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REVIEW

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The blood–brain and gut–vascular barriers: from the perspective of claudins

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ABSTRACT

In some organs, such as the brain, endothelial cells form a robust and highly selective blood-to-tissue barrier. However, in other organs, such as the intestine, endothelial cells provide less stringent permeability, to allow rapid exchange of solutes and nutrients where needed. To maintain the structural and functional integrity of the highly dynamic blood–brain and gut–vascular barriers, endothelial cells form highly specialized cell-cell junctions, known as adherens junctions and tight junctions. Claudins are a family of four-membrane-spanning proteins at tight junctions and they have both barrier-forming and pore-forming properties. Tissue-specific expression of claudins has been linked to different diseases that are characterized by barrier impairment. In this review, we summarize the more recent progress in the field of the claudins, with particular attention to their expression and function in the blood–brain barrier and the recently described gut–vascular barrier, under physiological and pathological conditions.

Abbreviations: 22q11DS 22q11 deletion syndrome; ACKR1 atypical chemokine receptor 1; AD Alzheimer disease; AQP aquaporin; ATP adenosine triphosphate; Aβ amyloid β; BAC bacterial artificial chromosome; BBB blood-brain barrier; C/EBP-α CCAAT/enhancer-binding protein α; cAMP cyclic adenosine monophosphate (or 3′,5′-cyclic adenosine monophosphate); CD cluster of differentiation; CNS central nervous system; DSRED discosoma red; EAE experimental autoimmune encephalomyelitis; ECV304 immortalized endothelial cell line established from the vein of an apparently normal human umbilical cord; EGFP enhanced green fluorescent protein; ESAM endothelial cell-selective adhesion molecule; GLUT-1 glucose transporter 1; GVB gut-vascular barrier; H2B histone H2B; HAPP human amyloid precursor protein; HEK human embryonic kidney; JACOP junction-associated coiled coil protein; JAM junctional adhesion molecules; LYVE1 lymphatic vessel endothelial hyaluronan receptor 1; MADCAM1 mucosal vascular addressin cell adhesion molecule 1; MAPK mitogen-activated protein kinase; MCAO middle cerebral artery occlusion; MMP metalloprotease; MS multiple sclerosis; MUPP multi-PDZ domain protein; PATJ PALS-1-associated tight junction protein; PDGFR-α platelet-derived growth factor receptor α polypeptide; PDGFR-β platelet-derived growth factor receptor β polypeptide; RHO rho-associated protein kinase; ROCK rho-associated, coiled-coil-containing protein kinase; RT-qPCR real time quantitative polymerase chain reactions; PDGFR-β soluble platelet-derived growth factor receptor, β polypeptide; T24 human urinary bladder carcinoma cells; TG2576 transgenic mice expressing the human amyloid precursor protein; TNF-α tumor necrosis factor α; WTwild-type; ZO zonula occludens.

Introduction of the blood-brain barrier and the gut-vascular barrier

The central nervous system (CNS) is a very sensitive structure and requires a homeostatic environment and protection from the constantly changing milieu in the blood stream, as well as from infectious agents and toxins. In the adult, maintenance of the CNS microenvironment and protection of the CNS itself is assigned to three barriers: the brain–cerebrospinal fluid barrier, the blood-cerebrospinal fluid barrier, and the blood–brain barrier (BBB). The BBB has been the most studied, with its discovery arising from the work of Paul Ehrlich, Max Lewandowski and Edwin Goldman. In 1885, Paul Ehrlich showed that when a dye was injected into animals it stained all of the tissues of the body but not the brain[1]. However, ironically, he attributed his findings to a lack of affinity of the dyes for the brain tissue, and never arrived at the idea of an active barrier function. Later, in 1900, Max Lewandowski observed that toxins did not pass across brain capillaries, and, based also on Ehlrich's experiments, he concluded with the concept of the BBB[1]. Finally, Edwin Goldman,

a student of Paul Ehrlich, injected dyes into the cerebrospinal fluid of the subarachnoid space of animals and saw that only the brain was stained, and not the body.[1] Then, in 1967, the combination of electron microscopy and intravenous injection of horseradish peroxidase into mice offered the opportunity to better describe the localization of the BBB in the capillaries and postcapillary venules of the CNS.[2] (Figure 1(a-c))

Likewise, in 2015, Spadoni and colleagues[9] injected mice intravenously with fluorescein isothiocyanate–dextran of different molecular sizes, and analyzed the leakage of the dye into the intestine. While molecules of 4 kDa passed through the endothelial barrier into the blood, molecules of 70 kDa did not. This indicated the concept of a gut–vascular barrier (GVB) that allows selective access of the intestinal luminal contents to the liver, which would thus provide tolerance against dietary antigens and commensal microbiota[9] (Figure 1(d-f)). Consistent with this, they reported leakage of 70 kDa dextran into the circulation during infection with *Salmonella enterica* serovar Typhimurium. This alteration of barrier permeability was due to *S. enterica*-induced down-regulation of endothelial Wnt signaling, which in turn produced an increase in plasmalemma vesicle-associated protein, a molecular component of the fenestrated endothelium that controls vascular permeability.[9]

The GVB provides a second intestinal barrier, and together with the intestinal–epithelial barrier, these guarantee the physical exclusion of gut microbiota from the systemic circulation (Figure 1(d)). The intestinal–epithelial barrier is a semipermeable barrier that is formed by the monolayer of enterocytes that covers the entire mucosa.[10] Furthermore, specialized enterocytes also limit bacterial invasion, such as the goblet cells that produce mucus, and the Paneth cells that secrete antimicrobial peptides.[11] Just as the intestinal–epithelial barrier selectively regulates what enters the host tissue, the intestinal vasculature must also be highly selective for what enters the circulation.[12]

The GVB shares many key morphological and functional characteristics with the well-known BBB (Figure 1). The main components of the BBB are the brain endothelial cells, which are in close contact with the pericytes, with both sheathed in the protective protein cover known as the basement membrane, which is produced by both cell types. Pericytes are recruited to vessels early in development, and they stabilize the vessels by their effects on brain endothelial cells, whereby they acquire more BBB characteristics. Brain endothelial cells and pericytes form the vascular unit of the BBB. The end-feet that project from the astrocytes that are attached on these endothelial cells and pericytes then function as a bridge between the vascular unit and the neurons, as well as promoting the maturation of the BBB. The highly coordinated activity that arises from these multiple cell types, which also include the vascular unit, glia and neurons, is known as the neurovascular unit (Figure 1(b)). The perivascular space then lies at the level of the postcapillary vessels, between the vascular unit and the astrocytes, and this is defined by the basement membrane and the parenchymal membrane that is produced by the astrocytes. This space contains antigen-presenting cells (i.e., microglia, macrophages), which have functions in immunosurveillance. Constant communication between the neurovascular unit and the other brain cell types assure the functions of the BBB as a physical, metabolic, transport and immunological barrier (for BBB reviews, see[13–16]).

