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Review

The *Adhesion* GPCRs: A unique family of G protein-coupled receptors with important roles in both central and peripheral tissues

T. K. Bjarnadóttir, R. Fredriksson and H. B. Schiöth*

Department of Neuroscience, Uppsala University, BMC, Box 593, 751 24, Uppsala (Sweden),
Fax: + 46 18 51 15 40, e-mail: helgis@bmc.uu.se

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Abstract. G protein-coupled receptors (GPCRs) are a diverse superfamily of membrane-bound receptors. The second largest subgroup of GPCRs, the *Adhesion* GPCRs, has 33 members in humans. Phylogenetic analysis of the entire repertoire of the seven transmembrane-domain (7TM) regions of GPCRs shows that the *Adhesion* GPCRs form a distinct family. *Adhesion* GPCRs are characterised by (1) long N termini with multiple functional domains often found in other proteins such as tyrosine kinases, integrins and cadherins, (2) highly complex genomic structure with multiple introns and splice variants and (3) a 7TM region that has no clear similarities with 7TM from other GPCRs. Several *Adhesion* GPCRs are known to have a role in the immune system but it is becoming more evident that many have important roles in the CNS. We speculate that the overall structural construction of the *Adhesion* GPCRs allows them to participate in different types of cell guidance.

Keywords. GPCR, EGF-7TM, receptor, CNS, immune system, N termini, functional domains.

Characteristics and overall repertoire of *Adhesion* GPCRs

G protein-coupled receptors (GPCRs), with their wide range of physiological roles, presently account for approximately 50 % of all newly introduced drug targets [1]. Based on phylogenetic analysis of the entire human GPCR repertoire, this superfamily can be divided according to the GRAFS classification system, into five major families: *Glutamate*, *Rhodopsin*, *Adhesion*, *Frizzled/Taste2*, and *Secretin* [2]. We use this nomenclature in this review and designate the receptor family in italics with the first letter in capitals (*i.e. Adhesion*). The most recently described is the *Adhesion* family, also referred to as family B [3], B2 [4], epidermal growth factor-seven span transmembrane (EGF-TM7) receptors [5], or the long N-terminal seven transmembrane receptors related to family B (LNB-TM7) family [6] by others. The main characteristic of the *Adhesion* family is their relatively long N termini, which can extend up to a few thousand amino acids and typically contains one or more functional domains, many of which have adhesive properties. These functional domains are generally unique for the *Adhesion* members and not found within other GPCR families [4, 7]. The *Adhesion* GPCRs are coded by many exons and their genomic structure is, in general, very complex, which is one of the reasons that most of these genes have been

* Corresponding author.

difficult to study and were described only relatively recently. Thus, the majority of *Adhesion* GPCRs are still orphans, where neither ligand nor function is known.

It is not entirely clear if the *Adhesion* GPCRs are truly functionally coupled to G proteins in the manner that is known for the typical *Rhodopsin* GPCR. It has, however, been suggested that the lectomedin receptor 1 (LEC1/LPHN1) interacts with the αo subunit of heterotrimeric G proteins as the two proteins co-purified in a two-step affinity chromatography [8]. Moreover, GPR56 has been shown to form complex with a G protein, αq/11 [9]. These reports suggest that members of the *Adhesion* GPCR are likely to signal through G protein-mediated pathways, although further functional proof would be valuable, *e.g.* by showing functional ligand coupling. There is also a lack of experimental data for the specific role of the seven transmembrane domain (7TM) in these proteins. The individual *Adhesion* GPCRs are listed as GPCRs by the Hugo Nomenclature committee and also at the IUPHAR receptor list. Many proteins listed there have not been experimentally shown to couple to a G protein. It would perhaps be more technically correct to call the GPCRs superfamily a family of "7TM proteins" but the GPCR nomenclature is much more established.

The first identified member of this family, the epidermal growth factor (EGF) module-containing receptor 1 (EMR1), was described in 1995 [10]. However, with the sequencing of the human genome [11] and development of bioinformatic tools, many novel GPCRs have been discovered. By 2003, the number of *Adhesion* GPCRs in humans was up to 30 [12, 13] and a year later the *Adhesion* family constituted 33 human and 31 mouse GPCRs, thought to account for the overall *Adhesion* repertoires in these species [14]. The family can be divided into several clans according to phylogenetic analysis (see Fig. 1). Interestingly, even though the phylogenetic analysis is based on 7TM regions alone, the clans show good agreement with the functional domains contained in the N termini. For example, receptors containing EGF domains group together in the same phylogenetic clan (III, in the phylogenetic tree, Fig. 1). The EGF clan contains EMR1–EMR4, the cell differentiating antigen receptor (CD97) and the EGF-TM7-latrophilin-related protein (ETL). The LEC clan contains the lectomedin receptors (LEC1–3). The CELSR clan (V) contains the cadherin EGF LAG seven-pass G-type receptors (CELSR1–3) and the BAI clan (I) contains the brain-specific angiogenesis inhibitor receptors (BAI1–3). The four remaining clans contain: GPR123–GPR125 (IV), GPR133 and GPR144 (VI), GPR110, GPR111, GPR113, GPR115, and GPR116 (VII), and finally GPR56, GPR97, GPR112, GPR114, GPR126, GPR128, the human epididymal gene product 6 (HE6), and the very long G protein-coupled receptor 1 (VLGR1) (II). Fairly little is known about the physiological role of many members of the *Adhesion* GPCRs. Those that have been studied the most are involved in the immune system. However, there is increasing evidence for a role of the *Adhesions* GPCRs in the CNS. The domains in the N termini make these receptors unique compared to all other GPCRs and the nature of these domains indicates that they interact with other cells or extracellular matrixes. Considering these domains, the *Adhesion* GPCRs could participate in functions similar or complementary to that of classical cell adhesion molecules such as integrins or cadherins. Also, considering that some of the *Adhesion* GPCRs contain domains similar to those found in tyrosine kinase receptors, they could participate in the same or similar pathways controlling cell fate and cell differentiation. Considering these unique features, it is likely that the physiological functions of the *Adhesion* GPCRs are fairly distinct and different from those of the other GPCR families.

Adhesive properties in the N termini and ligand binding of *Adhesion* GPCRs

The adhesive properties of *Adhesion* GPCRs are related to the functional domains situated in the long N termini. The 33 human GPCRs contain 14 different types of functional domains (Fig. 2 provides an overview), many of which are involved in: (i) protein-protein interactions in non-GPCRs such as the leucine-rich repeats (LRR) [15], (ii) cell-extracellular matrix or cell-cell adhesion like the Calx-Beta [16], the thrombospondin type 1 domain (TSP1) [17], laminin-type G (LamG) domains [18], galactose-binding lectin (GBL) domains [19], cadherin, and immunoglobulin domains [20]. Even though the adhesive role of the majority of these 14 domains has yet to be proven for *Adhesion* GPCRs, considerable evidence exists for the adhesive properties of the EGF domain in other proteins.

