-L, and Lin H-H (2016) Heparin interacts with the adhesion GPCR GPR56, reduces receptor shedding, and promotes cell adhesion and motility. *J Cell Sci* 129:2156–2169.
- Chong Z-S, Ohnishi S, Yusa K, and Wright GJ (2018) Pooled extracellular receptor-ligand interaction screening using CRISPR activation. *Genome Biol* 19:205.
- Cohen LJ, Esterhazy D, Kim S-H, Lemetre C, Aguilar RR, Gordon EA, Pickard AJ, Cross JR, Emiliano AB, Han SM, Chu J, Vila-Farres X, Kaplitt J, Rogoz A, Calle PY, Hunter C, Bitok JK, and Brady SF (2017) Commensal bacteria make GPCR ligands that mimic human signalling molecules. *Nature* 549:48–53.
- Cortijo C, Gouzi M, Tissir F, and Grapin-Botton A (2012) Planar cell polarity controls pancreatic beta cell differentiation and glucose homeostasis. *Cell Rep* 2:1593–1606.
- Davenport AP, Alexander SPH, Sharman JL, Pawson AJ, Benson HE, Monaghan AE, Liew WC, Mpmahanga CP, Bonner TI, Neubig RR, Pin JP, Spedding M, and Harmar AJ (2013) International Union of Basic and Clinical Pharmacology. LXXXVIII. G protein-coupled receptor list: recommendations for new pairings with cognate ligands. *Pharmacol Rev* 65:967–986.
- Davis NE, Beenken-Rothkopf LN, Mirsoian A, Kojic N, Kaplan DL, Barron AE, and Fontaine MJ (2012) Enhanced function of pancreatic islets co-encapsulated with ECM proteins and mesenchymal stromal cells in a silk hydrogel. *Biomaterials* 33:6691–6697.
- DeFronzo RA, and Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32 Suppl 2:S157–63.
- Demberg LM, Winkler J, Wilde C, Simon K-U, Schön J, Rothmund S, Schöneberg T, Prömel S, and Liebscher I (2017) Activation of adhesion G protein-coupled receptors: agonist specificity of stachel sequence-derived peptides. *J Biol Chem* 292:4383–4394.
- Duff MO, Olson S, Wei X, Garrett SC, Osman A, Bolisetty M, Plocik A, Celniker SE, and Graveley BR (2015) Genome-wide identification of zero nucleotide recursive splicing in *Drosophila*. *Nature* 521:376–379.
- Dunér P, Al-Amily IM, Soni A, Asplund O, Safi F, Storm P, Groop L, Amisten S, and Salehi A (2016) Adhesion G Protein-Coupled Receptor G1 (ADGRG1/GPR56) and Pancreatic β -Cell Function. *J Clin Endocrinol Metab* 101:4637–4645.
- Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpour S, Danielsson A, Edlund K, Asplund A, Sjöstedt E, Lundberg E, Szigartyo CA-K, Skogs M, Takanen JO, Berling H, Tegel H, Mulder J, Nilsson P, Schwenk JM, Lindskog C, Danielsson F, Mardinoglu A, Sivertsson A, von Feilitzen K, Forsberg M, Zwahlen M, Olsson I, Navani S, Huss M, Nielsen J, Ponten F, and Uhlén M (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol Cell Proteomics* 13:397–406.

- Fredriksson R, Lagerström MC, Lundin L-G, and Schiöth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 63:1256–1272.
- Frias JP, Nauck MA, Van J, Kutner ME, Cui X, Benson C, Urva S, Gimeno RE, Milicevic Z, Robins D, and Haupt A (2018) Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *Lancet*, doi: 10.1016/S0140-6736(18)32260-8.
- Friedman J (2016) The long road to leptin. *J Clin Invest* 126:4727–4734.
- Fu L-Y, and van den Pol AN (2010) Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. *J Neurosci* 30:10205–10219.
- Gan WJ, Do OH, Cottle L, Ma W, Kosobrodova E, Cooper-White J, Bilek M, and Thorn P (2018) Local integrin activation in pancreatic β cells targets insulin secretion to the vasculature. *Cell Rep* 24:2819–2826.e3.
- Grujic D, Susulic VS, Harper ME, Himms-Hagen J, Cunningham BA, Corkey BE, and Lowell BB (1997) Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. *J Biol Chem* 272:17686–17693.
- Gu G, Wells JM, Dombkowski D, Pfeffer F, Aronow B, and Melton DA (2004) Global expression analysis of gene regulatory pathways during endocrine pancreatic development. *Development* 131:165–179.
- Gupta R, Nguyen DC, Schaid MD, Lei X, Balamurugan AN, Wong GW, Kim J-A, Koltes JE, Kimple ME, and Bhatnagar S (2018) Complement 1q like-3 protein inhibits insulin secretion from pancreatic β -cells via the cell adhesion G protein-coupled receptor BAI3. *J Biol Chem*, doi: 10.1074/jbc.RA118.005403.
- Haitina T, Olsson F, Stephansson O, Alsö J, Roman E, Ebendal T, Schiöth HB, and Fredriksson R (2008) Expression profile of the entire family of Adhesion G protein-coupled receptors in mouse and rat. *BMC Neurosci* 9:43.
- Hamann A, Flier JS, and Lowell BB (1998) Obesity after genetic ablation of brown adipose tissue. *Z Ernährungswiss* 37 Suppl 1:1–7.
- Hamann J, Aust G, Araç D, Engel FB, Formstone C, Fredriksson R, Hall RA, Harty BL, Kirchhoff C, Knapp B, Krishnan A, Liebscher I, Lin H-H, Martinelli DC, Monk KR, Peeters MC, Piao X, Prömel S, Schöneberg T, Schwartz TW, Singer K, Stacey M, Ushkaryov YA, Vallon M, Wolfrum U, Wright MW, Xu L, Langenhan T, and Schiöth HB (2015) International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol Rev* 67:338–367.
- Hamoud N, Tran V, Aimi T, Kakegawa W, Lahaie S, Thibault M-P, Pelletier A, Wong GW, Kim I-S, Kania A, Yuzaki M, Bouvier M, and Côté J-F (2018) Spatiotemporal regulation of the GPCR activity of BAI3 by C1qL4 and Stabilin-2 controls myoblast fusion. *Nat Commun* 9:4470.
- Hamoud N, Tran V, Croteau L-P, Kania A, and Côté J-F (2014) G-protein coupled receptor BAI3 promotes myoblast fusion in vertebrates. *Proc Natl Acad Sci USA* 111:3745–3750.