Similarly, in the intestine, where the 'gut–vascular unit' consists of blood capillaries that are associated with the pericytes and enteric glial cells (Figure 1(e)). It is likely that these pericytes and enteric glial cells are essential for intestinal homeostasis, and that they can influence the endothelial barrier function, thus resembling the astrocytes in the brain. Indeed, it has been reported that transplantation of enteric glia into damaged spinal cord can accelerate repair of the vasculature at a site of injury and induction of the BBB properties.[17]

The GVB allows the diffusion from lumen to blood of molecules as large 4 kDa, which is eightfold the maximal size reported for the BBB. This will be because these barriers need to fulfill distinct, and diverse, functions. Indeed, the main role of the BBB is to avoid uncontrolled movement of any substances from the blood into the brain parenchyma, to protect the CNS from the constantly changing milieu of the blood stream.[18] To accomplish these functions, brain endothelial cells have low
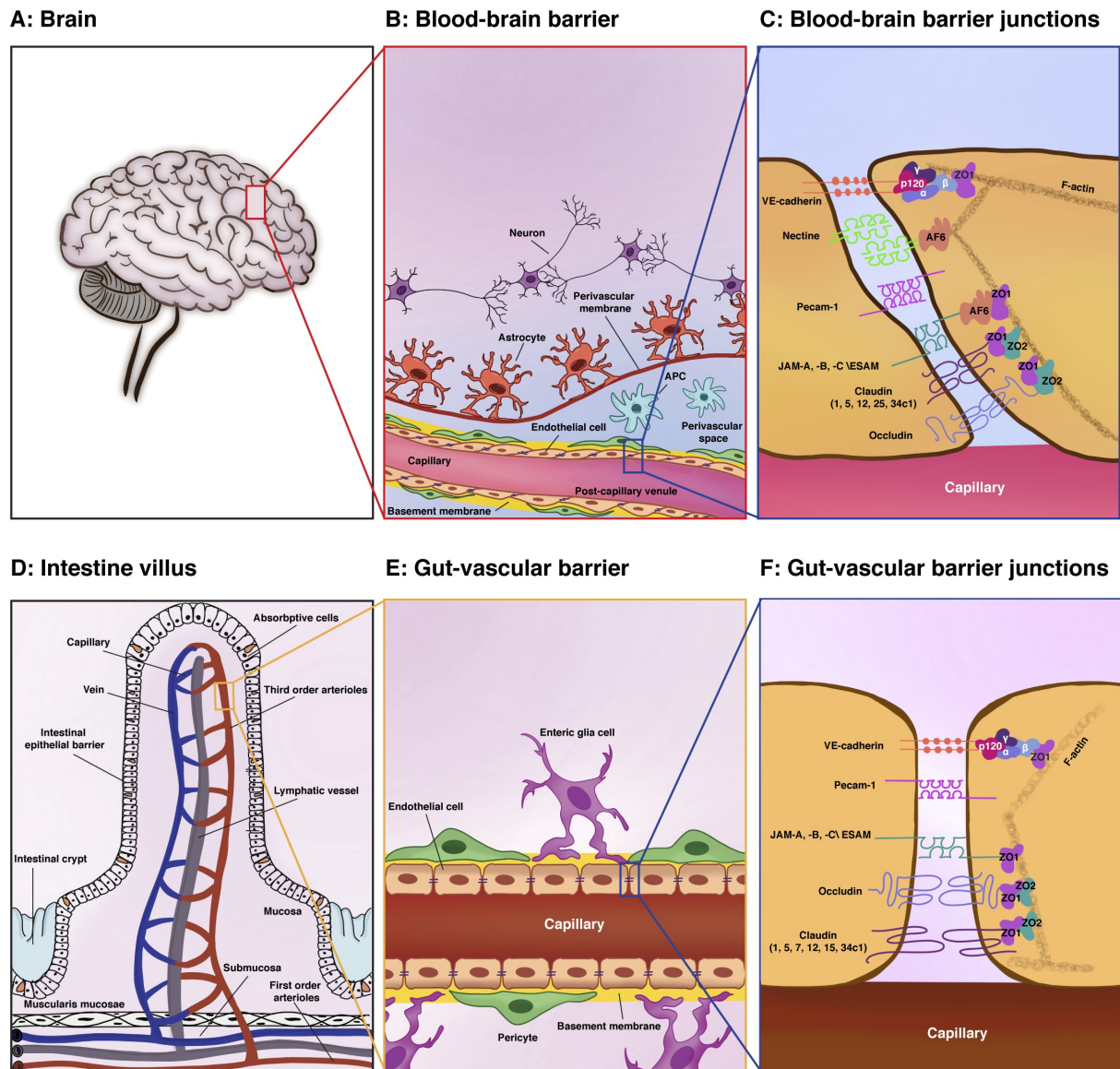
Figure 1. Structures of blood-brain and intestinal barriers. **A**. Diagram of the brain. Red square, enlargement shown in (B). **B**. Schematic representation of the components of the neurovascular unit at the level of the brain capillaries and the post-capillary venules. The blood–brain barrier is formed by endothelial cells that interact with perivascular elements, such as the basal lamina and the closely associated astrocytic end-feet processes, and perivascular neurons and pericytes. APC, antigen-presenting cell. Blue square, enlargement shown in (C). **C**. Brain endothelial cells are unique, in that they form complex tight junctions that involve claudins (1, 5, 12, 25, 34c1),[3–5] occludin and JAM-A/ESAM, where the interaction of several transmembrane proteins seal the paracellular pathway. ZO-1 and ZO-2 form the cytoplasmic plaque proteins that provide the physical support for the transmembrane proteins, and that also interact with the cytoskeleton either directly or indirectly. Adherens junctions consist of transmembrane (VE-cadherin) and cytoplasmic (catenins, p120, plakoglobin) plaque proteins. The cytoplasmic plaque proteins also form a bridge to interact with ZO-1 and the actin cytoskeleton, whereas nectin is secured to actin by afadin. **B, C**, Modified from.[6] **D**. Schematic representation of an intestinal villus and its vasculature. The epithelial and endothelial barriers are indicated. The submucosa contains the first-order arterioles that will become second-order arterioles and then capillaries, which are positioned just under the intestinal epithelium. Modified from.[7] Orange square, enlargement shown in (E). **E**. Schematic representation of the components of the gut–vascular unit at the level of the capillaries, showing the endothelial cells, pericytes and enteric glial cells. Blue square, enlargement shown in (F). **F**. Endothelial cells in the gut–vascular barrier are connected with each other through tight and adherens junctions. In the tight junctions, claudins (1, 5, 7, 12, 15, 34c1),[8] occludin, and JAMs/ESAM are linked to actin by the scaffolding proteins ZO-1/2. Adherens junctions are formed by homotypic binding of VE-cadherin. Catenins connect VE-cadherin to F-actin.

pinocytotic activity, lack fenestration, and are inter-connected with the complex and continuous tight junctions, thus controlling junction permeability. The tight junctions closest to the apical membrane maintain the BBB integrity by preventing diffusion of proteins between the luminal and abluminal membrane compartments and restricting the para-cellular pathway. This effectively prevents the passage of polar, hydrophilic drugs through the endothelial cell layer. Conversely, the intestinal endothelium is located underneath the epithelial layer, and it needs to be permeable to nutrients, due to the absorptive function of the gut (Figure 1 (d)). Thus, the endothelium in the GVB collaborates with enteroglial cells and pericytes to form tight and adherens junctions according to the needs of the overlying epithelial tissue. On the other hand, the GVB acts as the last and potentially most important line of defense to prevent pathogens from invading the bloodstream, and the subsequent systemic consequences.

Tight junctions include the transmembrane proteins claudins, occludin, junctional adhesion molecules (JAMs) and endothelial cell-selective adhesion molecule (ESAM), and the cytoplasmic zonula occludens proteins (ZO-1/2). Cingulin, 7H6, Afadin/AF6 and junction-associated coiled-coil protein (JACOP) are also cytoplasmic protein adaptors that connect ZO-1 to the cytoskeleton[19,20] (Figure 1(c)).

The tightness of the cerebral capillary junctions is reflected in their high trans-endothelial electrical resistance. Typically, electrical resistances of ~2000 Ω cm^2 are observed *in vivo* in pial microvessels on the surface of the brain as compared to 1 Ω cm^2 to 3 Ω cm^2 in mesenteric capillaries.[21,22] This difference in the trans-endothelial electrical resistance might be due to the distinctive composition of the tight junctions and adherens junctions in the BBB compared to the GVB.