Taking ligand binding into account, the members of the EGF clan are probably the most studied of the *Adhesion* clans. Overall, the human and mouse receptors have domain combinations of two to six EGF domains in their N termini (see Fig. 2). Most of these domains contain Ca^{2+} binding sites [21] within the following consensus sequence: (D/N)X(D/N)(E/Q)X(D/N)X(Y/F), where X are variable amino acids [22]. The Ca^{2+} binding sites are required for stabilisation of protein-protein interactions in EGF domains of some non-GPCR proteins [23]. Supposedly, they

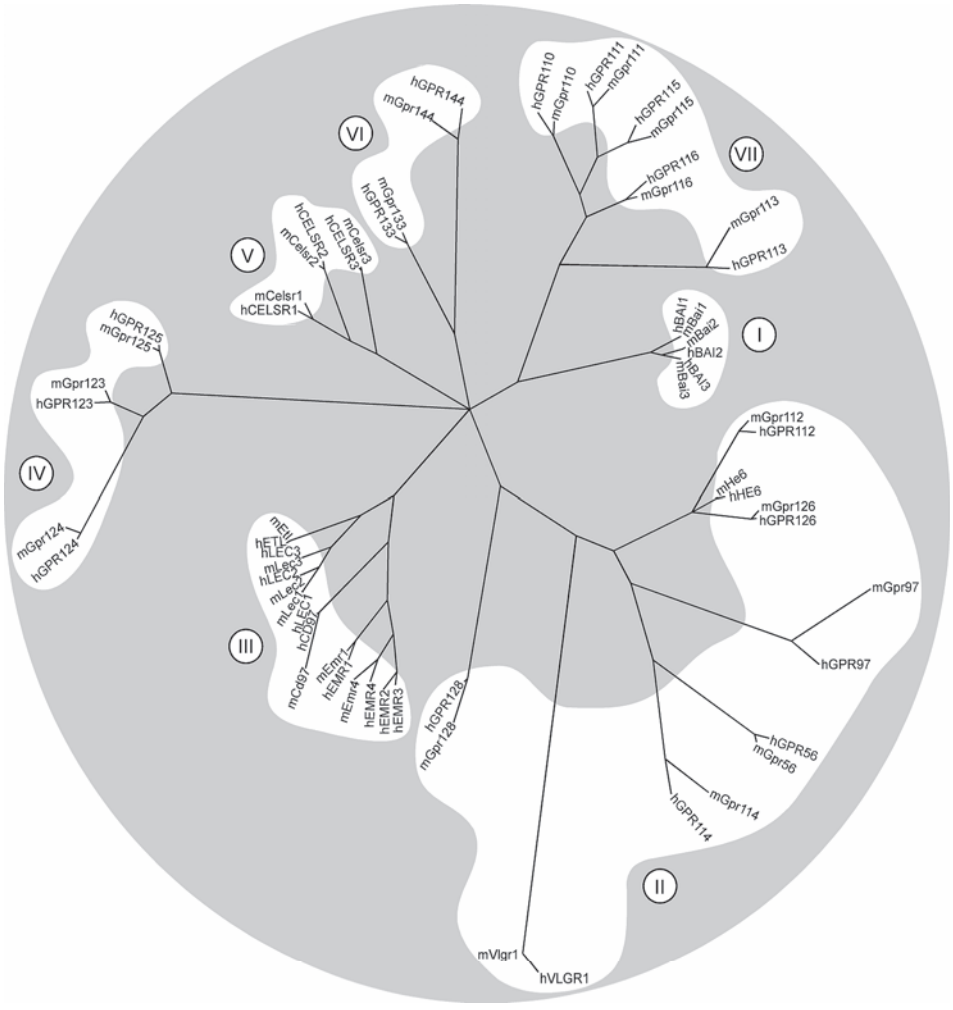

Figure 1. A maximum likelihood phylogenetic analysis of the 7TM regions of all human and mouse *Adhesion* GPCRs. The sequences were cut according to [5, 6, 12, 13]. Tree calculations were carried out using the Phylip package and 100 bootstraps, and branch lengths were calculated with Tree puzzle.

play a similar role in EGF-containing GPCRs by providing cell-adhesion to other cell surface molecules as already shown for some of the EGF clan members. The various CD97 splice variants contain between three and five EGF domains. Two of these domains require Ca^{2+} for interdomain stabilisation and, together with hydrophobic interactions, they mediate binding to a short consensus repeat (SCR) on decay-accelerating factor (DAF/CD55) [22, 24, 25]. DAF is found, for example, on surfaces of lymphocytes and erythrocytes, binding to CD97 functions to recruit immune cells by cell adhesion. Similarly, Ca^{2+}-dependent EGF domains are involved in CD97 and EMR2 binding to chondroitin sulphate (CS) glycosaminoglycans. Glycosaminoglycans in the extracellular matrix have been implicated in biological processes such as cell adhesion, proliferation, tissue repair, and immune responses [26]. The different ligand binding profiles for CD97 and EMR2 are quite remarkable since the two receptors are very similar and the EGF domains responsible for DAF binding in CD97 are 97 % identical to the EGF domain in EMR2. Thus, three amino acids are sufficient to alter the ligand binding profile [25].

Interestingly, the binding profile of CD97 differs greatly for the two ligands (DAF and CS). The first two EGF domains are involved in DAF binding, while the fourth EGF domain is required for the binding of CS (the same EGF domain required for CS binding of EMR2) [26]. In practice, only one of the CD97 splice variants, containing all five EGF-domains (EGF1–5), is able to bind both types of ligands [27]. This illustrates the great importance that functional domains can have with respect to ligand binding.

Although less is known about ligand binding of the other EGF-clan members, EMR3 has been shown to recognise a ligand on the surface of monocyte-derived macrophages and activated human neutrophils [28].