- Hauge M, Pio Ekberg J, Storm Engelstoft M, Timshel P, Madsen AN, and Schwartz TW (2017) Gq and Gs signaling acting in synergy to control GLP-1 secretion. *Mol Cell Endocrinol* 449:64-73.
- Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, and Gloriam DE (2017) Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov* 16:829-842.
- Herrera PL (2000) Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development* 127:2317-2322.
- Hilbig D, Dietrich N, Wandel E, Gonsior S, Sittig D, Hamann J, and Aust G (2018) The interaction of CD97/ADGRE5 with β -Catenin in adherens junctions is lost during colorectal carcinogenesis. *Front Oncol* 8:182. doi: 10.3389/fonc.2018.00182.
- Hoque M, Ali S, and Hoda M (2018) Current status of G-protein coupled receptors as potential targets against type 2 diabetes mellitus. *Int J Biol Macromol* 118:2237-2244.
- Huang G, Ge G, Wang D, Gopalakrishnan B, Butz DH, Colman RJ, Nagy A, and Greenspan DS (2011) $\alpha 3(V)$ collagen is critical for glucose homeostasis in mice due to effects in pancreatic islets and peripheral tissues. *J Clin Invest* 121:769-783.
- Huang Y-S, Chiang N-Y, Chang G-W, and Lin H-H (2018) Membrane-association of EMR2/ADGRE2-NTF is regulated by site-specific N-glycosylation. *Sci Rep* 8:4532.
- IDF (2017) IDF diabetes atlas, 8th edition. Brussels, Belgium.
- Iguchi T, Sakata K, Yoshizaki K, Tago K, Mizuno N, and Itoh H (2008) Orphan G protein-coupled receptor GPR56 regulates neural progenitor cell migration via a G α 12/13 and Rho pathway. *J Biol Chem* 283:14469-14478.
- Iismaa SE, Aplin M, Holman S, Yiu TW, Jackson K, Burchfield JG, Mitchell CJ, O'Reilly L, Davenport A, Cantley J, Schmitz-Peiffer C, Biden TJ, Cooney GJ, and Graham RM (2013) Glucose homeostasis in mice is transglutaminase 2 independent. *PLoS One* 8:e63346.
- Irving-Rodgers HF, Ziolkowski AF, Parish CR, Sado Y, Ninomiya Y, Simeonovic CJ, and Rodgers RJ (2008) Molecular composition of the peri-islet basement membrane in NOD mice: a barrier against destructive insulinitis. *Diabetologia* 51:1680-1688.
- Jean-Baptiste G, Yang Z, Khoury C, Gaudio S, and Greenwood MT (2005) Peptide and non-peptide G-protein coupled receptors (GPCRs) in skeletal muscle. *Peptides* 26:1528-1536.
- Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, Salisbury RJ, Blaylock J, Piper Hanley K, and Hanley NA (2013) Development of the human pancreas from foregut to endocrine commitment. *Diabetes* 62:3514-3522.
- Jensen J (2004) Gene regulatory factors in pancreatic development. *Dev Dyn* 229:176-200.
- Jeon J, Correa-Medina M, Ricordi C, Edlund H, and Diez JA (2009) Endocrine cell clustering during human pancreas development. *J Histochem Cytochem* 57:811-824.
- Ji B, Feng Y, Sun Y, Ji D, Qian W, Zhang Z, Wang Q, Zhang Y, Zhang C, and Sun Y (2018) GPR56 promotes proliferation of colorectal cancer cells and enhances metastasis via epithelial-mesenchymal transition through PI3K/AKT signaling activation. *Oncol Rep* 40:1885-1896.