Due to the endothelial cell heterogeneity between and within tissues with different functions, the vasculature of two segments of the intestine (i.e., colon, small intestine) has been compared to the brain vessels.[3] Exploiting the single-cell transcriptome dataset from Carmeliet and colleagues, we provide here evidence that in the adherens junctions of the brain, colon and small intestine endothelial cells, the gene expression levels are similar for VE-cadherin (*Cdh5*), α-catenin (*Ctnna1*), β-catenin (*Ctnnb1*), p120 (*Ctnnd1*) and plakoglobin (*Jup*). Similarly, in the tight junctions, the expression levels of junctional adhesion molecule-A (*F11r*), junctional adhesion molecule-B (*Jam-2*), endothelial cell adhesion molecule (*Esam*), zonula occludens-1 (*Tjp1*) and zonula occludens-2 (*Tjp2*) are comparable (Figure 2(a)). Instead, the expression levels for occludin (*Ocln*) and claudin-5 (*Cldn5*) are remarkably high in the brain. In addition, a number of the claudins, including claudins-7, −12, −15 and −34c1, show differential expression between the brain and the intestine (Figure 2(b)). Interestingly, these data on claudins distributions suggested that the diverse barrier functions of endothelial tissues reflect the use of the different claudins and indicate that claudin expression patterns might indeed be responsible for the known variations in permeability.

For instance, it is well known that the presence of high levels of claudin-5 in the BBB is responsible for the restriction of molecules and ions from blood to brain parenchyma.[23,24] However, the relationship between the expression of claudins and the protective role of the GVB to prevent the harmful elements from entering the blood stream from the intestine lumen remains to be defined.

In this review, we summarize the more recent progress on claudins, with particular attention to their expression and function in the BBB and the recently discovered GVB, under both physiological and pathological conditions. We will describe in detail each single barrier separately, because of the scarce availability of information for the GVB compared to the BBB. The reader is referred to several reviews for detailed discussions of the claudins in the other vascular barriers.[25–28]

Functional and structural properties of claudins

Claudins were originally purified from chicken liver by Mikio Furuse et al. in 1998.[29] They identified a protein of 22 kDa inside the tight junction fraction, and its purification and peptide sequencing allowed them to clone two full-length cDNAs that encoded two related proteins of 211 and 230 residues. These proteins were named claudins, which means 'close' in Latin.

Figure 2. Single-cell gene expression profiles of adherens and tight junctions in the brain and gut. Violin plots displaying the normalized expression levels for the VE-cadherin (*Cdh5*), α-catenin (*Ctnna1*), β-catenin (*Ctnnb1*), p120 (*Ctnnd1*) and plakoglobin (*Jup*) genes in adherens junctions (**A**) and for the junctional adhesion molecule-A (*F11r*), junctional adhesion molecule-B *Jam2*, endothelial cell adhesion molecule *Esam*, zonula occludens-1 (*Tjp1*), zonula occludens-2 (*Tjp2*), occludin (*Ocln*) and claudins (*Cldn*)-1, −5, −7, −12, −15 and −3*4c1* genes in tight junctions (**B**) in the brain, colon and small intestine (as indicated). Data were obtained from Kalucka, J. *et al.* (2020).

Since then, the number of proteins recognized as members of the claudin family has been growing continually, with 27 members already characterized in mammals.[30] However, more claudins are still being identified, such as claudin-34c1, for which there is experimental evidence at the transcript level for the vascular beds of different organs.[3]

As members of tetraspan tight junction proteins, claudins serve two main purposes: channel forming and sealing. These functions are not mutually exclusive for each claudin (i.e., claudins-4, −7, −8) as they strongly depend on the cell type in which these proteins are expressed.[26] Claudins control the paracellular permeability to ions, larger solutes and water by forming channels across tight junctions. Channel-forming claudins were initially identified through decreased transepithelial electrical resistance of overexpressing cell layers. For instance, claudin-2 forms channels for monovalent inorganic cations[31] and water,[32] while claudin-7 forms channels for Na^+.[33] Moreover, claudin-15 can act as a Na^+ channel and a Cl^- barrier *in vitro*, which depends on the cellular context.[34] Interestingly, claudin-12 appears to increase cell membrane Ca^{2+} permeability *in vitro*, and its expression is upregulated by vitamin D, which suggests a role for vitamin D in the uptake of Ca^{2+}.[35]

Sealing is another fundamental function of claudins. This property has been supported by several studies carried out on claudins distributions, which have suggested that the diverse barrier functions of a variety of endothelial tissues depend on the expression of their particular claudins. Claudin expression patterns might be responsible for the variations in permeability across a wide range of tissues, such as the BBB, GVB, blood–testis barrier, retinal barrier and thymic barrier. As knockout models for claudins-1 and −5 have been shown to be lethal, the role of claudins in the maintenance of vascular and epithelial barriers is undoubtedly crucial.[36,37] In the brain and in the retina, the presence of a barrier between the vascular lumen, the neuronal layers and the parenchyma maintains a regulated microenvironment and correct neuronal function. Indeed, claudin-5 is the predominant claudin expressed in endothelial tight junctions and has a pivotal role in the barrier properties of distinct tissues. For instance, in the BBB, claudin-5 has an important role in forming a barrier to small molecules, through its strong interactions with the members of the claudin family. Claudin-5 can form homophilic interactions (i.e., claudin-5/claudin-5) or it can bind to claudin-3[37,38] and claudin-1,[39] thus explaining the sealed barrier of this anatomic district. However, several recent reports have postulated numerous mathematical and computational configurations for ionic pores of claudin-5, although supporting experimental data are still not available.[40,41]

In the intestinal epithelium, claudins show differential expression patterns, and by their barrier function, these proteins prevent the unlimited passage of water and solutes, and the invasion of luminal antigens,[42] as was shown through the transepithelial electrical resistance, which has been shown to increase upon overexpression of claudins-1, −4, −5 and −7.[43] Interestingly, in 2001, Rahner et al. demonstrated the presence of claudins along the basolateral membrane of gastrointestinal epithelial cells, which suggested that the functions of the basal and lateral membrane compartments depend on the presence of specific claudin molecules.[44] It is plausible to assume that claudins have a role in the trafficking of endosomal vesicles from the basolateral membrane to apical tight junctions, which undergo continuous remodeling.[45] Furthermore, basolateral claudins might have accessory pore functions, to facilitate discretion of ion transport at tight junctions or to create signaling complexes that transfer information into the cells. Indeed, claudins-1, −2, and −7 are implicated in the regulation of the interfaces between cells and the extracellular matrix through the interactions with integrin molecules in focal adhesions.[46] Thus, claudins-1, −2, and −7 regulate normal cellular homeostasis under physiological conditions and facilitate adhesion of metastatic cells under pathological conditions. The diversity of transepithelial electrical resistance and paracellular ion flow is due to the differential expression of claudin members in the gastrointestinal tract. In humans, qRT-PCR analysis revealed the predominant expression of claudins-2 and −15 in the proximal part of the gastrointestinal tract,[47] while claudins-3, −4, −7, and −8 show higher expression in the more distal parts. Moreover, in the colon, claudin-1 is expressed and localized at the apex of epithelial cells,[48] while claudins-4 and −7 are detected in
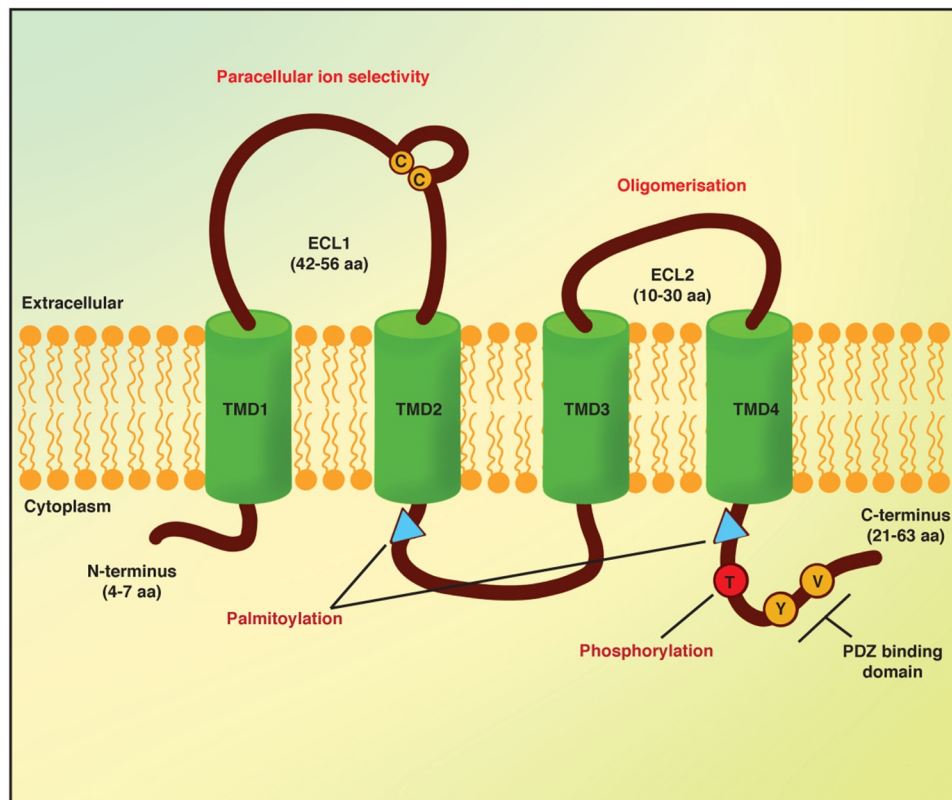
Figure 3. Schematic structure of claudins. Claudins consist of four transmembrane domains (TMD), two extracellular loops (ECL1, ECL2) and one intracellular domain, with the N-terminus and C-terminus located in the cytoplasm. Also shown: two highly conserved cysteines (C) that form a disulfide bond, two palmitoylation sites (triangles), conserved phosphorylation site Thr207 (T), and the C-terminal PDZ-binding motif, which includes a conserved tyrosine.