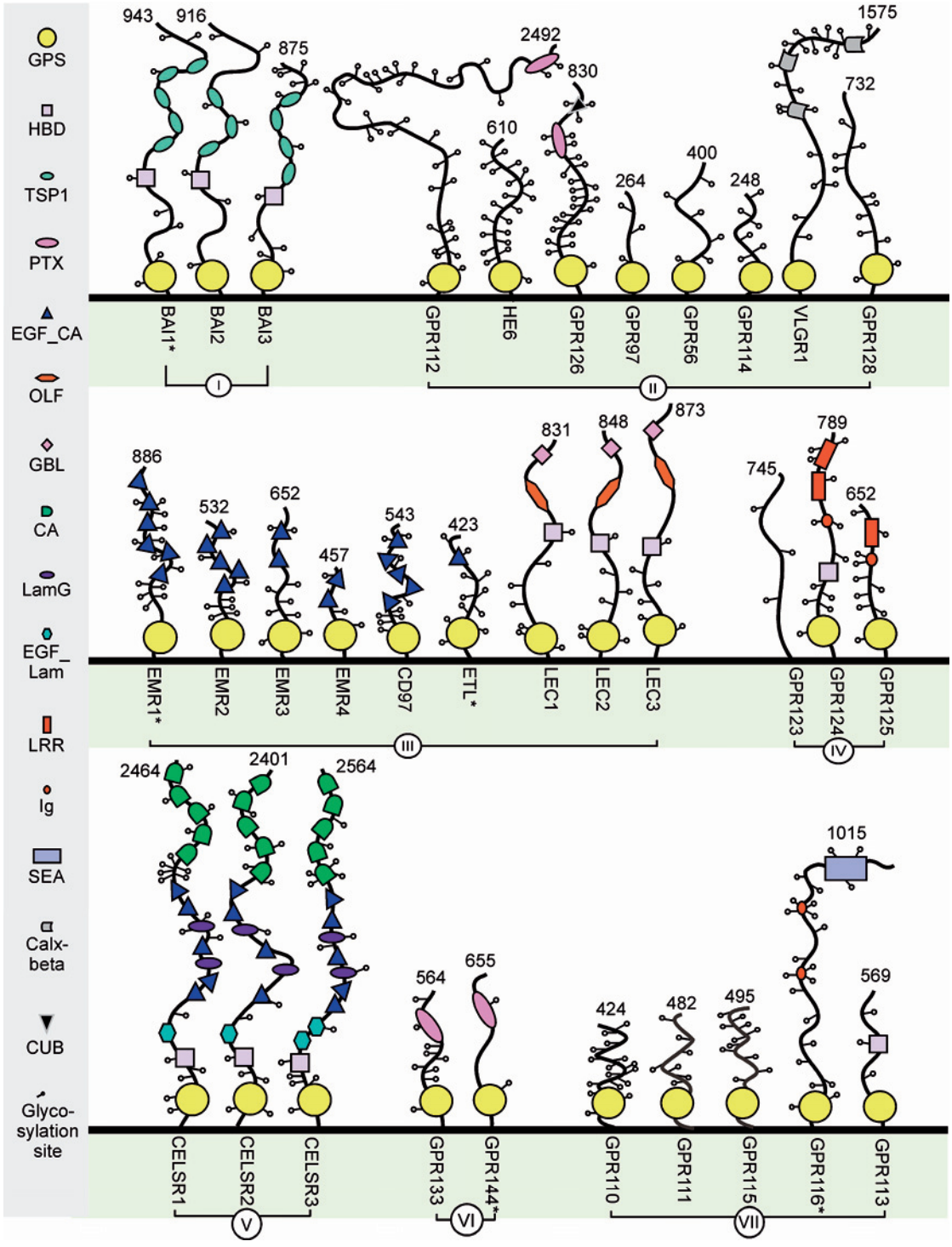

Figure 2. A schematic presentation of the functional domains found in the N termini of human *Adhesion* GPCRs according to RPS-blast, [5, 6, 12, 28]. The number of amino acids constituting each N termini is given and they are arranged (I–VII) according to the grouping in the phylogenetic tree (see Fig. 1). The following domains could be found GPS (GPCR proteolytic site), HBD (hormone binding domain), TSP1 (thrombospondin repeats, type 1), PTX (Pentraxin domain), EGF_CA (epidermal growth factor, calcium binding domain), OLF (olfactomedin domain), GBL (galactose binding lectin domain), CA (cadherin repeats), LamG (laminin G domain), EGF_Lam (laminin type Epidermal growth factor domain), LRR (leucine rich repeat), Ig (immunoglobulin domain), SEA (sea urchin sperm protein domain), Calx-beta domain, and CUB (C1r/C1 s urinary EGF and bone morphogenetic domain) domain. Possible N-glycosylation sites, with consensus sequences NXT or NXS (where X can be any amino acid), are also shown. Here we show the human N termini; however, the mouse N termini contain the same functional domains except for in the following cases: *mBai has four TSP1 domains, mEmr1 has 7 EGF_CA domains, mEtl has 2 EGF_CA domains, mGpr116 is missing SEA, and mGpr144 is missing PTX.

Moreover, a putative ligand for mouse EMR4 (mEMR4) has been identified on a B lymphoma cell line, making mEMR4 the first EGF-GPCR known to mediate interactions between myeloid cells and B cells [29]. To date, little is known about the ligand preferences and binding of the other *Adhesion* GPCRs.

Besides functional domains, most *Adhesion* GPCRs share a relatively high content of Ser/Thr residues in their N termini (about 20%) [5]. These residues are candidates for *O*- or *N*-glycosylation. *N*-glycosylation occurs exclusively at the consensus sequence NXS/NXT and is the most common post-translational modification of GPCRs, while O-glycosylation lacks a consensus sequence and is more difficult to study. The process of glycosylation is initiated in the ER and completed during transport through the Golgi. This is a fast process and thus there might not be enough time to glycosylate all possible sites. In GPCRs, *N*-glycosylation is functionally important for specific receptors to regulate cell surface transport or cell surface expression. Similarly, *O*-glycosylation can have an effect on maturation and plasma membrane expression and in some cases prevents cell surface expression [30]. When expressed in the membrane, the glycosylated regions often form stalk-like rigid structures and can thus serve as spacers between the 7TM helices and the ligand binding domains. Also, if glycosylations are found within the functional domains, the domain structure could be affected. Tissue-specific post-translational modification has been suggested for CD97, since the amount of *N*-glycosylation is shown to differ between smooth muscle cells and tumour cell lines. Moreover, the amount of *N*-glycosylation within the EGF domains of CD97 affects binding of both mAb and the ligand DAF. The lack of *N*-glycosylation of the relevant EGF domain in smooth muscle cells is predicted to lead to little, if any, affinity of CD97 for DAF. *O*-glycosylation seems to be less relevant for the molecular weight or interactions of CD97 [31].

The EMR2 has a chimaeric structure with the 7TM region of EMR3 (to which it is most related), whereas it has an EGF domain region nearly identical to CD97. Recent work on mammalian sequences of this EGF clan suggests a concerted evolution, apparently mediated by gene conversion, among EMR2 and the oppositely oriented and physically adjacent genes CD97 and EMR3 [32]. This concerted evolution may have continuously maintained the chimaeric structure of EMR2 since early mammalian radiation.