- Jin G, Sakitani K, Wang H, Jin Y, Dubeykovskiy A, Worthley DL, Tailor Y, and Wang TC (2017) The G-protein coupled receptor 56, expressed in colonic stem and cancer cells, binds progastrin to promote proliferation and carcinogenesis. *Oncotarget* 8:40606–40619.
- Johansson KA, Dursun U, Jordan N, Gu G, Beermann F, Gradwohl G, and Grapin-Botton A (2007) Temporal control of neurogenin3 activity in pancreas progenitors reveals competence windows for the generation of different endocrine cell types. *Dev Cell* 12:457–465.
- Kaido T, Yebra M, Cirulli V, and Montgomery AM (2004) Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1beta1. *J Biol Chem* 279:53762–53769.
- Kathiresan S, Melander O, Guiducci C, Surti A, Burtt NP, Rieder MJ, Cooper GM, Roos C, Voight BF, Havulinna AS, Wahlstrand B, Hedner T, Corella D, Tai ES, Ordovas JM, Berglund G, Vartiainen E, Jousilahti P, Hedblad B, Taskinen M-R, Newton-Cheh C, Salomaa V, Peltonen L, Groop L, Altshuler DM, and Orho-Melander M (2008) Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. *Nat Genet* 40:189–197.
- Kim J-E, Han JM, Park CR, Shin K-J, Ahn C, Seong JY, and Hwang J-I (2010) Splicing variants of the orphan G-protein-coupled receptor GPR56 regulate the activity of transcription factors associated with tumorigenesis. *J Cancer Res Clin Oncol* 136:47–53.
- Klepac K, Kilić A, Gnad T, Brown LM, Herrmann B, Wilderman A, Balkow A, Glöde A, Simon K, Lidell ME, Betz MJ, Enerbäck S, Wess J, Freichel M, Blüher M, König G, Kostenis E, Insel PA, and Pfeifer A (2016) The Gq signalling pathway inhibits brown and beige adipose tissue. *Nat Commun* 7:10895.
- Kolakowski LF (1994) GCRDb: a G-protein-coupled receptor database. *Receptors Channels* 2:1–7.
- Kong KC, Butcher AJ, McWilliams P, Jones D, Wess J, Hamdan FF, Werry T, Rosethorne EM, Charlton SJ, Munson SE, Cragg HA, Smart AD, and Tobin AB (2010) M3-muscarinic receptor promotes insulin release via receptor phosphorylation/arrestin-dependent activation of protein kinase D1. *Proc Natl Acad Sci USA* 107:21181–21186.
- Korpos É, Kadri N, Kappelhoff R, Wegner J, Overall CM, Weber E, Holmberg D, Cardell S, and Sorokin L (2013) The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. *Diabetes* 62:531–542.
- Kovacs P, and Schöneberg T (2016) The relevance of genomic signatures at adhesion GPCR loci in humans. *Handb Exp Pharmacol* 234:179–217.
- Krishnan A, Nijmeijer S, de Graaf C, and Schiöth HB (2016) Classification, nomenclature, and structural aspects of adhesion gpcrs. *Handb Exp Pharmacol* 234:15–41.
- Lang J, Ushkaryov Y, Grasso A, and Wollheim CB (1998) Ca²⁺-independent insulin exocytosis induced by alpha-latrotoxin requires latrophilin, a G protein-coupled receptor. *EMBO J* 17:648–657.
- Langenhan T, Aust G, and Hamann J (2013) Sticky signaling--adhesion class G protein-coupled receptors take the stage. *Sci Signal* 6:re3.
- Langenhan T, Piao X, and Monk KR (2016) Adhesion G protein-coupled receptors in nervous system development and disease. *Nat Rev Neurosci* 17:550–561.
- Lee J-W, Huang BX, Kwon H, Rashid MA, Kharebava G, Desai A, Patnaik S, Marugan J, and Kim H-Y (2016) Orphan GPR110 (ADGRF1) targeted by N-