both the tight junctions and the lateral membranes of the cell surface.[49] In the small intestine, claudin-7 is expressed along the basolateral and apical surfaces of epithelial cells, while in the ileum and colon, claudin-8 is distributed on the basolateral membrane of epithelial cells.[50,51]

Recent reports have demonstrated the unconventional localization of claudins in the nuclear district.[52] First, in 2005, Dhawan et al.[53] demonstrated the unexpected nuclear presence of claudin-1 in patients with human primary colon cancer and in cancer-related metastasis, while claudin-1 was localized on the basolateral membrane in the normal human colonic mucosa.[53] Similarly, numerous studies have shown localization of claudin-1 in the nucleus of melanoma cells and in thyroid carcinoma cells.[54,55] Later, the nuclear localization of other claudins was highlighted under pathological conditions: claudin-2 was identified in the nucleus in human lung adenocarcinoma cells. The nuclear distribution of claudin-2 increases cell proliferation

in human lung adenocarcinoma cells,[56] as for claudin-3 in breast cancer lines[57] and claudin-4 in endometrial cancer cells.[58]

The functions of the claudins are strictly dependent on their structures and post-translational modifications. Claudins constitute a protein family with a molecular mass that ranges from 18 kDa to 27 kDa. They are integral membrane proteins that are characterized by four hydrophobic transmembrane domains, a short N-terminus on the cytoplasmic side (~7 residues), two extracellular loops (ECLs; ECL1, ~52 residues; ECL2, 15–33 residues) and a C-terminus cytoplasmic domain that shows considerable length differences between claudins (21–63 residues)[29,59] (Figure 3).

The C-terminus cytoplasmic domain of claudins is predicted to be intracellular and it is fundamental for claudin stability and the intracellular transport of tight junctions.[60,61] Based on the length of the C-terminus domain, claudins can be subdivided into two groups: the 'classic' and 'non-classic'

claudins.[62] The classic claudins comprise claudins-1–9, −14, −17 and −19, and these show high structural similarity and have short C-terminus cytosolic domains. The non-classic claudins comprise claudins-10–12, −15, −16, −18, −21 and −24, which are characterized by long C-terminus domains, through which they can interact with scaffold proteins in the cytoplasm.[63] In claudins-1, −2, −4, −5 and −16, this domain can be phosphorylated, which controls some of their barrier functions (Figure 3). Furthermore, this phosphorylation is mediated by different kinases and is linked to claudin functional modulation as part of the tight junction assembly, as well as to control the function of the tight junctions. For instance, when claudin-5 is phosphorylated on Thr-207 by cAMP-dependent protein kinase A, barrier permeability in the brain endothelium is decreased,[64] while in the lung endothelium, it results in increased permeability of small molecules of the claudin-5–based barrier.[65] In addition, the permeability of the brain endothelium is increased by Rho/ROCK signaling, where its activation leads to phosphorylation of claudin-5 Ser and Tyr residues,[66] while phosphorylation by MAPK of Thr-203 in claudin-1 in lung endothelial cells decreases the barrier permeability.[67]

The region spanning from the fourth transmembrane domain to the C-terminus and the region of the intercellular loop after the second transmembrane domain are both characterized by a conserved pair of cysteines, which for claudin-14 can be palmitoylated (Figure 3). When this palmitoylation is impaired, claudin-14 loses its correct localization at the tight junction, which highlights the importance of this palmitoylation in the protein trafficking.[68]

Ubiquitination represents another post-translational modification that can regulate functional aspects of claudins. This is an ATP-dependent modification of proteins catalyzed by enzymes that activate, conjugate and favor the binding of ubiquitin to lysine residues of a target protein.[69] The fate of a protein depends on its ubiquitination, in terms of its trafficking (endocytosis) and lysosomal degradation.[70] For claudins, the C-terminus domain, and more specifically their PDZ domains, show the common requisites for ubiquitination. This process can occur on claudins-1, −2 and −4, and in this case, it also appears to affect their endocytosis and lysosomal degradation.[71]

Interactions with scaffolding proteins indirectly link claudins to the actin cytoskeleton and regulate claudins functions. It is very common for claudins to have a PDZ binding motif at the C-terminus domain, through which they can interact with scaffolding proteins ZO-1/2/3, PATJ and MUPP1.[72–74] Indeed, for most claudins, the end of the C-terminus contains a hydrophobic motif of two peptides that is crucial for binding to PDZ domains on several of the tight junction scaffolding proteins (Figure 3). However, this motif is not present in claudins-12, −22, −25 and −27.

The first, and longer, claudin extracellular loop (ECL1) is composed of 42–56 residues, and it is located between the first and second transmembrane domains. It contains a highly conserved signature motif across claudins, which suggests that its involvement in the main functions of this protein family. ECL1 defines the charge selectivity and magnitude of the small-ion permeability of the paracellular pathway[75] and shows a wide variation in the numbers and positions of its charged amino acids, which characterizes each of the different claudins. For instance, claudin-4 ECL1 shows the exchange of basic for acidic residues that leads to increased cation permeability, while conversely, claudin-15 ECL1 shows the replacement of acidic residues for basic residues, thus reversing the paracellular charge selectivity from binding to cations over anions.

The amino-acidic sequence of the second extracellular loop (ECL2) is highly conserved. Chimera studies have shown that ECL2 is not involved in the control of paracellular permeability,[74] while it is fundamental for interactions between claudins.[76] Several types of interactions can occur between claudins, as homophilic and heterophilic, in both *cis* and *trans*. This means that claudins can interact with each other along the plasma membrane of the same cell (*cis*-interaction) or of an opposing cell (*trans*-interaction). Moreover, any particular claudin can interact with the same family member (homophilic interactions) or with other family members (heterophilic interactions).

For instance, trans-interactions between self-associated molecules of claudin-5 are strongly

dependent on amino-acid substitutions in the binding core of ECL2, which supports a substantial contribution of ECL2 in the holding and narrowing function of this claudin.[77] Indeed, studies by Piontek and colleagues on claudin-5–transfected HEK cells suggested a role in the formation of tight junction strands via *trans*-interactions.[46] Based on their molecular modeling, they also hypothesized that ECL2 is folded in a helix-turn-helix motif and can form a dimer between different claudins of adjacent cell membranes through hydrophobic interactions of conserved aromatic residues. Moreover, studies carried out on claudin-2/claudin-2 dimers have shown that *cis*-interactions occur through the ECL2[78] domain. In addition, it has been demonstrated that ECL2 of claudins-3 and −4 is a receptor for *Clostridium perfringens* enterotoxin.[79,80] The C-terminus part of *C. perfringens* enterotoxin can bind claudins,[81,82] while the N-terminus part can shape pores in the plasma membrane and promote cell death.