The highly conserved GPCR proteolytic site

The GPCR proteolytic site (GPS) is the functional domain found conserved in almost all the *Adhesion* GPCRs (all except GPR123). In contrast to the other functional domains, the GPS has a different role from adhesion-yielding domains. The domain is found in a single copy adjacent to the TM region and contains four conserved cysteines, one glycine, and two conserved tryptophan residues [29]. This domain has proven to be essential in the proteolytic cleavage of N termini for a number of *Adhesions* such as CD97, ETL, EMR4, EMR2, and LEC1 [33–36]. Basically, a peptide bond (often between Leu/Ser or Leu/Thr) within the GPS domain is cleaved via an as-yet-unknow mechanism. After the cleavage, the N terminus is non-covalently linked to the TM region to form a two-subunit structure [36]. This proteolytic process has been shown to be essential for surface expression of LEC1. Since heterodimer structures exist for numerous other GPS-containing *Adhesions*, this mechanism may apply to all members of the family [37].

Conserved motifs in the transmembrane region

An alignment of the 7TM regions of the human *Adhesions* is shown in Figure 3. Three amino acids (His and Trp in TMIII and a Pro in TMIV), highlighted in black, are conserved throughout all 33 sequences. The EXXXXY motif in TMIII is well conserved and the Y is predicted to have the same location as the DRY motif in Rhodopsin GPCRs, important for providing structural constraints [38]. The W in TMIV is found in the majority of Rhodopsins, Secretins, and Frizzled family members but not in the Glutamate family [39]. The PALV motif in TMIV can be found in Secretin and Frizzled GPCRs. These common features with TM regions in other GPCRs are, over the entire region, very minor and contain comparably few alignable residues. From this, it can be concluded that the 7TM regions between *Adhesions* are less well conserved than for other GPCRs and that the TM region shows very little resemblance to the 7TM region from other GPCR families [2, 40].

Interaction with scaffolding proteins

Interactions between specific *Adhesion* GPCRs and specific scaffolding proteins have been shown to target these specific *Adhesion* GPCRs to certain subcellular structures. Interactions with shank proteins were found to be involved in the targeting of LEC1 and

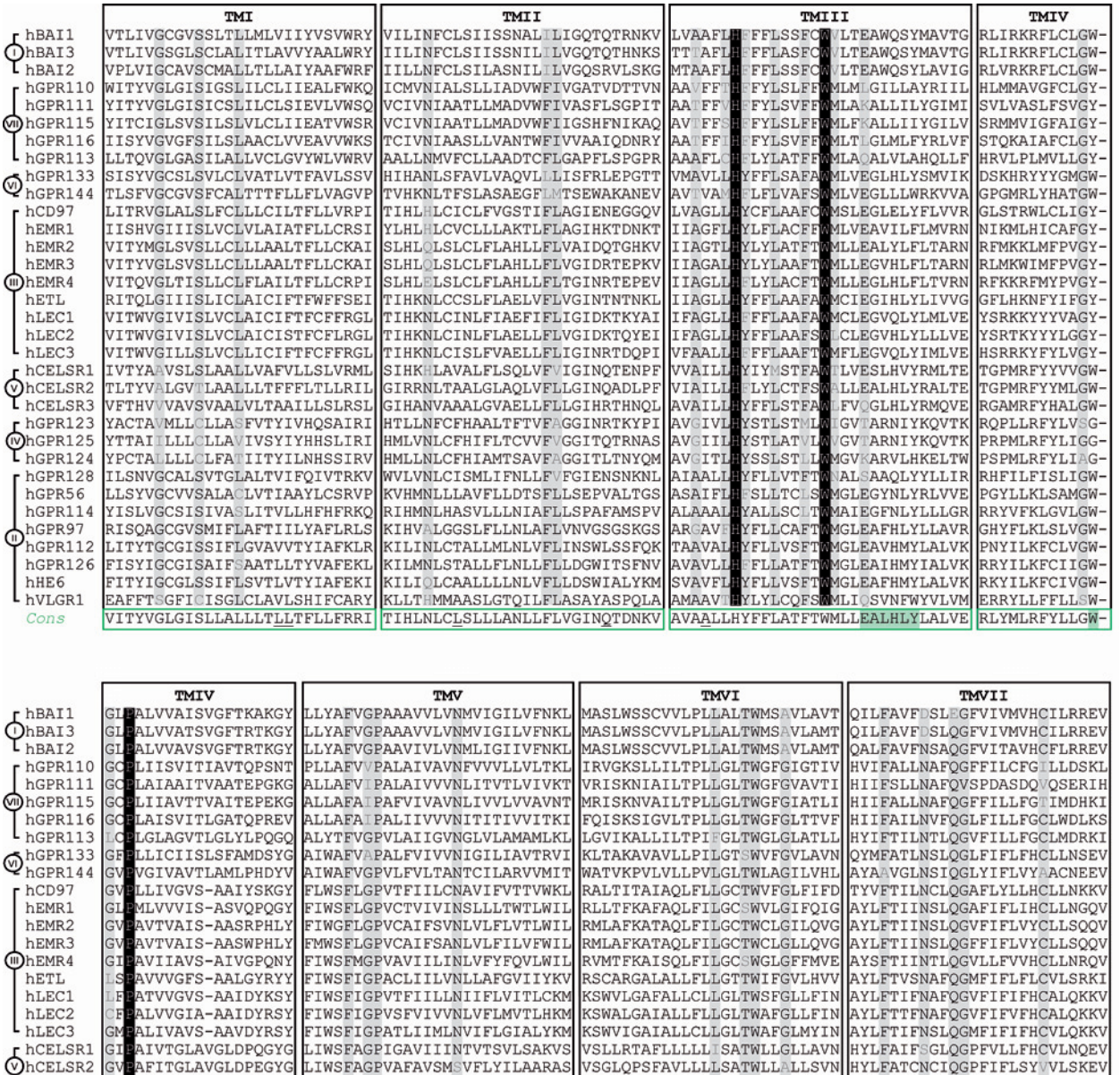

Figure 3. An alignment of the 7TM regions of human *Adhesion* GPCRs. Sequences were cut by RPS-blast according to [14]. The alignment was calculated using ClustalW and edited in Canvas. Numbers to the left of the sequence names indicate their phylogenetic grouping (I–VII), according to Figure 1. Amino acids highlighted in black are conserved throughout all the sequences, those in grey are conserved in 70 % of the sequences. A consensus sequence created from the amino acid most often occurring at each site is shown at the bottom (underlined residues represent cases were two amino acids occurred equally often throughout the alignment. In such cases the one occurring first was chosen for the consensus sequence). The green highlighted parts of the consensus sequence are motifs discussed in the text.

Table 1. EGF-clan tissue expression according to the available literature.