- docosahexaenylethanolamine in development of neurons and cognitive function. *Nat Commun* 7:13123.
- Li S, Jin Z, Koirala S, Bu L, Xu L, Hynes RO, Walsh CA, Corfas G, and Piao X (2008) GPR56 regulates pial basement membrane integrity and cortical lamination. *J Neurosci* 28:5817–5826.
- Liebscher I, and Schöneberg T (2016) Tethered agonism: A common activation mechanism of adhesion gpcrs. *Handb Exp Pharmacol* 234:111–125.
- Lin HV, and Accili D (2011) Hormonal regulation of hepatic glucose production in health and disease. *Cell Metab* 14:9–19.
- Little KD, Hemler ME, and Stipp CS (2004) Dynamic regulation of a GPCR-tetraspanin-G protein complex on intact cells: central role of CD81 in facilitating GPR56-Galpha q/11 association. *Mol Biol Cell* 15:2375–2387.
- Liu M, Parker RM, Darby K, Eyre HJ, Copeland NG, Crawford J, Gilbert DJ, Sutherland GR, Jenkins NA, and Herzog H (1999) GPR56, a novel secretin-like human G-protein-coupled receptor gene. *Genomics* 55:296–305.
- Llacua LA, Faas MM, and de Vos P (2018) Extracellular matrix molecules and their potential contribution to the function of transplanted pancreatic islets. *Diabetologia* 61:1261–1272.
- Low JT, Zavortink M, Mitchell JM, Gan WJ, Do OH, Schwieneing CJ, Gaisano HY, and Thorn P (2014) Insulin secretion from beta cells in intact mouse islets is targeted towards the vasculature. *Diabetologia* 57:1655–1663.
- Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, and Flier JS (1993) Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366:740–742.
- Luo R, Jeong S-J, Jin Z, Strokes N, Li S, and Piao X (2011) G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. *Proc Natl Acad Sci USA* 108:12925–12930.
- Luo R, Jeong S-J, Yang A, Wen M, Saslowsky DE, Lencer WI, Araç D, and Piao X (2014) Mechanism for adhesion G protein-coupled receptor GPR56-mediated RhoA activation induced by collagen III stimulation. *PLoS One* 9:e100043.
- Mace OJ, Tehan B, and Marshall F (2015) Pharmacology and physiology of gastrointestinal enteroendocrine cells. *Pharma Res Per* 3:e00155.
- Mamidi A, Prawiro C, Seymour PA, de Lichtenberg KH, Jackson A, Serup P, and Semb H (2018) Mechanosignalling via integrins directs fate decisions of pancreatic progenitors. *Nature*, doi: 10.1038/s41586-018-0762-2.
- Nair G, and Hebrok M (2015) Islet formation in mice and men: lessons for the generation of functional insulin-producing β -cells from human pluripotent stem cells. *Curr Opin Genet Dev* 32:171–180.
- Nakamura K, Fuster JJ, and Walsh K (2014) Adipokines: a link between obesity and cardiovascular disease. *J Cardiol* 63:250–259.
- Nie T, Hui X, Gao X, Li K, Lin W, Xiang X, Ding M, Kuang Y, Xu A, Fei J, Wang Z, and Wu D (2012) Adipose tissue deletion of Gpr116 impairs insulin sensitivity through modulation of adipose function. *FEBS Lett* 586:3618–3625.

- O'Sullivan ML, de Wit J, Savas JN, Comoletti D, Otto-Hitt S, Yates JR, and Ghosh A (2012) FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. *Neuron* 73:903–910.
- Ohashi K, Shibata R, Murohara T, and Ouchi N (2014) Role of anti-inflammatory adipokines in obesity-related diseases. *Trends Endocrinol Metab.* 25:348–355.
- Olaniru OE, and Persaud SJ (2018) Identifying novel therapeutic targets for diabetes through improved understanding of islet adhesion receptors. *Curr Opin Pharmacol* 43:27–33.
- Olaniru OE, Pingitore A, Giera S, Piao X, Castañera González R, Jones PM, and Persaud SJ (2018a) The adhesion receptor GPR56 is activated by extracellular matrix collagen III to improve β -cell function. *Cell Mol Life Sci* 75:4007–4019.
- Olaniru OE, Toczyska K, Cujba AM, Atanes P, Sancho R, Jones PM, and Persaud SJ (2018b) GPR56 is highly expressed by pancreatic progenitor cells and it regulates beta cell development and function. *Diabetologia* 61:S55.
- Otonkoski T, Banerjee M, Korsgren O, Thornell LE, and Virtanen I (2008) Unique basement membrane structure of human pancreatic islets: implications for beta-cell growth and differentiation. *Diabetes Obes Metab* 10 Suppl 4:119–127.
- Paavola KJ, and Hall RA (2012) Adhesion G protein-coupled receptors: signaling, pharmacology, and mechanisms of activation. *Mol Pharmacol* 82:777–783.
- Paavola KJ, Sidik H, Zuchero JB, Eckart M, and Talbot WS (2014) Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. *Sci Signal* 7:ra76.
- Paavola KJ, Stephenson JR, Ritter SL, Alter SP, and Hall RA (2011) The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity. *J Biol Chem* 286:28914–28921.
- Peiris H, Bonder CS, Coates PTH, Keating DJ, and Jessup CF (2014) The β -cell/EC axis: how do islet cells talk to each other? *Diabetes* 63:3–11.
- Persaud SJ (2017) Islet G-protein coupled receptors: therapeutic potential for diabetes. *Curr Opin Pharmacol* 37:24–28.
- Petersen SC, Luo R, Liebscher I, Giera S, Jeong S-J, Mogha A, Ghidinelli M, Feltri ML, Schöneberg T, Piao X, and Monk KR (2015) The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211. *Neuron* 85:755–769.
- Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Strausberg R, Dobyns WB, Qasrawi B, Winter RM, Innes AM, Voit T, Ross ME, Michaud JL, Descarrie J-C, Barkovich AJ, and Walsh CA (2004) G protein-coupled receptor-dependent development of human frontal cortex. *Science* 303:2033–2036.
- Poekes L, Lanthier N, and Leclercq IA (2015) Brown adipose tissue: a potential target in the fight against obesity and the metabolic syndrome. *Clin Sci* 129:933–949.
- Poelkens F, Lammers G, Pardoel EM, Tack CJ, and Hopman MTE (2013) Upregulation of skeletal muscle inflammatory genes links inflammation with insulin resistance in women with the metabolic syndrome. *Exp Physiol* 98:1485–1494.
- Porzio O, Massa O, Cunsolo V, Colombo C, Malaponti M, Bertuzzi F, Hansen T, Johansen A, Pedersen O, Meschi F, Terrinoni A, Melino G, Federici M, Decarlo N, Menicagli M, Campani D, Marchetti P, Ferdaoussi M, Froguel P, Federici G, Vaxillaire M, and Barbetti F (2007) Missense mutations in the TGM2 gene encoding transglutaminase 2 are found