The identification and characterization of the claudins family still leaves the need to explain how they participate in the generation of heterogeneity observed in diverse tissues, which emphasizes their crucial roles in the development and maintenance of vertebrate tissues.

Claudins of the blood–brain barrier

As indicated above, the BBB is formed by a tightly packed monolayer of non-fenestrated endothelial cells that line the brain capillaries and are enveloped by pericytes and perivascular astrocytes (Figure 1(a)). Cerebral capillaries account for 85% of the blood vessel length in the brain, which provides an endothelium surface area of ~12 m^2 for molecular exchange with the brain, with an approximate 1:1 ratio of capillaries to neurons.[83] Specialized endothelial cells along the cerebral vasculature are linked together mechanically by tight junction protein complexes that eliminate the paracellular space between the neighboring endothelial cells (Figure 1(c)).

A wide range of studies has suggested that claudins-1, −3, −5 and −12 are expressed in the BBB as integral components of tight junctions, with claudin-5 showing the highest expression in brain endothelial cells (Figure 1(c)). This expression of claudin-5 in brain endothelial cells has been shown to occur as early as embryonic day 12.5 in mice,[84] and to be maintained to adult life in different experimental models, including mice, rats and chickens.[84–86] *In-vitro* co-culturing of rat astrocytes with pig brain endothelial cells induced increases in claudin-5 expression under conditions of cell contact.[87] In the same context, glioma-conditioned medium has been shown to up-regulate claudins-1, −3 and −5 in the human ECV304 cell BBB *in-vitro* model; of note; however, these are not endothelium-derived cells as they originally came from human T24 urinary bladder carcinoma cells.[88] Moreover, brain endothelial cells from rats and chickens show gradual decreases in the expression of claudins-1 and −5, occludin and ZO-1 when cultured *in vitro*. In addition, in rats, the tight junction morphology *in-vitro* changes from a ratio of protoplasmic:extracellular faces of 55:45 to 16:84 *in vivo*. These data suggest a loss of BBB function as protoplasmic-face-associated tight junctions are linked to better sealing of the BBB.[85] Claudin-5 was connected to extracellular-face-associated tight junctions when Morita et al. transfected L-fibroblasts with a claudin-5 vector, which resulted in a switch in the tight junction morphology from protoplasmic face associated to extracellular face associated.[84] Furthermore, this study speculated that the switch of extracellular-face-associated tight junctions in the embryonic mouse brain (at E12.5) to protoplasmic-face-associated tight junctions in the adult is caused by early expression of claudin-5, with the other claudins expressed during maturation of the brain endothelial cells. Such early and high levels of claudin-5 in the BBB support the controlled permeability of the BBB. For this reason, to examine the involvement of claudin-5 in the development of the BBB, Nitta and colleagues generated *claudin-5* deficient (*claudin5*$^{-/-}$) mice.[37] Although these *claudin5*$^{-/-}$ mice showed the formation of normal tight junctions, with no bleeding or edema, and normal blood vessel development, their endothelia showed increased permeability to low-molecular-weight dyes. Tracer experiments in *claudin5*$^{-/-}$ mouse embryos (18.5E) revealed that small molecules ≤800 Da, such as Hoechst (452 Da) and gadolinium-diethylenetriamine penta-acetic acid

(742 Da), crossed the BBB, but microperoxidase (1.9 kDa) and tetramethylrhodamine-conjugated dextran (10 kDa) did not.[37] Moreover, size-selective, transient BBB opening was seen in siRNA-induced *claudin-5* knockdown mice. The permeation of small molecules of up to 742 Da from the brain microvessels, but not of molecules of 4.4 kDa, was seen for up to 48 h after the injection of the *claudin-5* siRNA in these mice, with no significant adverse effects seen. Furthermore, the administration of neuropeptide thyrotropin-releasing hormone (360 Da) into the tail vein of the mice after injection of the *claudin-5* siRNA inhibited the permeation for up to five times longer than that seen for the non-targeting siRNA control mice.[89] Finally, we recently showed that JAM-A promotes C/EBP-α binding to the *claudin-5* promoter, which induces *claudin-5* transcription and enhances the endothelial barrier function in mice. On the contrary, *JAM-A–null* mice have decreased claudin-5 expression and increased brain permeability to tracers with molecular weights <500 Da.[90]

Claudin-12 is another claudin that is expressed in mouse brain. In 2003, while studying the tight junction of their *claudin-5$^{-/-}$* mice embryos at E18.5, Nitta et al. also showed that this absence of *claudin-5* did not affect the expression of claudin-12, which was concentrated at ZO-1–positive tight junctions.[37] However, 5 y later, Ohtsuki et al. reported that *claudin-12* is not specific to brain endothelial cells in adult mice.[86] They isolated Pecam-1$^+$ and Pecam-1$^-$ cells from wild-type (WT) adult mouse brains using magnetic cell sorting. Here, they showed higher *claudin-12* mRNA levels in total brain tissue compared to these Pecam-1$^+$ and Pecam-1$^-$ cells. In agreement with the study of Ohtsuki et al. (2008), in 2019, Castro Dias et al. used knockout *claudin-12$^{LacZ/LacZ}$* reporter mice and demonstrated that according to the β-galactosidase signal in the brain, claudin-12 was expressed at very low levels in the endothelium and pericytes, and significantly more in the astrocytes and neurons.[91] They also used these *claudin-12$^{LacZ/LacZ}$* mice to show that none of the commercial antibodies can be used for Western blotting as bands of claudin-12 were seen for both WT and *claudin-12$^{LacZ/LacZ}$* mice. Furthermore, they did not see any changes in claudin-5, occludin and ZO-1 in freshly isolated brain endothelial cells; they thus concluded that claudin-12 is not needed for the tight junction function of the BBB. In 2008, Shimizu et al. described the barrier properties in terms of transporters and tight junctions using RT-qPCR and Western blotting in cultured pericyte cell lines from different organs, including the brain, lungs and peripheral nerves. Among the several transporters that they also found in the brain endothelial cells, the tight junctions of all three pericyte cell lines expressed claudin-12, ZO-1/2 and JAM-A, but not claudin-5.[92]

Claudin-1 has been shown to be expressed in rat and chicken brain endothelial cells *in vivo* and *in vitro*, and to be up-regulated by a glioma-conditioned medium in ECV304 cells.[85,88] However, Ohtsuki and colleagues demonstrated that *claudin-1* is 41.5-fold more enriched in endothelial cells from Pecam-1$^-$ compared to Pecam-1$^+$ mouse brains. Their data suggested that like claudin-12, claudin-1 is not specific for brain endothelial cells.[86]

Early studies on claudin-3 have demonstrated that it is expressed in brain endothelial cells. In particular, similar to claudins-1 and −5, claudin-3 is expressed and up-regulated in ECV304 cells treated with glioma-conditioned medium.[88] Moreover, it has been shown that claudin-3 is a target of Wnt/β-catenin signaling and is up-regulated *in vitro* upon β-catenin stabilization or Wnt3a treatment in mouse brain endothelial cells. Inactivation of β-catenin or inhibition of its signaling had the opposite effect. *In vivo*, stabilization of β-catenin increased claudin-3 expression in brain endothelial cells of embryos (E18.5) and pups at postnatal day 4 (P4). Inactivation of β-catenin *in vivo* decreased the expression of claudin-3 in the brain endothelial cells of pups at P4, P7 and P14.[93] Further experiments in adult mouse brains confirmed the expression and localization of claudin-3 at the tight junctions of brain endothelial cells.[94] However, Ohtsuki et al. reported that although *claudin-3* mRNA was detected in adult mouse brains, it was not endothelium specific.[86] The same study indicated the expression of other claudins as endothelium specific, including for *claudins-8, −10, −15, −17, −19, −20, −22* and *−23*, and that *claudins-10* and *−22* had comparable mRNA levels to *occludin*.

