Opportunities and challenges for drug discovery in modulating Adhesion G protein-coupled receptor (GPCR) functions

Andrey D. Bondarev, Misty M. Attwood, Jörgen Jonsson, Vladimir N. Chubarev, Vadim V. Tarasov & Helgi B. Schiöth


To link to this article: https://doi.org/10.1080/17460441.2020.1791075

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 10 Jul 2020.

View related articles

Citing articles: 1 View citing articles

Full Terms & Conditions of access and use can be found at https://www.tandfonline.com/action/journalInformation?journalCode=iedc20
Opportunities and challenges for drug discovery in modulating Adhesion G protein-coupled receptor (GPCR) functions

Andrey D. Bondarev, Misty M. Attwood, Jörgen Jonsson, Vladimir N. Chubarev, Vadim V. Tarasov and Helgi B. Schiöth

Department of Pharmacology, Institute of Pharmacy, I. M. Sechenov First Moscow State Medical University, Moscow, Russia; Department Of Neuroscience, Functional Pharmacology, Uppsala University, Uppsala, Sweden; Institute of Translational Medicine and Biotechnology, I. M. Sechenov First Moscow State Medical University, Moscow, Russia

ABSTRACT
Introduction: The G protein-coupled receptors (GPCR) superfamily is among the most widely exploited targets for therapeutics, with drugs mainly targeting the Rhodopsin, Glutamate and Secretin family receptors. The receptors of the Adhesion family, however, remain comparatively unexplored in this aspect. This review aims to discuss the druggability of Adhesion GPCRs (aGPCR), highlighting the relevant opportunities and challenges.

Areas Covered: In this review, the authors provide a disease-oriented summary of aGPCR involvement in humans and discuss the current status of characterizing therapeutic agents with a focus on new opportunities using low molecular weight substances.

Expert opinion: The small molecule antagonist dihydromunduletone and partial agonist 3α-acetoxydihydrodeoxyeudinin, along with the endogenous natural ligand synaptamidine currently comprise some of the most important discoveries made in an attempt to characterize aGPCR druggability. The small molecule modulators provide important insights regarding the structure-activity relationship and suggest that targeting the tethered peptide agonist results in a nonselective pharmacological action, while synaptamidine may be considered a potentially attractive tool to achieve a higher degree of selectivity.

1. Introduction

The G protein-coupled receptors (GPCRs) are well recognized as one of the most commonly employed targets for therapeutics. According to Hauser and colleagues [1], nearly 34% of all drugs approved by the Food and Drugs Administration (FDA) as of July 2017 target various GPCRs, accounting for almost 27% of the global market share. The GPCR superfamily includes ~800 receptor members of which about half are olfactory receptors [2,3]. The superfamily has been sub-divided into classes and families based on evolutionary homology and split into five families according to the GRAFS classification: Glutamate (class C), Rhodopsin (class A), Adhesion (class B2), Frizzled (class F) and Secretin (class B1) [2]. Most of the 398 non-olfactory GPCRs are considered druggable and there are at least 475 approved drugs that target more than 100 GPCRs and at least 320 new agents are in clinical trials that target almost 70 novel GPCRs [1,3].

The Rhodopsin family, the largest and most diverse GPCR family, is currently characterized by the greatest number of both established and investigational targets with more than 150 GPCRs being targeted by therapeutic agents [1]. The other GPCR families are also characterized by significant therapeutic potential with at least 14 of the 15 members of the Secretin family and 11 of the 22 Glutamate family members being targeted by 34 and 21 different drugs, respectively [1]. A growing number of targets are associated with the relatively small Frizzled family (11 members) with four GPCRs being targeted by 10 different agents [1]. The Adhesion family, despite having relatively long been recognized as suitable targets for therapeutics [3], remains largely unexplored in this context with no FDA approved drugs as of late 2019. Nevertheless, recent years have seen growing efforts to further characterize the druggability of Adhesion GPCRs (aGPCRs), including small molecule modulators screening studies, revitalizing the overall interest. This creates a need for studies that could provide further insights on the future of this unique receptor family given the current evidence and what could potentially be done to maximize the efficiency of discovery efforts in future.

In the present review, we aim to provide insights on the opportunities that the aGPCRs can provide for pharmacological development, as well as the important challenges that may hamper it. To achieve this, we will provide a disease-oriented summary of the currently available evidence on aGPCR involvement in humans and will use examples of recently discovered modulatory molecules to illustrate the current status of targeting aGPCRs in therapeutics. To assess if any aGPCRs had...
reached clinical trials as targets yet, we mined the recently updated GPCRDB database [4], specifically its subset dedicated to trends in clinical trials [1]. To provide evidence on the aGPCR involvement in human disease and advancements made in elucidating modulatory agents, we collated the relevant data from recent (2014 onwards) reviews [5–7].

2. Overview of the Adhesion GPCR family

The aGPCR family is an evolutionarily ancient receptor family within the GPCR superfamily, encompassing 33 members in humans, divided into nine subfamilies [2,8]. The different main branches of the GPCR family are suggested to have originated from a common ancestor [9] supported with detailed analysis of the 7-transmembrane (7TM) domains. There is also considerable evidence that the well-known Secretin family, which includes drug targets mediating the effects of several hormones, has descended from the ancient aGPCR receptor branch [10]. Interestingly, the aGPCR family is also identified in the fungi, where it notably lacks the N-terminal domains [11].

The aGPCR family receptors show broad expression patterns [8] and, as their name suggests, play numerous roles in cell-cell and cell-matrix adhesion. It is a unique receptor family that is characterized by the structurally diverse N- and C-terminal domains [8], the ability of individual receptors to recognize and interact with multiple ligands, and distinctive receptor activation mechanisms due to the mixed nature of aGPCR N-termini-regulated signaling. The aGPCR nomenclature is known to be diverse as well, in this review we adhere to the relatively recently proposed nomenclature [5] using the ADGR system (ADhesion G protein Receptor) for naming the receptors.