- in patients with early-onset type 2 diabetes. Mutation in brief no. 982. Online. *Hum Mutat* 28:1150.
- Prömel S, Langenhan T, and Araç D (2013) Matching structure with function: the GAIN domain of adhesion-GPCR and PKD1-like proteins. *Trends Pharmacol Sci* 34:470–478.
- Pucci A, and Batterham, RL (2018) Mechanisms underlying the weight loss effects of RYGB and SG: similar, yet different. *J Endocrinol Invest* doi: 10.1007/s40618-018-0892-2
- Qiu J, Bosch MA, Tobias SC, Krust A, Graham SM, Murphy SJ, Korach KS, Chambon P, Scanlan TS, Rønnekleiv OK, and Kelly MJ (2006) A G-protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. *J Neurosci* 26:5649–5655.
- Qiu J, Xue C, Bosch MA, Murphy JG, Fan W, Rønnekleiv OK, and Kelly MJ (2007) Serotonin 5-hydroxytryptamine_{2C} receptor signaling in hypothalamic proopiomelanocortin neurons: role in energy homeostasis in females. *Mol Pharmacol* 72:885–896.
- Ravier MA, Leduc M, Richard J, Linck N, Varrault A, Pirot N, Roussel MM, Bockaert J, Dalle S, and Bertrand G (2014) β -Arrestin2 plays a key role in the modulation of the pancreatic beta cell mass in mice. *Diabetologia* 57:532–541.
- Regard JB, Sato IT, and Coughlin SR (2008) Anatomical profiling of G protein-coupled receptor expression. *Cell* 135:561–571.
- Riedel MJ, Asadi A, Wang R, Ao Z, Warnock GL, and Kieffer TJ (2012) Immunohistochemical characterisation of cells co-producing insulin and glucagon in the developing human pancreas. *Diabetologia* 55:372–381.
- Roberts CK, Hevener AL, and Barnard RJ (2013) Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. *Compr Physiol* 3:1–58.
- Rochlin K, Yu S, Roy S, and Baylies MK (2010) Myoblast fusion: when it takes more to make one. *Dev Biol* 341:66–83.
- Rønnekleiv OK, Fang Y, Zhang C, Nestor CC, Mao P, and Kelly MJ (2014) Research resource: Gene profiling of G protein-coupled receptors in the arcuate nucleus of the female. *Mol Endocrinol* 28:1362–1380.
- Rossi M, Zhu L, McMillin SM, Pydi SP, Jain S, Wang L, Cui Y, Lee RJ, Cohen AH, Kaneto H, Birnbaum MJ, Ma Y, Rotman Y, Liu J, Cyphert TJ, Finkel T, McGuinness OP, and Wess J (2018) Hepatic G_i signaling regulates whole-body glucose homeostasis. *J Clin Invest* 128:746–759.
- Rothwell NJ, and Stock MJ (1983) Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin Sci* 64:19–23.
- Salisbury RJ, Blaylock J, Berry AA, Jennings RE, De Krijger R, Piper Hanley K, and Hanley NA (2014) The window period of NEUROGENIN3 during human gestation. *Islets* 6:e954436.
- Salzman GS, Ackerman SD, Ding C, Koide A, Leon K, Luo R, Stoveken HM, Fernandez CG, Tall GG, Piao X, Monk KR, Koide S, and Araç D (2016) Structural basis for regulation of GPR56/ADGRG1 by its alternatively spliced extracellular domains. *Neuron* 91:1292–1304.
- Santos R, Ursu O, Gaulton A, Bento AP, Donadi RS, Bologa CG, Karlsson A, Al-Lazikani B, Hersey A, Oprea TI, and Overington JP (2017) A comprehensive map of molecular drug targets. *Nat Rev Drug Discov* 16:19–34.