Receptor	Tissue expression	References
CD97	Adrenal gland, bladder, brain, dendritic cells, heart, intestine, kidney, leukocytes (granulocytes, monocytes, T cells, B cells), liver, lung, lymph monocytes, T cells, B cells), liver, lung, lymph node, pancreas, placenta, prostate, skeletal muscle, skin, spleen, stomach, testis, thymus, tonsils, uterus	[21, 34, 48, 49, 99, 100]
EMR1	Haematopoietic cells (monocytes, macrophages, myeloid cells)	[10, 50]
EMR2	Bone marrow, leukocytes (monocytes, granulocytes), liver, lung, lymph nodes, placenta, skin, spleen, thymus, tonsils	[34, 48]
EMR3	Bone marrow, heart, kidney, leukocytes (neutrophils, monocytes), liver, lung, lymph node, macrophages, pancreas, placenta, skeletal muscle, spleen, thymus, tonsils	[26]
EMR4	Kidney, liver, lung, macrophages, spleen, thymus	[29]
ETL	Brain, heart (cardiomyocytes), liver, lung (bronchiolar smooth muscle cells), muscle (vascular smooth muscle cells), kidney, spleen	[62]

LEC2 to synaptic membranes [41, 42]. Shank 1 and LEC1 are primarily expressed in the brain and both proteins seem to co-enrich in the postsynaptic density structures. Furthermore, shank 1 induces a clustering of LEC1 in transfected cells, thus providing evidence that these proteins interact in vivo [42]. Interaction between BAI1 and BAIAP2 is been proposed to play a role in growth cone guidance [43]. The expression profile of BAIAP2 is almost identical to that of BAI1 and appears to be active mainly in neurons. Moreover, there is evidence that interaction between BAI1 and BAP3 could participate in regulating release of neurotransmitters [44]. It is also interesting that the C-terminal end motif ETTV is conserved in the homologues GPR124 and GPR125 (termed TEM5 and TEM5-like) [45] and that this motif may be involved in interaction with the PDZ domain of the human homologue of Drosophila discs large tumour suppressor (hDLG) protein [46]. Considering the other *Adhesion* GPCRs, only the BAI1–3 and the GPR133 contain a C-terminal PDZ-interacting motif. This motif is evolutionarily conserved [45] and, together, the results support the notion that the *Adhesion* GPCRs have complex interactions with intracellular proteins, something that would be very valuable to study further.

Tissue expression and possible functions

The EGF clan – components of the immune system

With respect to ligand binding and tissue expression, the EGF clan is probably the best-studied group of *Adhesion* GPCRs. Results of several studies on the tissue expression pattern of these receptors can be seen in Table 1. Most of these observations were carried out on human tissue using Northern blotting [28, 47–49], *in situ* hybridisation [34] or by RT-PCR [10, 28]. Others relied on rat or mouse tissues and either of the methods mentioned above [29, 34, 50]. According to extensive human and mouse EST and mRNA searches both at NCBI dbEST and UCSC [51], 80 % (144 of 179) of the mouse EMR1 ESTs and 25 % (7 of 28) of the human EMR2 ESTs are found in leukocytes. The highest number of ESTs was found for CD97 with 22 % (41 of 183) from immune system-related tissues, such as bone marrow, leukocytes, spleen, and stem cells. Only a few ESTs were found for EMR3 and EMR4. However, some were also found in immune system-related tissues including spleen, bone marrow, and haematopoietic stem cells for EMR3 and spleen and stem cells for EMR4. The ETL receptor expression profile was more distributed between different tissues according to the EST data. This indicates that EGF-clan members are mainly expressed by cells of the immune system and smooth muscle cells. Accordingly, CD97 has a physiological function within the immune system, inducing localised inflammatory responses [52] and CD97 has an essential role in the migration of neutrophils [53]. CD97alpha, the extracellular domain of CD97, promotes angiogenesis *in vivo* and CD97 may contribute to angiogenesis associated with inflammation and tumour progression [54]. Furthermore, CS, the ligand of CD97 and EMR2, has been implicated in the pathogenesis of rheumatoid arthritis, which raises the question of whether CD97 could be involved in this disease. These speculations were supported by localisation of the ligands, DAF and dermatan sulphate (DS), as well as their receptors, CD97 and EMR2, in rheumatoid synovial tissue. Recent work found that CD97 neutralisation increases resistance to collagen-induced arthritis in mice [55]. Supposedly, CD97 (present on all leukocytes) functions as a primary DS receptor, whereas EMR2 (expressed on macrophages and dendritic cells) may serve as a second DS receptor contributing to the increase of macrophages and dendritic cells seen in rheumatoid synovial tissue [56]. Moreover, DAF seems to play an important role in some

Table 2. Tissue expression of the BAI clan according to the available literature.

Receptor	Tissue expression	References
BAI1	Brain (amygdala, caudate nucleus, cerebral cortex, corpus callosum, frontal lobe, hippocampus, medulla, occipital pole, putamen, substantia nigra, subthalamic nucleus, temporal lobe, thalamus), colon, heart, kidney, leukocyte, liver, lung, ovary, pancreas, placenta, prostate, skeletal muscle, small intestine, spinal cord, spleen, testis, thymus	[44, 63, 67, 68]
BAI2	Brain (amygdala, caudate nucleus, cerebral cortex, corpus callosum, frontal lobe, hippocampus, medulla, occipital pole, putamen, substantia nigra, subthalamic nucleus, temporal lobe, thalamus), heart, skeletal muscle, spinal cord, thymus	[63, 71]
BAI3	Brain (amygdala, caudate nucleus, cerebral cortex, corpus callosum, frontal lobe, hippocampus, medulla, occipital pole, putamen, substantia nigra, subthalamic nucleus, temporal lobe, thalamus), heart, spinal chord	[63, 71]

malignant tumours since expression of alternatively spliced DAF mRNA has been observed in invasive breast cancer [57] and both DAF and CD97 expression are up-regulated in prostate cancer compared with normal tissue [58]. CD97 is also found on smooth muscle cells where they are expressed in distinct subpopulations, which contributes to smooth muscle cell diversity [59]. Direct stimulation of DAF on T cells with CD97 can modulate T cell activation but does not seem to interfere with CD55-mediated complement regulation, providing support for a multifaceted role for CD55 in human T cell activation [57]. CD97 expression in tumour cells is regulated by environmental factors and variations of individual cell properties can be linked to individual patterns of tumour cell invasion [60].