The aGPCR family consists of the following members: ADGR1–4 (Latrophillin subfamily/subfamily I), ADGRE1–5 (EGF–7TM subfamily/subfamily E), ADGRA1–3 (subfamily A), ADGRC1–3 (CELSR subfamily/subfamily C), ADGRD1 and 2 (subfamily D), ADGRF1–5 (subfamily F), ADGRB1–3 (BAI subfamily/subfamily B), ADGRG1–7 (subfamily G) and ADGRV1 (subfamily V). The receptors’ structures are schematically depicted in Figure 1. Apart from the long and structurally diverse N-terminal domains, the other unique features of aGPCRs are the presence of the GPCR autoproteolysis–inducing (GAIN) domain [12] and the GPCR proteolysis site (GPS) motif [13]. The GPS is found within the GAIN domain and both are responsible for signaling activation through a tethered peptide agonist (also referred to as the Stachel sequence) [14]. The aGPCRs architecture layout varies depending on the receptors’ ability to undergo GPS-mediated autoproteolysis (Figure 2) [15,16]. All aGPCRs are characterized by the three-partite topology-based structure compartmentation, which includes extracellular (ECD) and intracellular domains (ICD) separated by 7TM domains (Figure 2(a)). Receptors with cleavable GAIN domains are capable of undergoing autoproteolysis and can additionally produce the two-partite cleavage-based structure layout, containing N-terminal (NTF) and C-terminal (CTF) fragments (Figure 2(b)).

The receptors of aGPCR family are primarily known to interact with cellular and matricellular ligands [15] via four mechanisms [7] that can be grouped into two categories based on the spatial configuration of signaling activation. The first category includes mechanisms I and II, with both involving receptor-ligand interactions in cis-configuration, which result in signaling induction within the aGPCR-expressing cell. The second category includes mechanisms III and IV, which includes ligand-receptor interactions in cis- and trans-configurations, which can induce signaling in cells expressing the aGPCR (cis) or in cells either in contact with or distant from the cell expressing the receptor (trans). Mechanism I involves the interaction of an aGPCR with a soluble ligand which then induces Stachel sequence-mediated activation in a manner comparable to the other GPCRs families [17]. Examples of ligands involved are WNT-7 [18], chondroitin sulfate B [19], C1qL4 [20], L-phenylalanine [21], collagen III [22] and the cellular prion protein C (PrP C) [23]. Mechanism II is an example of matricellular interactions that occurs between aGPCRs and proteins in the extracellular matrix such as collagen III [22], collagen IV [24], and laminin-211 [25] which result in activation through mechanical perturbations. Mechanism III involves the activation and subsequent release of an aGPCR’s NTF region [26] which acts as a ligand that can modulate distant cell functions [27]. This mechanism is currently only known to be represented by vasculostatins, such as Vstat120 [28]. Mechanism IV involves cell–cell interactions between aGPCRs and other membrane proteins, such as neurexins [29], CD55 [30], CD90 [31], Lasso/tenuerin-2 [32], CD81 [33], stabulin-2 [20], and LPAR1 [34]. Table 1 summarizes the currently available evidence regarding cleavability, NTF interactions and signaling partners of aGPCRs.

The aGPCRs have been shown to play roles in many different biological processes and here we provide an overview of some of these features. On the cellular level, the C subfamily receptors, being vertebrate homologs to the Drosophila melanogaster Flamingo receptor, are involved in planar cell polarity [36]. Several aGPCRs are reported to be involved in the cytoskeletal organization regulation: the C subfamily receptors are involved in mediating the node cilia localization and rotational axis determination [37,38], while the B subfamily was found to be involved in the dendritic spine formation [39]. Several aGPCRs are involved
in cellular adhesion interactions; in vitro studies have shown such involvement for ADGRE5 [40], ADGRE2 [41], and ADGRG1 [42,43]. Additionally, some receptors are reported to be involved in cellular migration, such as ADGRG3 [44], ADGRA2 [45], ADGRE5 [46], and the C subfamily receptors [47]. A number of aGPCRs have been shown to play roles in cell cycle regulation: ADGRL2 was reported to be involved in the epithelial–mesenchymal transition within the atrioventricular canal [48], ADGRG6 is noted to be important for the myelinating Schwann cells differentiation [49], while the B subfamily receptors, particularly ADGRB1, affect cell proliferation and survival [50], and cell-cell fusion [51,52].
On the whole organism level, the aGPCR family is currently known to be associated with the immune, cardiovascular, respiratory, nervous, musculoskeletal, reproductive, renal, intestinal, sensory, endocrine and gastrointestinal systems; the first four are currently characterized by the largest known extent of aGPCR involvement. In the immune system, the subfamily E receptors ADGRE1, 2, 3, and 5, as well as ADGRB1 and ADGRG1 have been shown to be expressed in myeloid and lymphoid cells and have been implicated in innate and adaptive immune processes [53]. In the cardiovascular system, the L subfamily and ADGRG6 were found to be involved in cardiac development; ADGRAL1 was reported to be expressed in the rat heart during development [54], while ADGRGL2 is involved in the valve formation, as reported in [48]. ADGRG6, based on the results of animal studies, is presumed to affect the cardiac integrity and function [55,56]. Additionally, ADGRG5 and ADGRAL4 were shown to be involved in tumor angiogenesis [57,58]. In the respiratory system, ADGRA2, ADGRAL1, ADGRG6, and ADGRF5 were found to play roles in lung development and functioning, as well as alveolar surfactant homeostasis [59]. The aGPCR involvement in the nervous system includes the subfamilies L (ADGRAL1, 2 and 3), C (ADGRGC1 and 3) and G (ADGRG1 and 6) which were found to be implicated in a wide range of processes, such as neural tube closure, neuronal exocytosis, synapse formation and functioning, regulation, as well as communication within neurons [60].