- Santulli G, Lombardi A, Sorriento D, Anastasio A, Del Giudice C, Formisano P, Béguinot F, Trimarco B, Miele C, and Iaccarino G (2012) Age-related impairment in insulin release: the essential role of $\beta(2)$ -adrenergic receptor. *Diabetes* 61:692–701.
- Sato M, Dehvari N, Oberg AI, Dallner OS, Sandström AL, Olsen JM, Csikasz RI, Summers RJ, Hutchinson DS, and Bengtsson T (2014) Improving type 2 diabetes through a distinct adrenergic signaling pathway involving mTORC2 that mediates glucose uptake in skeletal muscle. *Diabetes* 63:4115–4129.
- Scheideler M, Herzig S, and Georgiadi A (2017) Endocrine and autocrine/paracrine modulators of brown adipose tissue mass and activity as novel therapeutic strategies against obesity and type 2 diabetes. *Horm Mol Biol Clin Investig* 31.
- Shih HP, Panlasigui D, Cirulli V, and Sander M (2016) ECM signaling regulates collective cellular dynamics to control pancreas branching morphogenesis. *Cell Rep* 14:169–179.
- Shih HP, Wang A, and Sander M (2013) Pancreas organogenesis: from lineage determination to morphogenesis. *Annu Rev Cell Dev Biol* 29:81–105.
- Shima Y, Kawaguchi S, Kosaka K, Nakayama M, Hoshino M, Nabeshima Y, Hirano T, and Uemura T (2007) Opposing roles in neurite growth control by two seven-pass transmembrane cadherins. *Nat Neurosci* 10:963–969.
- Silva J-P, Lelianaova V, Hopkins C, Volynski KE, and Ushkaryov Y (2009) Functional cross-interaction of the fragments produced by the cleavage of distinct adhesion G-protein-coupled receptors. *J Biol Chem* 284:6495–6506.
- Silva J-P, Lelianaova VG, Ermolyuk YS, Vysokov N, Hitchen PG, Berninghausen O, Rahman MA, Zangrandi A, Fidalgo S, Tonevitsky AG, Dell A, Volynski KE, and Ushkaryov YA (2011) Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. *Proc Natl Acad Sci USA* 108:12113–12118.
- Simundza J, and Cowin P (2013) Adhesion G-protein-coupled receptors: elusive hybrids come of age. *Cell Commun Adhes* 20:213–226.
- Sinacore DR, and Gulve EA (1993) The role of skeletal muscle in glucose transport, glucose homeostasis, and insulin resistance: implications for physical therapy. *Phys Ther* 73:878–891.
- Smith JS, and Rajagopal S (2016) The β -Arrestins: Multifunctional Regulators of G Protein-coupled Receptors. *J Biol Chem* 291:8969–8977.
- Sriram K, and Insel PA (2018) G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs? *Mol Pharmacol* 93:251–258.
- Stacey M, Chang G-W, Davies JQ, Kwakkenbos MJ, Sanderson RD, Hamann J, Gordon S, and Lin H-H (2003) The epidermal growth factor-like domains of the human EMR2 receptor mediate cell attachment through chondroitin sulfate glycosaminoglycans. *Blood* 102:2916–2924.
- Stephenson JR, Paavola KJ, Schaefer SA, Kaur B, Van Meir EG, and Hall RA (2013) Brain-specific angiogenesis inhibitor-1 signaling, regulation, and enrichment in the postsynaptic density. *J Biol Chem* 288:22248–22256.
- Tuteja N (2009) Signaling through G protein coupled receptors. *Plant Signal Behav* 4:942–947.
- Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigartyo

- CA-K, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist P-H, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, and Pontén F (2015) Proteomics. Tissue-based map of the human proteome. *Science* 347:1260419.
- Vallon M, and Essler M (2006) Proteolytically processed soluble tumor endothelial marker (TEM) 5 mediates endothelial cell survival during angiogenesis by linking integrin $\alpha(v)\beta_3$ to glycosaminoglycans. *J Biol Chem* 281:34179–34188.
- Van Deijnen JH, Van Suylichem PT, Wolters GH, and Van Schilfgaarde R (1994) Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man. *Cell Tissue Res* 277:115–121.
- Villasenor A, Chong DC, and Cleaver O (2008) Biphasic Ngn3 expression in the developing pancreas. *Dev Dyn* 237:3270–3279.
- Virtanen I, Banerjee M, Palgi J, Korsgren O, Lukinius A, Thornell LE, Kikkawa Y, Sekiguchi K, Hukkanen M, Kontinen YT, and Otonkoski T (2008) Blood vessels of human islets of Langerhans are surrounded by a double basement membrane. *Diabetologia* 51:1181–1191.
- Volynski KE, Silva J-P, Leliana VG, Atiqur Rahman M, Hopkins C, and Ushkaryov YA (2004) Latrophilin fragments behave as independent proteins that associate and signal on binding of LTX(N4C). *EMBO J* 23:4423–4433.
- Wang X-J, Zhang D-L, Xu Z-G, Ma M-L, Wang W-B, Li L-L, Han X-L, Huo Y, Yu X, and Sun J-P (2014) Understanding cadherin EGF LAG seven-pass G-type receptors. *J Neurochem* 131:699–711.
- Ward Y, Lake R, Yin JJ, Heger CD, Raffeld M, Goldsmith PK, Merino M, and Kelly K (2011) LPA receptor heterodimerizes with CD97 to amplify LPA-initiated RHO-dependent signaling and invasion in prostate cancer cells. *Cancer Res* 71:7301–7311.
- Weston MD, Luijendijk MWJ, Humphrey KD, Möller C, and Kimberling WJ (2004) Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* 74:357–366.
- White JP (2016) Control of skeletal muscle cell growth and size through adhesion gpcrs. *Handb Exp Pharmacol* 234:299–308.
- White JP, Wrann CD, Rao RR, Nair SK, Jedrychowski MP, You J-S, Martínez-Redondo V, Gygi SP, Ruas JL, Hornberger TA, Wu Z, Glass DJ, Piao X, and Spiegelman BM (2014) G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy. *Proc Natl Acad Sci USA* 111:15756–15761.
- WHO (2016) Obesity and overweight. Fact sheets. www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
- Wilde C, Fischer L, Lede V, Kirchberger J, Rothmund S, Schöneberg T, and Liebscher I (2016) The constitutive activity of the adhesion GPCR GPR114/ADGRG5 is mediated by its tethered agonist. *FASEB J* 30:666–673.
- Winer S, Tsui H, Lau A, Song A, Li X, Cheung RK, Sampson A, Afifiyan F, Elford A, Jackowski G, Becker DJ, Santamaria P, Ohashi P, and Dosch H-M (2003) Autoimmune islet destruction in spontaneous type 1 diabetes is not beta-cell exclusive. *Nat Med* 9:198–205.
- Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang A-H, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S,