Recently, Haseloff and colleagues compared the gene expression profiles of claudins in human and

mouse brain endothelia obtained by laser capture microdissection and in primary brain endothelial cells and immortalized brain endothelial cells-3.[95] In contrast to *in-vitro* BBB models, *claudin-5* mRNA *in vivo* is just one of another six high-abundance, tetraspanning tight junction protein transcripts. The mRNAs for *claudins-11*, −12 and −25 and for *occludin* (in human, mouse), and for *claudins-1* and −27 (in human) are in the same quantitative range within species, which suggests that a complex of complementary proteins (mainly claudins) operates within the paracellular space of the BBB *in vivo*. This joint operation is lost *in vitro*, where *claudin-5* becomes dominant, and the absence of several highly abundant *claudins* might also account for the relatively low tightness of *in-vitro* BBB models.[96]

Single-cell RNA sequencing has provided the opportunity to investigate the transcriptional profiles of individual cells, and to thus categorize cells more accurately in terms of specific clusters. Two recent studies used single-cell RNA sequencing to better describe the BBB and the endothelium throughout the mouse body,[3–5] where with the exception of *claudin-5* the various claudins have shown discrepancies with older data. In the first studies,[4,5] four vascular and vessel-associated cell types of transgenic reporter mouse models were used. *Cldn5(BAC)-GFP* was used to define endothelial cells, *Pdgfrb (BAC)-EGFP;Cspg4-DsRed* to define mural cells, *Tagln-Cre;R26-stop-tdTomato* to define astrocytes, and *Pdgfra-H2BGFP* to define perivascular fibroblast-like cells. In total, 3,436 single-cell transcriptomes were analyzed from adult mice, of which around 1,500 were from endothelial cells, 1,000 from pericytes, and the remaining ~1,000 from other cells, such as vascular smooth muscle cells, astrocytes, oligodendrocytes, microglia and perivascular and meningeal fibroblast-like cells. Twenty-one sequences of *claudins* mRNA were detected, 13 of which were expressed in <10 individual cells, and were thus considered to be background noise. *Claudin-3* was detected in four cells, one of which was an endothelial cell. *Claudin-1* was detected in only a subpopulation of fibroblasts, *claudin-5* was specific to endothelial cells, *claudin-10* only to astrocytes, *claudin-11* to oligodendrocytes and fibroblasts, *claudin-14* only to a few oligodendrocytes, and *claudin-15* to a few

pericytes and smooth muscle cells, while *claudins-12* and −25 were ubiquitously expressed.

The second single-cell RNA sequencing study examined endothelial cell heterogeneity across 11 tissues, including the brain.[3] In the CD45⁻CD31⁺ brain endothelial cells were identified that were selected by sorting for only four different claudins, among which *claudins-1*, −12 and −34c1 were low, while *claudin-5* was 10-fold to 100-fold more expressed. While *claudins-1*, −5 and −12 have been reported to be expressed in brain endothelial cells, *claudin-34c1* has only been mentioned in this one study to date.

Due to intra-tissue endothelial cell heterogeneity and to differentiate putative functions in the vessels, endothelial cells have been divided into the following nine vessel-related phenotypes, based on marker gene expression analysis: artery; artery sheer stress; capillary; capillary arterial; capillary venous; choroid plexus; interferon; large artery; and large vein. *Claudins-1*, −12 and −34c1 showed similar expression levels across all of the phenotypes of endothelial cells, with the exception of the absence of *claudin-12* in large artery (Figure 4). *Claudin-5* showed similar expression for all of the endothelial cell phenotypes, apart from a small decrease for the artery sheer stress, and a 50% decrease in the choroid plexus (Figure 4).

For claudin-3 expression in brain endothelial cells, the data of Castro Dias et al. (2019) are in agreement with both of these single-cell sequencing studies. To clarify things for the debate, they generated mice with a total knockout of *claudin-3*. These mice showed an intact BBB, with no indications of claudin-3 mRNA and protein in the brain endothelial cells as assessed by RT-qPCR and Western blotting. They concluded that in previous claudin-3 immunofluorescence studies, the antibodies used had cross-reacted with other unknown antigen(s) that were also present in the *claudin-3* knockout mice.[97]

Claudins in the gut–vascular barrier

The main function of the gastrointestinal circulation is to deliver the required oxygen for the secretory, absorptive and motor activities of the intestine, with the 'return delivery' of CO_2 for its removal, and also of the absorbed nutrients and

Brain

Figure 4. Single-cell gene expression profiles of claudins in the brain. Violin plots displaying the normalized expression values of claudins (*Cldn*)-*1*, −*5*, −*12* and −*34c1* in the nine vessel-related phenotypes in the brain. Data were obtained from Kalucka, J. *et al*. (2020).

water for all of the organs of the body, using both the blood and the lymphatic vasculature. The intestines are divided into the small and large intestines. The small intestine is subdivided into the duodenum, jejunum and ileum (Figure 5). The large intestine starts with the cecum and continues with the colon, to end with the rectum and anus.[7] The blood supply to the intestines comes from different vessels, but all of these derive from the aorta, which is divided into the celiac trunk, the superior mesenteric artery, and the inferior mesenteric artery[98] (Figure 5). The arterial blood vessels enter the intestine via the surface of the serosa, which is in contact with the mesentery (the structure that gives support to the vasculature in the peritoneum). Then, these arterial blood vessels enter the villi, to

Figure 5. Vascular bed in the gastrointestinal tract. The small (duodenum, jejunum, ileum) and large (cecum, colon, rectum, anus) intestines are perfused by different vessels, although all of them derive from the aorta, which is divided into the celiac trunk, superior mesenteric artery and inferior mesenteric artery. Modified from.[7]

give rise to the branched network made of arterioles and venules, and the capillaries that penetrate the three main tissue layers of the mucosa, muscularis and submucosa[99] (Figure 1(d)). From the muscular layer, the first-order arterioles are in the submucosa, and the second-order arterioles derive from these, which end up as the third-order arterioles. These last penetrate into the mucosa and along to the tip of the villi, where they become capillaries and form a mesh-like structure.[100] In contrast, for the microvasculature of the colon, the branches of the arterioles and capillaries pass along the luminal surface of the mucosa and form a network around the glands, and are much closer to the epithelium than in the small intestine[101] (Figure 1(e)).

In 2015, Spadoni et al. described a novel anatomical structure in the murine and human intestines that they called the GVB.[9] The GVB consists of the blood capillaries located just below the intestinal epithelium (for review, see[102]). The GVB is composed of closely interacting intestinal vascular endothelial cells, pericytes and enteroglial cells (Figure 1e). Among the tight junctions here, claudin-5 is expressed primarily in lymphatic endothelial tight junctions. Claudin-12 has been associated with other cell types in the lamina propria.[35] Moreover, the transcriptional profile of CD45−CD31+CD105+LYVE1− blood endothelial cells purified from the small intestine of mice confirmed the expression of *claudin-12* and revealed the expression of the other claudins, such as *claudins-2*, −3, −4, −7, −15 and −23[8] (Figure 1(f)). While the expression of some of these proteins, such as claudin-12, has been validated by immunofluorescence, the expression of these other claudins needs to be further analyzed.[9]