Other systems are less characterized but are believed to play notable roles due to their expression profiles and/or functional implications. The subfamilies B (ADGRB1 and 2) and G (ADGRG1 and 6), ADGRV1, as well as ADGRG5 are associated with the musculoskeletal system; ADGRB1 and 2, along with ADGRG1 and ADGRG5 are expressed in myoblasts and were found to play role in muscle growth [61], while ADGRV1 and ADGRG6 are found to be implicated in bone metabolism and growth [62]. The G subfamily receptors ADGRG1 and 2 are also expressed in the male reproductive system, particularly the testis, and linked to fertility [63,64]. ADGRC1, ADGRG6, some subfamily F receptors (ADGRF1, 3 and 5) and ADGRG5 are associated with the renal system through either expression profiles or developmental and/or functional involvement [65]. In the integumentary system,
ADGRG1 and the subfamily F receptors (ADGRF2, 4 and 5) were identified to be present in the murine epidermis [8,66], with ADGRG1 being a crucial component in hair follicle polariza-
tion [67]. Additionally, ADGRG1, along with ADGRG6 and ADGRV1 were identified to be expressed in the vestibular, auditory, and visual systems, and have been implicated in their functionality and development [68]. The subfamily C (ADGRG2 and 3), together with ADGRG1 and ADGRF5 are implicated in the proper functioning of the endocrine system, particularly pancreas [69]; ADGRG2 is also linked to the lipids metabolism [70]. ADGR1, ADGR14, and ADGRG1 are reported to be strongly expressed in the pancreatic islet cells [69]. In the gastrointestinal (GI) system, ADGR5 is reported to be noticeable in the human intestinal epithelium [71]. Several other receptors are reported to exhibit noticeable presence in the GI tissue as well, including the G subfamily ADGRG7, ADGRG1, ADGRG2, and ADGRG6 [69].

Important to mention, other vertebrate species, such as rodents (mice, rats) or zebrafish, which are commonly employed in drug discovery studies, are characterized by several important differences in the aGPCR repertoire. Phylogenetic studies point out an overall high degree of homology, with rodents showing homology with 31 human aGPCRs (except ADGRE2 and ADGRE3) [68], while zebrafish has homologs to 24 human receptors, except for ADGRF1, ADGRF2, ADGRF5, ADGRG5, ADGRE1, ADGRE2, ADGRE3, and ADGRE4 [72]. Furthermore, while the overall expression and functional profiles of aGPCRs in these species are reported as comparable to humans, the exact localization and physiological functions of individual homologs often vary, as covered in details in the respective studies [68,72]. Additionally, the aGPCRs are characterized by interspecies splice variant differences, as covered in [7] on the example of ADGRG1.

Such a wide range of biological functions expectedly makes the aGPCR family a very tempting target for therapies; a notion increasingly supported by studies showing aGPCR involvement in human diseases [59,60,62,65,69,73]. The structural features also support aGPCR druggability, with several strategies to modulate the receptors’ activity reported in the literature [6]. In the next section, we will discuss some of the more notable human diseases with documented aGPCR involvement, putting emphasis on the therapeutic value of such an involvement.

3. Adhesion GPCRs as therapeutic targets – clinical implications

The clinical significance of the aGPCR family is recognized predominantly due to their strong involvement in organ development and functional maintenance, as well as cellular processes associated with malignancy. Current evidence shows that aGPCRs are associated with a fairly wide range of neurologic and neoplastic disorders. Interesting recent implications have also been identified for musculoskeletal, metabolic, and cardiovascular disorders. Table 2 presents a summary of the current knowledge on aGPCR’s suggested involvement in human disease.

3.1. Adhesion GPCRs in neurology

Several neurodevelopmental disorders, such as bilateral frontotoparietal polymicrogyria (BFPP), neural tube defects (NTDs), attention deficit hyperactivity disorder (ADHD), and Tourette syndrome (TS) have been associated with aGPCRs [60]. Additionally, evidence exists on the aGPCR involvement in some paretic and epileptic disorders. BFPP is an autosomal recessive disease associated with a variety of serious neurologic symptoms including mental and motor retardation, seizures, ataxia, and esotropia, and has been linked with mutations in ADGRG1 [102,103]. This receptor is significantly involved in cerebral cortex development, modulating the processes through ADGRG1 – collagen III interactions which results in RhoA activation from coupling to Gα12/13 [104]. In total, 22 disease-relevant mutations within the ADGRG1 gene in humans had been characterized, acting via various mechanisms, most notably abolishing the collagen III-binding ability of ADGRG1 through the missense changes to the ligand-binding domain [104,105]. Two missense mutations in the GPS motif are reported to abolish the autoproteolytic cleavage in the receptor, preventing its surface expression, while a missense change to the TM domain is reported to be associated with the cobblestone-like cortex histological features [104,106].