- Schrauwen P, and Spiegelman BM (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* 150:366–376.
- Wu MP, Doyle JR, Barry B, Beauvais A, Rozkalne A, Piao X, Lawlor MW, Kopin AS, Walsh CA, and Gussoni E (2013) G-protein coupled receptor 56 promotes myoblast fusion through serum response factor- and nuclear factor of activated T-cell-mediated signalling but is not essential for muscle development in vivo. *FEBS J* 280:6097–6113.
- Xu L, Begum S, Hearn JD, and Hynes RO (2006) GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis. *Proc Natl Acad Sci USA* 103:9023–9028.
- Zyryanova T, Schneider R, Adams V, Sittig D, Kerner C, Gebhardt C, Ruffert H, Glasmacher S, Hepp P, Punkt K, Neuhaus J, Hamann J, and Aust G (2014) Skeletal muscle expression of the adhesion-GPCR CD97: CD97 deletion induces an abnormal structure of the sarcoplasmatic reticulum but does not impair skeletal muscle function. *PLoS One* 9:e100513.

Fig 1: Adhesion GPCR architecture.

Adhesion GPCRs have unusually long extracellular N-terminal fragments (NTF) that contain several adhesive motifs, which are important in cell adhesion and cell-matrix interactions. The NTF is joined non-covalently to the C-terminal fragment at the GPCR proteolytic site (GPS) within the GPCR autoproteolysis-inducing (GAIN) domain. The stachel sequence is buried in the GAIN domain and is exposed upon ligand binding.

Fig 2: Activation of aGPCRs.

Dissociation of the N-terminal fragment from the C-terminal transmembrane domain is the main activation mechanism for most aGPCRs. Binding of activating ligands, which include the extracellular matrix proteins collagen III and laminin-211, leads to detachment of the N-terminal fragment of cleavable aGPCRs from the C-terminal fragment at the GPS. This exposes a stachel sequence that induces tethered agonism and subsequent downstream signalling. The stachel sequence may also be exposed by mechanical force or cellular vibrations. Receptor activation is coupled to intracellular effects through coupling to members of the heterotrimeric GTP-binding protein family (G_{α_s} , G_{α_i} , G_{α_q} and $G_{\alpha_{12/13}}$).

Fig 3: Expression pattern of aGPCRs in metabolically active tissues.

The relative expression of aGPCR transcripts in human brain, liver, gastrointestinal tract, skeletal muscle, pancreas and adipose tissue are indicated as RPKM (reads per kilobase of transcript per million mapped reads). + = ≤ 1 ; ++ = 1-5; +++ = 5-10; ++++ = 10-15; >++++ = >15 RPKM. Data were obtained from published RNAseq analysis (Fagerberg *et al.*, 2013).

Fig 4: Schematic summary of the signalling pathways downstream of aGPCRs that have been implicated in metabolic cell development and function.

a) CELSR3 promotes differentiation of pancreas endocrine progenitors to β -cells in mice via JNK activation and phosphorylation of c-jun. CELSR3 knockout mice have severe β -cell differentiation deficiency, which leads to glucose intolerance in adulthood.

b) Activation of a $G\alpha_s$ -coupled aGPCR such as LPHN1 in β -cells increases cAMP generation, which promotes insulin exocytosis through activation of protein kinase A (PKA) and/or exchange proteins activated by cAMP (EPACs). Similarly, GPR56 activation by collagen III in β -cells increases insulin secretion through $G\alpha_q$ -mediated signalling. Conversely, activation of a $G\alpha_i$ -coupled aGPCR such as BAI3 by its agonist C1q3 inhibits insulin exocytosis by decreasing cAMP production.

c) Deletion of GPR116 in pre-adipocytes in vitro impaired their capacity to differentiate into mature adipocytes, and GPR116 knockout mice are glucose intolerant and insulin resistant.

d) BAI3 recruits the ELMO/DOCK1 complex in myoblasts, allowing their fusion to generate multinucleated myofibres during myogenesis. This function is differentially regulated by stabilin-2 and C1ql4 regulation of BAI3: whereas stabilin-2 activates BAI3, leading to coupling to $G\alpha_i$ to promote myoblast fusion, C1ql4 inhibits it.