Recently, Carmeliet and coworkers performed single-cell RNA sequencing of endothelial cells from 11 adult mouse tissues.[3] Among these, colon and small intestine endothelial cells showed high expression of gene sets involved in vascular basement membrane/collagen deposition, vascular
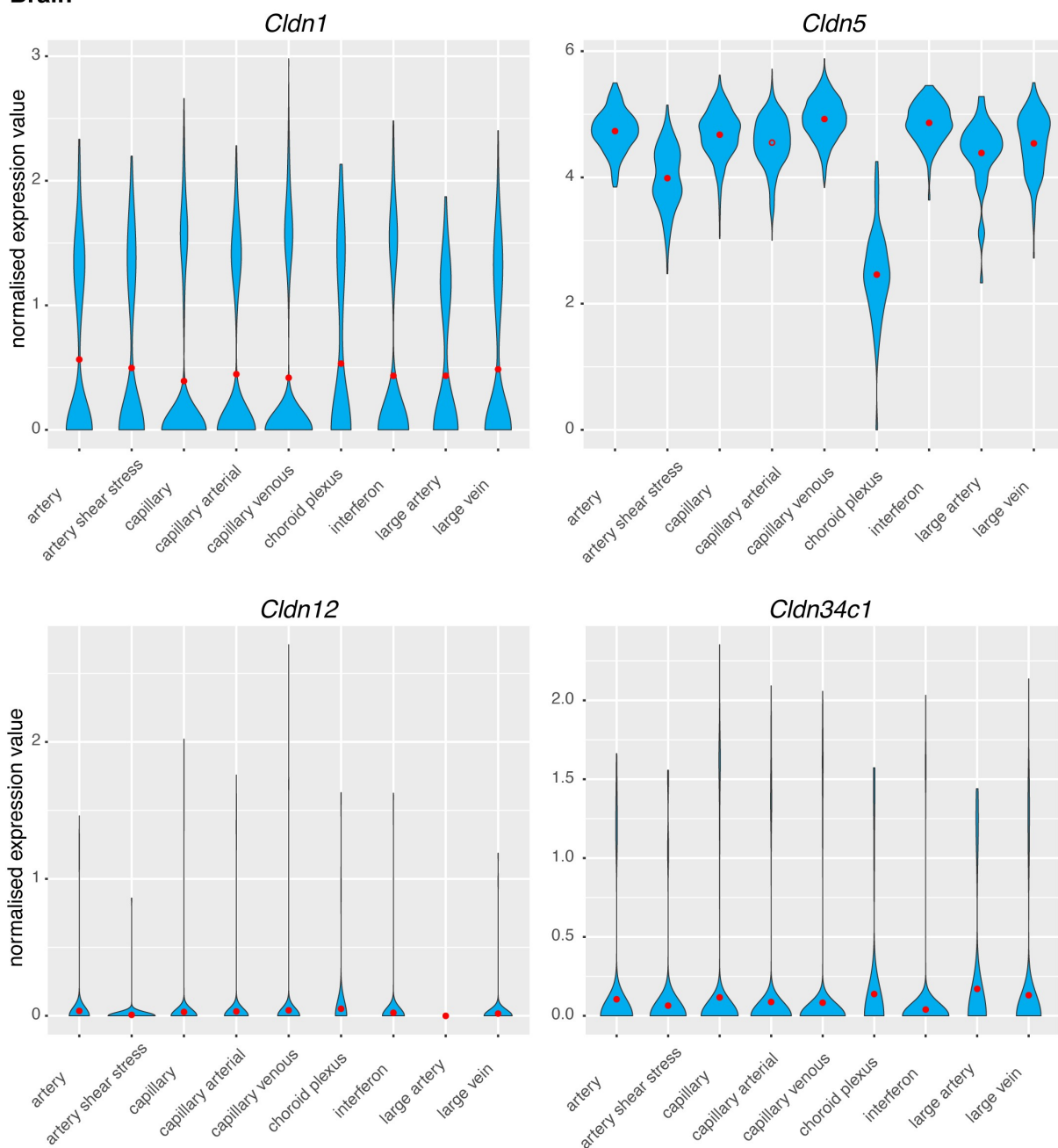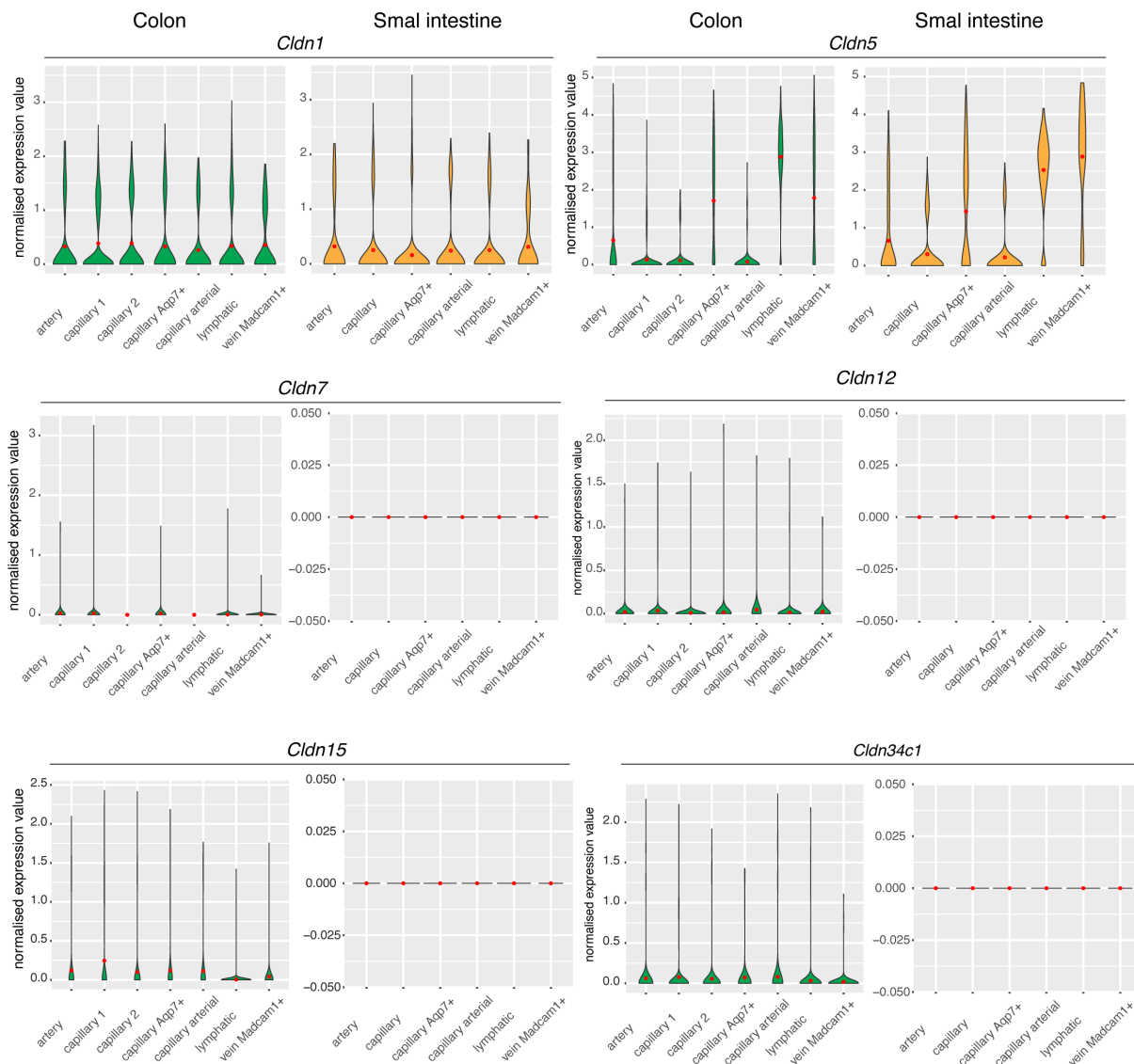
Figure 6. Single-cell gene expression profiles of claudins in the intestine. Violin plots displaying the normalized expression values of claudins *(Cldn)-1*, −5, −7, −12, −15 and −34c1 in seven different endothelial cell populations in the colon and small intestine. Data were obtained from Kalucka, J.*et al.* (2020).

endothelial growth factor signaling, endothelial cell migration, and vascular barrier integrity and maintenance,[103,104] in agreement with their known roles in the maintenance of the gut vascular barrier.[9] Based on marker-gene expression, the clustering of the different endothelial cell populations identified in the intestine showed the following phenotypes: arterial endothelial cells, with the large artery and artery endothelial cell subclusters; capillary endothelial cells, with all of the capillary endothelial cell subclusters and capillary–arterial endothelial cells; lymphatic endothelial cells, with all of lymphatic endothelial cell subclusters; and

endothelial cells restricted to only some tissues (Madcam1+ veins, Aqp7+ capillaries) (Figure 6).