The NTDs linked to aGPCR include spina bifida, anencephaly and craniorachischisis. NTDs are a group of severe birth defects with complex genetic, nutritional, and environmental etiology, affecting the central nervous system and resulting in neural tube closure failure which exposes the neuroepithelium to stress that leads to degeneration and neural deficit [107]. Spina bifida, characterized by the caudal neural tube fusion failure, is the most common type of such defects, while craniorachischisis and anencephaly, both types of cranial NTDs, are associated with the failure to initiate neural tube closure in the hindbrain and are the most severe [107]. The group was found to be associated with mutations in subfamily C aGPCRs, particularly ADGRG1, which is significantly expressed in the central nervous system during early development stages [60]. In craniorachischisis, among the identified disease-associated mutations include various missense substitutions in the ADGRG1 gene that contribute to disrupted ADGRG1 plasma membrane localization [108]. Additionally, a unique novel missense gain-of-function mutation (p.P870 L) was identified in Chinese patients and found to result in the increased PCP and Wnt pathways activation [109]. In spina bifida, particularly its severe form myelomeningocele, two TG dinucleotide repeat mutations on the ADGRG1 gene coding region sequence were identified, resulting in altered membrane localization and interrupted ADGRG1 – VANGL-2 recruitment and interaction, which are critical for the planar cell polarization [110]. Additionally, 11 missense single nucleotide mutations were predicted through in silico modeling to affect the receptor’s cell-cell binding, structure and membrane localization, and the G protein-coupled receptor kinase and protein kinase B phosphorylation site [110]. An earlier study has additionally identified a related set of 15 mutations found to contribute to myelomeningocele and several other more commonly occurring forms of the disease, such as lipomyelo-
cele and lipomyelomeningocele [111]. ADGRG2 and 3 were
identified to be associated with the NTDs as well, although less notably. Three mutations in the ADGRC2 were predicted to be damaging in silico and associated with spina bifida and anencephaly in Chinese patients, while absence of the ADGRC3 was identified to potentially cause craniarachischisis in the same population [109]; no functional implications have yet been investigated, however.

ADHD, a heritable, chronic disease, is associated with a range of neurobehavioral symptoms such as hyperactivity, inattention, and impulsivity [112] and had been shown to be associated with the L subfamily AGPCRs, specifically ADGRL3 [60]. This receptor exhibits the highest expression profile in the ADHD-linked amygdala, caudate nucleus, cerebellum, and cortex regions of the brain [113]. The receptor’s disease involvement was identified to result from a range of single nucleotide polymorphism mutations (SNPs) that generally confer increased susceptibility to the disease [114–117]. Notably, four SNPs were found to be associated with the increased risks of developing ADHD and better responsiveness to treatment [114,115]. Interestingly, one of these SNPs, rs6551665, was also found to be associated with autism spectrum disorders among white Brazilians of European descent [116]. Additionally, an SNP in patients of Chinese Han ethnicity was found to strongly affect the susceptibility to environmental risk factors, such as maternal stress [117]. No other ADGRL receptor has yet been strongly associated with ADHD; however, microdeletions in ADGRL1 were linked to an overactive behavioral phenotype associated with mental retardation and hyperactivity [118].

TS is another aGPCR-mediated neurobehavioral disorder, manifested with multiple motor and one or more vocal tics of multifactorial etiology and an early age of onset, persisting for more than a year and frequently associated with other neurobehavioral disorders, such as ADHD, obsessive-compulsive disorder (OCD) and several similar conditions [119]. Based on the results of two recent whole exome studies [120,121], ADGRC3 was identified as a high-confidence elevated risk gene, associated with OCD as a comorbidity. The mutations identified so far comprise three nonsynonymous single nucleotide variants and a single frameshift substitution
A smaller-scale study among Chinese patients has provided additional evidence, identifying damaging ADGRC3 variants linked to more severe tic symptoms [122].

Among the paroxysmal disorders, progressive spastic paraparesis is currently associated with the aGPCR family and linked to ADGRB2 in a patient with neurodegenerative disease [123]. A single disorder-relevant gain-of-function mutation had been identified so far, resulting in increased signaling via Gαo and Gaq coupling and cell surface expression [123]. Additionally, the mutation was found to disrupt the ADGRB2 interaction with brain-specific endophilin A1 [123].

In epilepsy, rare and ultra-rare ADGRV1 haploinsufficiency was found to be associated with myoclonic seizures, based on a study involving four patients with 5q14.3 deletions from a 95-patient cohort [124]. Specifically, three ultra-rare disease-causing mutations and a single polymorphism variant were identified to be the most damaging based on in silico testing and predicted to be associated with up to 6% of myoclonic seizure cases [124]. Functionally, ADGRV1 was found to be involved in the GABA-ergic interneuron development in the embryonic cortex [125] and the wide-spread GABA-ergic cortical cell dysfunction could be a potential epileptogenic mechanism [124].

From the therapeutic development point of view, ADGRL3 and ADGRG1 may be considered the most interesting potential aGPCR targets in neurologic diseases. The ADGRL3 involvement in ADHD treatment responsiveness may provide means to improve the treatment options in resistant patients. ADGRG1, being currently characterized best in terms of its biology [7], is a very attractive potential drug target candidate overall.

### 3.2. Adhesion GPCRs in neoplasms

Previous studies have associated the aGPCR family with neoplastic conditions, such as hematologic malignancies; pulmonary, gastrointestinal, hepatic, pancreatic, and renal tumors; endocrine and reproductive neoplastic conditions; and musculoskeletal and a number of central nervous system (CNS) tumors, as summarized in [73]. The aGPCR family is believed to be involved in cancer through multiple mechanisms, including tumor angiogenesis [126,127] and tumor cell migration/invasion [128,129] modulation. The aGPCR ECD interactions are also believed to be implicated in tumorigenic activity, possibly through their hallmark adhesive properties that affect the tumor cells viscoelasticity [130].

To date, the most robust overall evidence exists on ADGRE5 and ADGRG1, both being among the earliest aGPCRs to be identified in human cancer cells [131,132]. ADGRE5 had been previously described to be associated with CNS tumors, such as glioblastoma multiforme [133], thyroid [131], gallbladder [134], GI, pancreatic [128,135], and prostate [136] cancers, acute lymphoblastic leukemia [137,138], acute myeloid leukemia [139] and musculoskeletal tumors [140,141], often showing high expression profiles and association with poor outcome. Recent studies have additionally identified functional implications in ovarian tumors: ADGRE5 was found to contribute to the metastatic ovarian cancer cells resistance to paclitaxel via the NF-κB/miR-503-5p and JAK2/STAT3 pathways [142]. Additionally, ADGRE5 was recently identified as an MYC target gene that is specifically expressed in Burkitt lymphoma cells [143].