Table 1

Adhesion GPCR		Expression in human		Ligands or N-terminal interactors	Ligand action	Metabolic phenotypes in KO mice
Sub-group	Current nomenclature (previous nomenclature)	Pancreas	Islets			
I	ADGRL1 (LPHN1)	Yes	Yes (+++)	α -latrotoxin		
	ADGRL2 (LPHN2)	Not detected	Yes (+)	α -latrotoxin		
	ADGRL3 (LPHN3)	Yes	Not detected	Fibronectin leucine rich transmembrane protein 3		
	ADGRL4 (ELTD1)	Not detected	Yes (++)			
II	ADGRE1 (EMR1)	Not detected	Not detected			
	ADGRE2 (EMR2)	Not detected	Yes (+)	Chondroitin sulphate ^a		
	ADGRE3 (EMR3)	Not detected	Not detected			
	ADGRE4 (EMR4)	Not detected	Not detected			
	ADGRE5 (CD97)	Not detected	Yes (+)	CD55, $\alpha_5\beta_1$ integrin		
III	ADGRA1 (GPR123)	Not detected	Yes (+)			
	ADGRA2 (GPR124)	Not detected	Yes (+)	Glycosamino-glycans ^a		
	ADGRA3 (GPR125)	Yes	Yes (+)			
IV	ADGRC1 (CELSR1)	Not detected	Yes (+)			
	ADGRC2 (CELSR2)	Not detected	Yes (+)	N-terminal homophilic interaction		Normal endocrine cell differentiation (Cortijo <i>et al.</i> , 2012)
	ADGRC3 (CELSR3)	Not detected	Yes (+)	N-terminal homophilic interaction		Impaired β -cell differentiation, glucose intolerance (Cortijo <i>et al.</i> , 2012)

V	ADGRD1 (GPR133)	Yes	Yes (+)			
	ADGRD2 (GPR144)	Not detected	Not detected			
VI	ADGRF1 (GPR110)	Yes	Not detected	Synaptamide (Lee <i>et al.</i> , 2016)		
	ADGRF2 (GPR111)	Not detected	Not detected			
	ADGRF3 (GPR113)	Not detected	Yes (+)			
	ADGRF4 (GPR115)	Not detected	Yes (+)			
	ADGRF5 (GPR116)	Not detected	Yes (+)			Hyperlipidaemia, impaired glucose tolerance and insulin resistance, decreased adipocyte size (Nie <i>et al.</i> , 2012)
VII	ADGRB1 (BAI1)	Not detected	Not detected	Phosphatidyl-serine, lipopolysaccharide, $\alpha_v\beta_5$ integrin, Nogo receptor-like 2 and 3 (Chong <i>et al.</i> , 2018)		
	ADGRB2 (BAI2)	Not detected	Yes (+)			
	ADGRB3 (BAI3)	Not detected	Yes (+)	C1qI1 &3, stabilin-2 (Hamoud <i>et al.</i> , 2018)	↓ insulin secretion (Gupta <i>et al.</i> , 2018)	
VIII	ADGRG1 (GPR56)	Yes	Yes (+++)	Collagen III ^a , TG2 ^a , heparin, progastrin	↑ insulin secretion, ↓ apoptosis (Dunér <i>et al.</i> , 2016; Olaniru <i>et al.</i> , 2018a)	Normal glucose homeostasis (Olaniru <i>et al.</i> , 2018a)
	ADGRG2 (GPR64)	Yes	Not detected			
	ADGRG3 (GPR97)	Not detected	Yes (+)			
	ADGRG4 (GPR112)	Not detected	Yes (+)			

	ADGRG5 (GPR114)	Not detected	Yes (+)			
	ADGRG6 (GPR126)	Not detected	Yes (+)	Collagen IV ^a , laminin ^a , prion protein		
	ADGRG7 (GPR128)	Not detected	Not detected			
IX	ADGRV1 (VLGR1)	Yes	Not detected			

Table 1: Adhesion GPCR expression in human pancreas and islets.

Expression of aGPCR proteins in human pancreas and islets is taken from immunohistochemistry data from the Human Protein Atlas Project, in which all antibodies were validated for sensitivity and reliability (www.proteinatlas.org/about/antibody+validation) (Uhlen *et al.*, 2015). aGPCR mRNA expression in human islets is taken from qPCR and RNA-Seq data (Amisten *et al.*, 2013; Uhlen *et al.*, 2015).

Key: Yes (+++): strongly expressed; Yes (++) : moderately expressed; Yes (+): slightly expressed; Not detected: not detected or no available data, but not necessarily a proof of absence; blank cells: unknown or not yet investigated; ^a: endogenous ligands/binding partners of aGPCRs which are components of the ECM (Stacey *et al.*, 2003; Vallon and Essler, 2006; Xu *et al.*, 2006; Luo *et al.*, 2011; Paavola *et al.*, 2014; Petersen *et al.*, 2015).

Figure 1

Figure 2

Figure 3