Considering the expression patterns and preliminary results for the other EGF-clan members, it seems likely that they play a role in mediating immune responses (except for ETL). The EMR2 receptor is up-regulated during differentiation and maturation of macrophages; conversely, it is down-regulated during dendritic cell maturation [61]. The alternative splicing and glycosylation of the EMR2 receptor seems to be regulated during myeloid differentiation. EMR2 can be specifically up-regulated by LPS and IL-10 via an IL-10-mediated pathway in monocytes and macrophages, and is also detected in subpopulations of myeloid cells, including macrophages and neutrophils in inflamed tissues, suggesting that EMR2 plays a role in the migration and adhesion of myeloid cells during cell differentiation, maturation and activation. The EMR3 recognises a ligand on macrophages and neutrophils. It has been suggested that by modulating myeloid-myeloid interactions, the receptor can amplify or reduce immune and inflammatory responses [27]. Recent studies show that EMR3 is a marker for mature granulocytes [61]. Similarly, mouse EMR4 is the first receptor known to mediate the cellular interaction between myeloid cells and B cells, giving it potential for a specific role in recruiting B cells [29]. In contrast to the general expression of EGF members, ETL is primarily found in smooth muscle cells. Its expression is developmentally regulated in rat and human heart, suggesting an important role in the growth phase and maturation of cardiac muscle [62]. Thus, even though the EGF-clan members predominantly seem to carry out functions in the immune system, there is a possibility that their roles are not restricted to only one function. For example, CD97 is also found in various peripheral tissues such as kidney, stomach and pancreas, giving a possibility for cell-adhesion roles outside the immune system.

The BAI clan – inhibitors of angiogenesis, specifically in glioblastomas

Experiments for localising tissue expression of the BAI clan have also mostly been carried out using Northern blot analysis and human tissue [44, 63] and the results are listed in Table 2. EST and mRNA findings from searches both at NCBI dbEST and UCSC are in accordance with these results since 35–70 % of the human and mouse BAI1 ESTs, 57–65 % of human and mouse BAI ESTs, and 39–53 % human and BAI3 ESTs were found in brain [51].

Non-GPCR genes containing thrombospondin type 1 repeats (TSP1) act to inhibit angiogenesis [64]. Recently, evidence has emerged that the TSP1 domains in BAI1 also have an anti-proliferative action on endothelial cells [65]. The N terminus of BAI1 is released after proteolytic cleavage at the conserved GPS site. This secreted fragment, named Vasculostatin, dramatically reduces *in vivo* angiogenesis [66]. Furthermore, BAI1 transcripts are induced by p53, the most frequently altered gene in human glioblastoma (common primary brain tumours in adults), and expression of BAI1 was absent or significantly reduced in seven out of nine glioblastoma cell lines tested [67, 68]. Thus, BAI1 seems to play a key role in regulating the vascularisation of tumours [69]. BAI3 has also been found to be absent or significantly reduced in five of nine glioblastoma cell lines examined. However, despite this and the similar tissue specificity among the three BAI receptors, only BAI1 is transcriptionally regulated by p53. This suggests that

Table 3. Tissue expression of the CELSR clan according to the available literature.

Receptor	Tissue expression	References
CELSR1	Brain (area postrema, choroids plexus, lateral, third, and fourth ventricle), eye, kidney, lung, spinal cord, spleen	[73, 75]
CELSR2	Brain (cerebral cortex, cerebellum, hippocampus, brain stem, olfactory bulb), eye, kidney, lung, spinal cord, spleen	[74, 75]
CELSR3	Brain (cerebellum, olfactory bulb), eye, spinal cord	[74–76]

Table 4. The LEC-clan tissue expression according to the available literature.

Receptor	Tissue expression	References
LEC1	Brain (cortex, hippocampus, striatum), fibroblasts, heart, kidney, liver, lung, pancreas, placenta, skeletal muscle, spleen	[8, 77, 78, 101, 102]
LEC2	Brain, colon, heart, kidney, liver, lung, ovary, pancreas, placenta, prostate, skeletal muscle, spleen, testis	[77, 78, 103]
LEC3	Brain, heart, kidney, lung, pancreas, placenta, spleen, testis	[77, 78, 103]

the BAI receptors share similar roles in inhibiting angiogenesis in glioblastomas, even though BAI1 seems transcriptionally regulated in a manner different of the other two [63]. BAI2 and BAI3 participate in ischaemia-induced brain angiogenesis [70]. BAI2 has been found to affect vascular endothelial growth factor and thereby influences inhibition of angiogenesis [71, 72].

Although all three members of the BAI clan are found in various sub regions of the brain, they are also found in tissues of the periphery. In fact, the BAI2 transcripts expressed in heart and skeletal muscle are longer than those found in brain [63]. Thus, it is quite possible that these longer transcripts are splice variants, and it cannot be excluded that different splice variants are expressed in different tissues where they may regulate angiogenesis.

The CELSR clan – involved in developmental processes

The tissue expression of the CELSR-clan receptors has mainly been studied in mouse and rat, using Northern blots, in situ or RT-PCR [73–76]. Comparison of EST and mRNA search results with literature findings (shown in Table 3) strengthens the conclusion that these receptors have important expression patterns in the brain. A total of 57 % (25 of 44) of CELSR1, 43 % (42 of 97) of CELSR2, and 58 % (18 of 31) of CELSR3 mouse ESTs were found in brain [51]. During mouse embryonic development, the CELSR family exhibits restricted but distinct patterns of expression suggestive of functional roles in a number of developmental processes such as the early development of the hindbrain as well as the early peripheral nervous system (PNS) [76]. Due to the adhesion domains of the CELSRs, which have potential for recognising and interacting with other cell types or extracellular matrix, it is also tempting to speculate that these proteins also have a role in neuronal growth and plasticity in the adult brain. One possibility, not yet investigated, is that the CELSRs could be involved in axon guidance and hence could be important for processes such as memory formation and learning. It is also possible that the CELSRs could have a role in the regeneration of synapses after neuronal injury. In addition, CELSR1 is thought to be involved in cell-cell signalling in the adult brain [73].

The LEC clan – LEC1 has a role in synaptic cell-adhesion and exocytosis

Results from the literature regarding tissue expression of the LEC clan can be found in Table 4. According to these, LEC1 is primarily expressed in the brain but is also present in peripheral tissues. LEC3 is also highly abundant in the brain, whereas LEC2 has no preferential expression in brain but is present rather uniformly in peripheral tissues. LEC2 expression was found in liver, placenta, heart, lung, kidney, pancreas, spleen, and ovary [77, 78]. From EST data, 14–28 % of the human and mouse EST for LEC1 are found in the brain, as are 25–45 % of human and mouse LEC2 ESTs, and 8–22 % of human and mouse LEC3 ESTs [51]. In addition, peripheral expression of LEC2 was supported by nine human and one mouse EST found in the lung, one from each species in kidney, and a single human EST in placenta. However, no LEC2 ESTs were found in heart, ovary, or spleen. The discrepancy between findings can be explained by the fact that even though EST data are accumulating rapidly, the EST databases still contain large variability in sequence quality as well as variations in the representation of sequences, tissues and cell states [79] and this limits the accuracy of expression patterns.