ADGRG1 was initially identified in metastatic melanoma [132]; other documented neoplastic involvement includes gliomas [144], non-small cell lung cancer (NSCLC) [145], ovarian, cervical, breast, pancreatic, renal tumors [146], esophageal carcinoma [147] and leukemias [148,149]. A notable therapeutic implication here is the receptor’s contribution to mesenchymal differentiation and radioresistance inhibition in glioblastoma through the NF-κB signaling pathway [150]. Another example of such aGPCR therapeutic implications is ADGRA2’s role in overcoming resistance to gefitinib in NSCLC cells through its downregulation or inhibition [151].

Among the other notable recent advances in characterizing the aGPCR involvement in cancer is ADGRF1’s role as a high-risk marker in NSCLC via two SNPs [152]. The earliest evidence suggesting the role of ADGRF1 in cancer was when the receptor was found to be overexpressed in lung and prostate adenocarcinomas [153]. Additionally, a recent in vitro study [154] has shown that ADGRF1 is overexpressed in the Aldefluor® and anti-HER2 therapy-resistant breast cancer cell populations. Moreover, knocking-down ADGRF1 might inhibit the HER2+ breast cancer cells regardless of anti-HER2 drug sensitivity without affecting the efficacy and/or potency of lapatinib [154].

To summarize, the most notable aGPCR therapeutic implication in neoplasms is the role of ADGRF1, ADGRE5, ADGRA2, and ADGRG1 in treatment responsiveness. Additionally, cancer-associated mutations are identified in every aGPCR subfamily as per the Catalog of Somatic Mutations in Cancer [155], making neoplastic indications a very robust direction to work with in efforts to identify aGPCR drug targets. Table 2 includes several other neoplasms, where aGPCRs are reported to be involved.

### 3.3. Adhesion GPCRs in musculoskeletal disorders

The musculoskeletal disorders associated with aGPCR mutations in human are arthrogryposis multiplex congenita (AMC), adolescent idiopathic scoliosis (AIS), and the aggressive form of periodontitis (AD), which are all associated with mutations in ADGRG6 [62]. AMC is a heterogeneous group of rare disorders, characterized by congenital multiple joint contractures arising from phenotypes that are affected by mutations within the motor neurons, the neuromuscular junction and/or the skeletal muscles [156]. The available evidence indicates that a range of homozygous mutations within ADGRG6 results in the particularly severe forms of the disorder; a missense substitution in the GAIN domain of the receptor which results in significantly impaired autoproteolysis is of note [157]. Functionally, ADGRG6 signaling was found to be implicated in Schwann cell development and functioning, which is critical for myelination within the peripheral nervous system [158].

AIS is the most common form of spinal development disorders, characterized by lateral spine curvature of multifactorial etiology, and becomes apparent during the pubertal growth spurt [159]. The disease is recognized to have a strong genetic basis with several genome-wide association studies identifying susceptibility loci in populations of various
Ancestral differences, notably SNP mutations in ADGR6 [160–164]. The receptor was found to be highly expressed in cartilage and there is evidence indicating its role in skeletal growth, supported by genome-wide scans for human adult stature [165]. A notable functional involvement is significant association of two SNPs with an increased susceptibility to AIS possibly through expression levels of ADGR6 in adipose tissues [160–162]. Another SNP, identified in Chinese patients, is possibly implicated in the ADGR6 gene expression in paravertebral muscles [160,162]. Additionally, a recent study has showed that a novel SNP found in the Chinese Han patients results in the inhibited inclusion of exon 6, possibly implicated in chondrogenic differentiation, based on an in vitro study using human mesenchymal stem cells [163].

AD is a severe form of periodontal tissue inflammation, characterized by early age of onset and the more apparent genetic nature of its etiology [166]. A single SNP mutation in ADGR6 was identified in Japanese patients with AD, which resulted in impaired signal transactivation as well as producing no effect on the mRNA expression of the calcification-related genes bone sialoprotein, osteopontin, and Runx2 [167]. This implicates that the mutation may negatively influence the homeostasis of periodontal ligament tissues by affecting the regulation of the cytodifferentiation of human periodontal ligament cells [167].

The involvement of ADGR6 in AMC, AIS, and AD may provide an interesting opportunity to expand therapeutic options in these diseases to include pharmacological approach; better characterization of the ADGR6 pathobiology is vital for further exploration of this direction. Additionally, given how most of the disease-relevant mutations here had been identified in patients of Chinese and Japanese ancestries, it may create opportunities for tailored interventions.

3.4. Adhesion GPCRs in miscellaneous disorders

For the purpose of this review, the term ‘miscellaneous disorder’ encompasses conditions affecting the sensory, dermal, endocrine, and cardiovascular systems. The respective conditions are type 2 C Usher syndrome (USH2 C), vibratory angioedema (VA), type 2 diabetes (T2DM), congenital heart disorders (CHDs), coronary artery disease (CAD), and myocardial infarction (MI). USH2 C is an autosomal recessive development disorder found to be associated with ADGRV1 and characterized by typically moderate congenital hearing loss and retinitis pigmentosa [168,169]. The ADGRV1 receptor is expressed in the eye and cochlear tissues and believed to be associated with ankle links, present in the vestibular and auditory bundles, and play important roles in their development as a component of connecting filaments [170]. A number of disease-relevant mutations in ADGRV1 are reported and mapped across the N-terminal domain and typically result in a truncated protein [171,172]. Among the most recent discoveries is a nonsense mutation that is predicted to create a premature stop codon near the receptor’s N-terminus which prevents 7TM translation [173]. There are currently no conclusive data on receptor–ligand interactions for ADGRV1; however, it has been found that extracellular Ca²⁺ may interact with ADGRV1 to activate protein kinases A and C via Gαs and Gαq, respectively [174]. Another study has shown that the ICD fragment of ADGRV1 has a particularly strong inhibitory effect on the adenylate cyclase pathway as the Y6244fxS1 mutation in the ICD improved the receptor’s Gα coupled [175].