Thus, even though no ESTs were found for LEC2 in heart, ovary, or spleen, this does not exclude its expression in these regions. LEC1 is at least 50-fold more abundant in the brain than in any other tissue [78]. Additionally, LEC1 can bind α-latrotoxin, which

serves a role in recruiting the toxin to synapses and stimulating exocytosis. Thus, the restricted localisation of LEC1 in nerve terminals suggests it carries out highly specialised functions in synaptic cell adhesion in the brain [80], which could couple through a G-protein to exocytosis of synaptic vesicles. LEC2 on the other hand, has been shown to be unable to function as a α-latrotoxin receptor when expressed in liver membranes and similar effects have been described for LEC3, suggesting that the two indeed have functions distinct from those of LEC1 [78]. With its widespread expression, LEC2 could be a part of the PNS, whereas LEC3, found enriched in the brain, seems involved in effects other than exocytosis.

The remaining *Adhesion* GPCRs

Expression patterns of the group of GPR123, GPR124 and GPR125 have been studied. Recent real-time PCR data suggest that GPR123 is predominantly expressed in CNS [61]. *In situ* hybridisation data also showed high expression of GPR123 in all brain regions containing large pyramidal cells such as cortical layers 5 and 6, the piriform cortex, hippocampal pyramidal cell layers and the subiculum. Pyramidal cells are important excitatory projection neurons in the cortex and hippocampus that most likely release glutamate as their neurotransmitter. The layer 5 pyramidal neurons project to the motor-related subcortical nuclei, brainstem and spinal cord, while layer 6 large pyramidal and small spindle-like pyramidal cells project efferent fibres to the thalamus. GPR123 expression was also found in a majority of thalamic nuclei that are important for different sensory afferents and other brain regions. GPR123 was also found in several sensorimotor control structures such as the cortex, thalamus, cerebellum, inferior olive and spinal cord. Moreover, expression of GPR123 was shown in the ventromedial hypothalamic nucleus. A recent report also indicates that GPR124 is expressed on the surface of endothelial cells and that this protein binds to several glycosaminoglycans [81]. It is proposed that the soluble part of GPR124 is shed by endothelial cells during angiogenesis and that it binds to glycosaminoglycans that are present on the extracellular matrix and cell surface proteoglycans. Moreover, further proteolytic processing of soluble GPR124 may lead to exposure of its RGD motif, thereby mediating endothelial cell survival by linking integrin v3 to glycosaminoglycans. GPR125 seems to be expressed both centrally and peripherally according to Northern blot and EST data. Expression of GPR124 has been found in skeletal muscle, prostate, testis, and colon and lower in heart, placenta, and small intestine [46].

Little is known about the remaining *Adhesion* GPCRs. So far, expression studies have been described for GPR56 (also known as TM7XN1), GPR113, GPR116 (also known as Ig-hepta), GPR126 and HE6. GPR56, GPR126 and HE6 group together according to phylogeny (see Fig. 1) as do GPR113 and GPR116. GPR56 expression has been studied in human and rat using Northern blotting, RT-PCR and *in situ* approaches [82, 83]. The highest level of mRNA was detected in the thyroid gland but it was also present in specific brain areas and a wide variety of peripheral tissues [82]. Neither human nor mouse searches yielded any thyroid gland ESTs. However, 15–17 % of the mouse and human ESTs for GPR56 were found in brain and the rest were widespread in the periphery [51]. Recent study shows that overexpression of GPR56 suppresses tumour growth and metastasis, whereas reduced expression of GPR56 enhances tumour progression. It was shown that GPR56 binds specifically to tissue transglutaminase, TG2, which is a widespread component of tissue and tumour stroma. GPR56-TG2 interactions may suppress tumour growth and metastasis [84].

HE6 expression has been studied by Northern blotting in human and rat tissue. The receptor was found expressed in epididymis tissue [85]. Targeted mutation of the mouse HE6 results in male infertility and this protein may be target for male contraception [86]. Most of mouse EST from searches in NCBI dbEST and UCSC database come from breast 35 % (8 out of 23) and the human ESTs from placenta 35 % (6 of 17). Additionally, 29 % (5 of 17) of human ESTs come from the male reproductive organs, further supporting the expression of HE6 in the reproductive organs.

GPR126 (also known as VIGR or DREG) expression, studied by Northern blots in human tissue and by RT-PCR in mouse, was shown to be selective to endothelial cells derived from larger vessels but not from micro-vessels [87], and at high levels in the heart during embryogenesis and in the adult lung [88]. Considering EST data, only two mouse GPR126 ESTs are found in the lung and none are detected in the heart. However, 52 % (15 of 29) of the human ESTs came from placenta [51].

RT-PCR and *in situ* experiments indicated that GPR113 was expressed both in testis and taste cells (circumvallate papillae) of mouse [89]. Only four human ESTs were found and all four came from male reproductive system, which is consistent with findings in the mouse testis.

High expression levels of GPR116 (Ig-hepta) were found in rat lung using Northern blot [90] with lower expression levels detected in kidney and heart. For humans, ESTs for all three tissues were identified even though only 12 % (6 of 49) of the total human ESTs came from lung, 8 % from kidney, and 4 % from heart. Other ESTs come from various peripheral and CNS

tissues, suggesting a very widespread distribution of GPR116. GPR116 was recently suggested to be processed into multiple fragments including proEGF2 region that is converted to EGF2 by the processing enzyme furin [86].

Unfortunately, EST and mRNA searches at both NCBI dbEST and UCSC database for other members of the two groups discussed above resulted in relatively few EST hits (1–6), making the results difficult to interpret. However, an exception is the very long GPCR 1 (VLGR1) with 166 human ESTs and 51 mouse ESTs. A majority (27%) of the mouse ESTs came from the brain, whereas the human ESTs seem widespread in tissues such as adrenal gland, brain, breast, eye, gastrointestinal, kidney, lung, and pancreas. It cannot be excluded that tissue-specific VLGR1 splice variants exist in various tissues since it is the longest of the *Adhesions* and is coded for by over 80 exons.

Although EST data as a means of identifying specific expression patterns of receptors are at best indicative, they can still prove valuable, especially for the many receptors where tissue expression has not been studied specifically. The likelihood of the expression profile from EST data corresponding to true expression is highly correlated to the number of ESTs found. A vast number of ESTs could be found for some of these receptors and here the EST data are likely to provide a relatively good indication of the true expression pattern. On the other hand, for other receptors, searches yielded only few EST hits, so here the data are obviously less conclusive. Even so, each EST hit alone should be considered important. When the *Rhodopsin* GPR119 receptor was first discovered [91], EST searches resulted in only one single EST from pancreas. Today, there are evidence that GPR119 is predominantly expressed in pancreatic β-cells and plays a pivotal role in inducing insulin secretion from pancreatic β-cells as well as the intact pancreas [92]. Thus, the relevance of few EST hits should not be underestimated.