VA is a rare autosomal dominant immediate-type cutaneous physical hypersensitivity disorder characterized by local erythematous and edematous lesions due to mast cell degranulation resulting from vibratory stimulus [176,177]. The disorder was found to be associated with ADGRE2, which is expressed in the myeloid cells, particularly on neutrophils, macrophages, and mast cells and possibly implicated in detecting and responding to the physical dermal stimuli through inducing myeloid migration [176]. The only mutation identified so far is a missense substitution which results in an autoproteolysis-deficient ADGRE2 variant through destabilizing interactions between the receptor’s extracellular and transmembrane subunits [178].

Several aGPCRs, namely ADGRGC3, ADGRF5, ADGRB3, and ADGRG1, appear to have a notable presence in the pancreatic islets with presumed roles in their development and insulin secretion [69]. ADGRF5- and ADGRGC3-knockout mice have both shown phenotypes which are characterized by glucose intolerance and impaired β-cell differentiation [179,180], with the ADGRF5-knockout mutant also presenting with insulin resistance [179]. ADGRB3–C1q/3 interactions mediate insulin secretion inhibition [181] and ADGRG1–collagen III interactions mediate stimulation of the β-cells secretory function and additionally regulate apoptosis, cell proliferation, and adhesion [182]. While an ADGRG1-knockout mice study showed no negative impact on glucose homeostasis [182], human T2DM subjects showed loss-of-function changes in ADGRG1 within the pancreatic islets, with particularly decreased expression of two reported ADGRG1 gene splice variants, both encoding the N-terminal end of the collagen III-binding region [183].

CHDs are the most common type of birth defects, presenting as structural and functional cardiac abnormalities before birth and classified into cyanotic heart disease, left-sided obstruction defects, and septation defects [184]. The underlying genetic basis is ever-expanding, with a recent study speculating on the possible role of the GPCR superfamily [185,186]. While currently an isolated case in patients of Chinese ethnicity, the C subfamily was found to be involved in CHDs through a number of potentially disease-specific mutations. In addition to the previously mentioned role in the NTDs, the gain-of-function p. P870 L in ADGRCl1 is also associated with perimembranous ventricular septal defect (SD) and patent foramen ovale (PFO) [109]. Several other CHDs were additionally found to be associated with the receptor: atrial and ventricular SDs, patent ductus arteriosus (PDA), double outlet right ventricle, pulmonary stenosis (PS), muscular portion ventricular SD and transitional endocardial defect [109]. Two mutations in ADGRCl2 were found to be associated with Ebstein’s anomaly, tricuspid incompetence, P5 and atrial SDs [109]. In ADGRCl3, three mutations were found to be
associated with ventricular and atrial SDs, PDA, PFO, tetralogy of Fallot [109].

In CAD, the leading cause of mortality worldwide with numerous genetic underlying mechanisms [187], an SNP mutation in ADGRE3 was found to be associated with increased susceptibility based on a longitudinal exome-wide association study among Japanese patients [188]. Interestingly, the CC minor genotype of this SNP was found to confer protection against CAD in the studied population [188]. The disease-causing mechanism of the SNP is assumed to alter the ADGRE3 functionality in vascular inflammation [188]. A related study among the Japanese patients with early-onset cardiovascular and renal diseases [189] has additionally identified potential association of ADGRL3 in MI and chronic kidney disease.

In this category, T2DM is of major interest, due to the apparent involvement of multiple aGPCRs and the presence of ADGRG1 loss-of-function mutations in human patients. The involvement of ADGRE3, ADGRL3, and the C subfamily in cardiovascular disorders may open up interesting therapeutic implications as well, including personalized interventions.

As target identification and validation is usually the first and one of the most critical steps in a drug discovery process [7], the growing evidence on therapeutically notable involvement of aGPCRs in humans is encouraging for drug development, as it provides us with an increasing range of potential molecular targets, associated with diverse pathologies. It also provides important insights on the receptors’ pathobiology, giving us an idea on how diseases may affect the receptors’ functionality which is important for targeted assays [7].

As the therapeutic potential of aGPCRs grows more and more evident, it becomes important to investigate the opportunities for the next step of drug discovery, which typically includes a lead compound identification. So far, the efforts have yielded several interesting compounds with the aGPCR-modulating properties. The final chapter of this review will discuss the current evidence on these agents and will attempt to speculate on what they can bring to advance the field of aGPCR drug discovery.

4. Pharmacologic modulation and challenges in drug discovery

The ongoing attempts to de-orphanize the aGPCR family have resulted in the identification of several compounds with varying pharmacological activity toward aGPCRs (Figure 3). Notably, small molecule agonists have been identified for the receptors ADGRG1, ADGRG3, and ADGRG5. The earliest such molecules to be reported, beclomethasone, ezetimibe, zeronal, and flunarizine, were identified as ADGRG3 activators. With beclomethasone, the modulatory activity was elucidated through the (35)S]GTPγS incorporation assay, showing the activation induction through G0-protein coupling [190]. In the other three compounds, the β-arrestin recruitment assay has showed G protein-independent β-arrestin recruitment [191]. Receptors ADGRG1 and ADGRG5 were found to be partially activated by gedunin and khivurin derivatives, most notably the C1, C3 and C15-substituted gedunin derivative 3α-acetoxydihydrodihydrogedunin, which was elucidated through high-throughput SRE-luciferase screening to target the 7TM

Figure 3. Adhesion G protein-coupled receptors as therapeutic targets. The figure illustrates the currently recognized druggable sites as per [6] and schematically shows the molecular mechanisms of action for modulators included in the review. The colors signify sites that may be employed to develop aGPCR therapeutics: the extracellular domains of the N-terminal region are an attractive target for engineered antibodies (brown); the GAIN domain (green) may be potentially modifiable by protease modulators; the tethered peptide interaction site (red) is potentially targetable by peptide molecules; the 7TM domain, which is represented with seven blue transmembrane regions, is commonly targeted by small-molecule modulators (orange); and the PDZ binding motif in the C-terminal region is potentially targetable by small molecules to disrupt the PDZ scaffold protein interactions in some aGPCRs [6]. Abbreviations: GAIN, GPCR autoproteolysis-inducing domain; 7TM, 7-transmembrane domain; PDZ, PSD95/Dlg1/Zo-1 domain.
domain as an orthosteric agonist that resulted in G₁₃ activation [192]. Interestingly, 3-α-acetoxydehydaroxyedumunin was also reported to inhibit the activity of ADGRF1 [192]. The structure-activity relationship has showed the presence of acetox moieties at positions C₃ and C₇ of the gedunin core to be vital for activity, resulting in the most potent compound [192].

Only a single small molecule antagonist has been described so far, a rotenone derivative called dihydromunduletone, which was elucidated through the high-throughput SRE-luciferase screening to target ADGRG1 and ADGRG5 [193]. The compound is characterized by the 2-phenylacetophenone core providing a high degree of flexibility and the ability to adopt multiple conformations [193]. The inhibition is achieved through targeting the 7TM domain with dihydromunduletone which presumably acts as a competitive antagonist to the tethered-peptide molecule through binding to the receptor’s orthosteric site, resulting in the decreased G₁₃ activation [193]. Chemically, in addition to the already mentioned 2-phenylacetophenone, the phenolic hydroxyl moiety was also reported to be of note, which contributes to the drug–receptor interaction [193].

Engineered antibodies have been characterized for ADGRG1, ADGRE5, and ADGRE2. The use of anti-ADGRE5 monoclonal and polyclonal antibodies was shown to result in the receptor’s neutralization, resulting in decreased granulocyte migration [194,195]. An anti-ADGRE2 antibody-mediated ligation was found to result in neutrophil adhesion and migration increase, superoxide production augmentation, and proteolytic enzyme degranulation [41]. Additionally, an anti-ADGRG1 polyclonal antibody was created that transcriptionally activates the receptor and inhibits the neural progenitor cells migration, except in knockdown mutants [196]. We have not been able to identify any recent development here; a possible explanation could be the overall difficulty in producing functional and conformationally active receptor antigens, an issue characteristic to the GPCR superfamily as a whole [197]. Nevertheless, antibodies are recognized as a very attractive class of therapeutics in the context of GPCR drug discovery as a whole, offering better receptor specificity and selectivity [198].

The proposed natural ligand [7] synaptamide may also provide an interesting insight on how the activation of an aGPCR member can be used therapeutically. Synaptamide is an endogenous docosahexaenoic acid metabolite that is dependent on the dietary intake of omega-3 fatty acid and shown to activate ADGRF1 in a cAMP-dependent manner and was reported to do so independently of the receptor’s autophoretolysis or tethered-agonist activity [199]. Initially identified in CNS development through neurogenesis, neuritogenesis, and synaptogenesis promotion, a recent study has established that the anti-inflammatory properties of synaptamide are mediated by ADGRF1 [200]. An interesting aspect of synaptamide is its structural similarity to anandamide, a metabolite of arachidonic acid that is capable of binding to cannabinoid receptors [199]. Another noteworthy detail is that synaptamide’s bioactivity is reported to be heavily dependent on the integrity of the receptor’s N-terminal domain [201].

Overall, among the particularly notable advances in recent years to be considered is the characterization of two small molecular weight modulators 3-α-acetoxydehydaroxyedumunin and dihydromunduletone. Both modulators affect ADGRG1 and ADGRG5; such a lack of selectivity is in line with a recent study [202] showing that the aGPCRs may be cross-activated via a tethered agonist. Both receptors are also characterized by a shared amino acid sequence of their peptide agonists [193], a property which appears to affect selectivity as well. In 3-α-acetoxydehydaroxyedumunin, the presence of inhibitory activity against ADGRF1 [192] is an important observation that could also provide insights on how to achieve better selectivity. Further elucidation of the molecules’ structure-activity relationship is therefore vital for a better understanding of how to reach a satisfactory level of receptor selectivity in a small molecule modulator. It is also notable that both molecules were elucidated using common screening assay techniques, which could facilitate future drug discovery efforts. The earliest experiences in explicating beclomethasone, ezetimibe, zeranol, and flunarazine should not be overlooked either, as they provide a set of opportunities for a wider range of screening techniques in the aGPCR drug discovery. The structures of small molecular weight modulators and synaptamide are presented on Figure 4.

5. Conclusion
There have been important advances in elucidating the signaling mechanisms of several aGPCRs providing optimism about pursuing drug discovery for these receptors with similar approaches as for many other GPCRs. There also are continuous efforts to establish the relationship between the aGPCR family and human disease providing important details that are useful in identifying possible disease targets. The aGPCR drug development itself, however, is still in its infancy; while there are therapeutically attractive compounds identified, none have progressed significantly beyond the initial screening as of late 2019. Nevertheless, there are several important research avenues that have been identified, such as the tethered peptide agonist promiscuity, which may prove valuable for future drug discovery efforts.