Alternative splicing and role of introns

The *Adhesion* GPCRs have very complex genomic structure and a vast number of exons. There are several examples of domain changes in the N termini through alternative splicing [21, 25, 93] and the fact that their introns are frequent and long make them very interesting to study with respect to non-classical transcription. The increasing number of ESTs and the possibility of using genome assembly to aid alignment of expression and gene databases has provided good opportunities to study alternative splice variants [79, 94]. In a recent study, we used ESTs and full-length mRNA sequences to systematically analyse splice variants for the *Adhesion* GPCRs. We have analysed over 1600 human mRNA and EST sequences aligned to the genome. We identified over 50 novel alternative splice variants and it is clear that splice variants are very frequent and can be found for a majority of the *Adhesion* GPCRs. Most of the functional variants that have now been described differ in length of the N termini when compared with the most typical slice variant (template). Several of the splice variants have N termini that differ in functional domains as compared to the template. The group of CD97, ETL, and EMR1–4 has one or multiple EGF domains in their N termini. It has been shown that EGF domains 1 and 2 of CD97 play a significant role in interaction with the ligand CD55 (DAF) [24, 25]. Similarly CD97, as well as EMR2, bind chondroitin sulphates through EGF domain 4 [26]. Other functional domains, such as the cadherin repeat (CA) and laminin G domains, are also considered to take part in protein-protein interactions. Splice variants that lack these domains are unable to bind these ligands, so it is therefore apparent that alternative splicing can influence how these receptors interact with other proteins. The splice variants for GPR56 and GPR124 lack the GPS domain [51]. Both these receptors are still orphans, so the functional relevance of this is difficult to study at this stage. One possibility is that the alternative splicing regulates surface expression of GPR56 and GPR124. Such a mechanism has previously been shown to operate on the LEC1 receptors where the alternative splicing mechanism regulates the surface expression [37]. A high number of so-called non-functional splice variants for the *Adhesion* GPCRs, which do not have the entire 7TM region intact, have been found [51]. It is difficult to assess if these non-functional splice variants have a functional role but there are increasing numbers of reports that suggest a physiological function of a splice variant that initially looked non-functional [95, 96]. It is interesting in this context that after alternative splicing of mRNA, some introns are considered to be able to escape degradation to make non-protein-coding (nc) RNA, a part of the regulatory network of the cell [97]. It is interesting that several introns with high EST support exist among the *Adhesion* GPCRs, *e.g.* in the cases of BAI3 and LEC3 [51]. It is possible that such clusters could be considered candidate precursors for functionally active ncRNA, which could communicate with other genes or gene products to carry out functions in chromatin modification, transcriptional control, regulation of alternative splicing or some other ncRNA functions.

Table 5. The tissue expression of GPR56, GPR113, GPR116, GPR126, and HE6 according to the available literature.

Receptor	Tissue expression	References
GPR56 (TM7XN1)	Bladder, brain (amygdala, hippocampus, hypothalamic nucleus, caudate nucleus, corpus callosum, cortex, paraventricular nucleus, substantia nigra, subthalamic nucleus, thalamus), heart, kidney, lung, pancreas, prostate, skeletal muscle, testis, thyroid gland, uterus	[82, 83]
GPR113	Circumvallate papillae, testis	[89]
GPR116 (Ig-hepta)	Lung (alveolar walls), heart, kidney (intercalated cells in the collection duct of kidney)	[90]
GPR126	Endothelial cells, heart, lung	[87, 88]
HE6	Epididymis tissue	[85]

Perspectives

The *Adhesion* GPCRs are a truly a unique family among the GPCRs. Their 7TM regions form a separate phylogenetic cluster among GPCRs [2] and their functional characteristics differ very much from the Secretin GPCRs that are receptors for peptides hormones. It is the only GPCR family that has a long N terminus containing multiple functional domains and it is the only family that shows similarities between GPCRs and other membrane protein families such as tyrosine kinase receptors, cadherins and integrins. The physiological relevance of the domains in the N termini of *Adhesion* GPCRs is only poorly understood. It is, however, clear that the *Adhesion* GPCRs seem to be found in most vertebrate species and, for example, the chicken has 18 *Adhesion* GPCRs that resemble those in humans [40]. An *Adhesion* like GPCR is found in dictyostelium [98] and our preliminary studies of the evolutionary history of the receptors suggest an ancient presence (Lagerstrom et al., unpublished). Much more work is needed to understand the overall evolutionary history of the *Adhesion* GPCRs and this is complicated by their complex genomic structure. The most well-studied receptors physiologically are the EMR1–3 and the related CD97. These have been found to bind cell surface molecules like DAF as well as extracellular matrix molecules to mediate cell guidance. These receptors have, so far, been shown to be mainly important in the immune system, but the overall function of *Adhesion* GPCRs is likely to be much broader. Tissue expression data have shown that of the 20 most studied *Adhesion* receptors (See Tables 1–5), 10 have CNS as one of their main sites of expression. Of these, the BAI receptors are important for control of angiogenesis in the brain, but also in other tissues, while the function in CNS of all the others is virtually unknown. Published expression data on the remaining *Adhesion* receptors is so far scarce. EST data, although based on very few ESTs, indicate that these are expressed in the CNS and several (e.g. GPR123, GPR124 and GPR125) appear to have the brain as a major site of expression. A recent in situ hybridisation study has shown that GPR123 has a very specific expression in the brain, with high expression in thalamic nuclei and regions containing large pyramidal cells in mouse [45]. Also, a recent RT-PCR study on a large number of rat tissues for all the *Adhesion* GPCRs has shown that a large number of the newly discovered *Adhesion* GPCRs (Haitina et al., unpublished) have abundant expression in CNS tissues, confirming many of the EST results that have been discussed above. Thus, the *Adhesion* GPCRs may play a very important physiological role in the brain. It is possible that the adhesive properties of these receptors could have a role in axon guidance and neuronal plasticity. As such, they could be important for functions such as neuronal repair and memory formations, but could also be relevant for degenerative diseases such as Parkinson's and Alzheimer's disease. The importance of plasticity in the adult brain as well as adult neurogenesis was long underestimated but has recently been shown to be more important than previously thought. Recent data suggest that treatment of diseases such as depression and anxiety could be dependent on neurogenesis and plasticity and that antidepressant drugs are able to induce those processes. It is possible that *Adhesion* GPCRs could play a role in this context. It would be very valuable if more endogenous ligands as well as selective agonists and antagonists could be discovered.

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