6. Expert opinion
The evidence that we have discussed throughout the review provides several important insights on the overall concept of aGPCR druggability. First, it is the strong association of the receptors in rare development disorders. From the broader therapeutic development perspective, the aGPCR potential is evident the most in neurology and cancer. A particularly interesting direction here that should be further investigated is the mechanism of aGPCR-mediated treatment responsiveness and/or drug resistance in ADHD, ovarian and breast cancer, NSCLC, and glioblastoma. This may provide important insights on the mechanisms of such resistance and could even potentially result in the emergence of strategies to improve the currently available treatment options for these chronic and often hard-to-treat diseases. Furthermore, several disease-associated mutations, currently identified in people of certain ethnicities, make it tempting to speculate on the possibility of personalized therapeutics development. There is also growing
evidence on the aGPCR involvement in chronic non-communicable diseases, such as T2DM, cancer, and cardiovascular disorders, which warrants closer attention. Among the important issues is the heavy involvement of aGPCRs in developmental processes and their broad distribution/physiological functions. This poses significant risks of side effects and makes satisfactory selectivity an especially important aspect of aGPCR drug development.

The drug screening efforts have also provided several valuable insights. Dihydrourdinulaton and 3-α-acetoxydihydroursine, the two low molecular weight ADGRG1/ADGRG5 modulators, are the first and currently only described molecules of this kind. Both molecules are characterized by nonselective action due to their mechanism of action involving interaction with tethered peptide agonist. The tethered peptide agonist was found to be able to cross-activate receptors across different subfamilies [202], a property that should be carefully considered in future drug development efforts. A potentially interesting tool in this context could also be synaptamide, a selective endogenous ligand to ADGRF1, capable of activating the receptor independently of the tethered peptide agonist. This compound appears to possess features that contribute to its selectivity to ADGRF1, given its striking similarity to the unrelated anandamide. The compound’s further characterization could shade light on how to achieve greater selectivity. The characterized structural features of both dihydourdinulaton and 3-α-acetoxydihydroursine should not be overlooked either, as they may provide information on how to achieve specific pharmacological action. It should also be noted that antibodies appear to have particularly interesting implications for selective action considering their previous use against other GPCRs; this class of therapeutics needs to be investigated further as well.

A notably persistent issue in our way to fully understand the druggability potential of individual aGPCR receptors is the insufficient understanding of signaling mechanisms and/or ligands involved; an issue relevant to even some of the receptors with known pathogenetic involvement [7]. Another important potential obstacle is the difficulty of applying the classical concepts of pharmacology, such as agonism, orthostericism and allosterism, to the aGPCRs. The receptors often follow a two-step activation model where the ligand-NTF interaction and the Stachel signaling/basal activity are involved in their biology [7]. It is important to recognize, however, that some of these features of the aGPCRs, particularly the Stachel-activated signaling and constitutive activity, can potentially provide an invaluable help in identifying more possible signaling pathways, thereby promoting assay development, an important tool for drug discovery. The structural features seem to contribute to diverse G protein-coupled signaling (further explored in [7]), an important feature to keep in mind for assay screening studies as well. It is also crucial to keep into account the disease-relevant changes when developing further screening efforts; this is especially relevant for ADGRG1 and ADGRG5, as these receptors already have small molecule modulators.
identified and it would be beneficial to try and explore their activity in a pathologic environment.

Overall, the current evidence suggests some receptors hold the highest potential to become a therapeutic target, particularly ADGRG1 and ADGRF1. ADGRG1 is currently characterized by the largest extent of pathogenetic involvement and both of the recently characterized small molecule modulators target this receptor. Additionally, this receptor is characterized by a relatively simple structure of its N-terminal region, a property that appears to positively impact the druggability. ADGRF1 is another example of a suitable receptor, characterized by a slightly more complex structure. While it does not have as extensive pathogenetic involvement as ADGRG1, it seems to provide implications for selectivity. It is likely to be beneficial for the global efforts to concentrate on the well-studied receptors first to advance the aGPCR drug discovery.

To conclude, it is evident that the key to fully exploring the aGPCR druggability is the full characterization of receptors’ biology. Therefore, characterizing the biology, as well as the pathobiology of these proteins should continue being one of the most important directions in the field. The characterization of ADGRG6 extracellular region in zebrafish [203] is one of the recent such milestones that should build up even greater interest in this exciting protein family. Last but not least, the interactions of small molecules with receptors require further elucidation as well, in order to facilitate more extensive structure-activity relationship studies.

Funding

This manuscript has been supported by Vetenskapsrådet, Hjärnfonden, and Novo Nordisk Fonden.

Declaration of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

ORCID

Helgi B. Schióth http://orcid.org/0000-0001-7112-0921

References

Papers of special note have been highlighted as either of interest (+) or of considerable interest (+•) to readers.


•• This paper provides the most comprehensive summary on the aGPCR biology to date.

•• This paper provides the most recent insights on the aGPCR druggability overall.

•• This paper provides several interesting perspectives on ADGRG1 druggability.


• This paper provides insights on how mutations in ADGRL3 affect the responsiveness to pharmacologic ADHD treatment.


• This paper provides insights on how mutations in ADGRL3 affect the responsiveness to pharmacologic ADHD treatment.


• This paper provides evidence on the ADGREG5 tumorigenicity and treatment responsiveness.


• This paper presents evidence on protective role of ADGRG1 in cancer and treatment responsiveness.


• This paper presents evidence on how ADGRA2 contributes to the drug resistance in cancer.


This paper provides the information on 3-α-acetoxydroxydideoxygudedin and several other recently discovered partial aGPCR agonists.


This paper covers the only currently known small molecule aGPCR antagonist.


   "This paper discusses the applicability of antibodies as GPCR modulators."
   "This paper summarizes the most recent overall evidence regarding synaptamide.
   "This paper provides evidence on how synaptamide is involved in the ADGRF1-mediated anti-inflammatory activity."
   "This paper provides evidence on tethered peptide promiscuity.