Here, *claudin-5* was widely expressed in the distinct vascular beds of both the colon and the small intestine. Specifically, both of these showed high transcript levels of *claudin-5* mainly in the lymphatic endothelial cells. Moreover, *claudin-5* was expressed in venous endothelial cells that showed enriched expression of endothelial venule markers (*Madcam, Lrg1, Ackr1*) as defined as Madcam1+ veins.[105,106] These represent a specialized subtype of endothelial cells that are adapted for recruiting/trafficking of

lymphocytes from the blood to the tissue interstitium[107] (Figure 6). A remarkable level of claudin-5 transcript was detected in capillary endothelial cells with elevated transcript levels of genes involved in the uptake and metabolism of glycerol and fatty acids (*Aqp7, Tcf15, Cd36, Fabp5*). These are defined as Aqp7+ capillaries, and they might be involved in the removal of glycerol from enterocytes into the portal system, to bypass the lacteal system (Figure 6). Arteries, capillaries and arterial capillaries also expressed detectable mRNA levels of *claudin-5*, which suggested an essential role of claudin-5 in the maintenance of the structural integrity of the gut vasculature and the control of the diffusion of molecules through the intercellular space.

Although *claudin-1* was expressed at similar levels in the distinct vascular beds of both the colon and small intestine, *claudin-12* was revealed only in the colon, which appears to reflect a function in ion transport rather than in endothelium sealing[35] (Figure 6). Interestingly, the *claudins-7*, −15 and −34c1 transcripts showed very little, if any, expression in the colon vasculature, but not in the small intestine vascular bed, in agreement with the roles of these claudins as channels that strictly control the paracellular movement of solutes[63] (Figure 6).

Claudin-related diseases of the central nervous system

The main property of the intact BBB is its controlled permeability through the barrier itself for molecules, cells and microorganisms, via the paracellular and transcellular routes. Reactive oxygen species, metalloproteases (MMPs), angiogenic factors, pathogens and immune cell activation and infiltration can cause impairment of the paracellular and transcellular routes, and consequently, CNS damage. This can lead to the development of CNS diseases or their exacerbation, depending on the occurrence of the disease relative to the BBB impairment.[108]

Here, we summarize the involvement of claudins in several diseases in which claudins dysregulation and mislocalisation increase the permeability of the BBB to peptides, proteins and immune cells, which can lead to, or exacerbate, diseases. These diseases are: Alzheimer's disease (AD), ischemic stroke, multiple sclerosis (MS) and schizophrenia.

Alzheimer's disease

Alzheimer's disease is a chronic neurodegenerative disease that causes severe dementia that is characterized by memory loss, impaired reasoning, and personality alterations. The pathological hallmarks of this disease include the formation of amyloid beta (Aβ) plaques in the brain parenchyma and around blood vessels and neurofibrillary tangles in the neurons, which are composed of hyperphosphorylated tau proteins.[109,110] Histological analysis of postmortem human AD brains has shown BBB breakdown as defined by plasma albumin and immunoglobulins around amyloid plaques.[111] In 2019, Nation and colleagues presented evidence of BBB impairment by measuring soluble platelet-derived growth factor receptor-β (sPDGFRβ) as a biomarker of damaged capillaries in patients with AD. However, they showed the circulation of sPDGFRβ by cerebrospinal fluid independent of amyloid plaques and tau status by positron emission tomography, which suggested vascular dysfunction as a component of AD pathology.[112] Aβ toxicity and neuroinflammation have been associated with down-regulation of tight junction proteins in patients with AD who suffer from cerebral amyloid angiopathy at the capillary level. Two studies by Carrano and colleagues showed that in postmortem brains of patients with cerebral amyloid angiopathy, at the capillary level there was a remarkable reduction, or even complete loss, of claudin-5, among the other tight junction proteins. The capillaries were positive for Aβ plaques or were associated with activated microglia that were positive for nicotinamide adenine dinucleotide phosphate oxidase 2, an enzyme that is responsible for reactive oxygen species production.[113,114] Although Viggars et al. correlated increased levels of albumin and fibrinogen to more progressed AD pathology in human postmortem brains, the expression levels of claudin-5, occludin and ZO-1 were not affected.[115] Nevertheless, BBB impairment is well accepted as a pathological characteristic of human AD pathology.[112]

Table 1. Expression of claudins in the blood-brain and gut-vascular barriers under both physiological and pathological conditions.

Claudin type	Expression (Blood-brain barrier)	Alteration	Reference	Expression (Gut-vascular barrier)	Alteration	Reference
Claudin-1	Protein detected in endothelial cells of adult rat and chicken in vitro and in humans	Decreased when in vitro, and in human AD and AD rat model	63, 100	Protein detected in rat intestinal microvascular endothelial cells	Decreased expression upon TNF-α treatment	137
	Protein detected in human ECV cells in vitro	Increased by glioma-conditioned media	66			
	mRNA and protein detected in endothelial cells in mouse and human	Decreased mRNA in MCAO mice	73, 99			
	mRNA and protein detected in endothelial cells in mouse and human	Increased mRNA and protein in MCAO mice and in human stroke	15, 113			
Claudin-2	No	Unknown		mRNA detected in small intestine and colon vascular bed of mice	Unknown	15, 84
Claudin-3	mRNA and protein in endothelial cells in mouse	Decreased mRNA in MCAO mice and lost in EAE mice	72, 73, 112	Protein detected in rat intestinal microvascular endothelial cells	Increased expression upon TNF-α treatment	137
	Protein detected in ECV cells in vitro	Increased by glioma-conditioned media	66	mRNA detected in small intestine vascular bed of mice	Unknown	84
	mRNA and protein detected in endothelial cells of adult mouse and in vitro	Increased by Wnt3a	63			
Claudin-4	No	Unknown		mRNA detected in small intestine and colon vascular bed of mice	Unknown	84
				Protein detected in human jejunal samples	Increased expression in patient with chronic intestinal pseudo-obstruction	135
Claudin-5	Protein detected in endothelial cells of adult rat, chicken and human	Decreased when in vitro, in human AD and schizofrenia	63, 95, 96, 100, 123, 128, 129	mRNA detected in small intestine and colon vascular bed of mice	Unknown	15, 84
	Protein detected in endothelial cells in adult rat	Decrease in hypoxic, in MCAO, and in EAE rats	108, 111, 121			
	mRNA and protein detected in endothelial cells in mouse and human	Increased mRNA and protein in MCAO mice and in human stroke	15, 68, 73, 75, 76, 113			
	Protein detected in endothelial cells of mouse embryo (E12.5), (18.5) and in adult	Unknown	62, 59			
	Protein detected in endothelial cells of adult pig in vitro	Increased upon co-culture with rat astrocytes in contact	65			
	Protein detected in ECV cells in vitro	Increased by glioma-conditioned media	66			
	Protein detected in endothelial cells of adult mouse	Decreased in AD, AD/Glut-1-knockout mouse model, EAE mouse model	67, 99, 100, 119, 120			
Claudin-7	No	Unknown		mRNA detected in small intestine and colon vascular bed of mice	Unknown	15, 84
Claudin-8	mRNA detected in endothelial cells of adult mouse	Unknown	64	No	Unknown	
Claudin-10	mRNA detected in endothelial cells of adult mouse	Unknown	64, 75, 76	No	Unknown	
Claudin-11	mRNA and protein detected in endothelial cells in mouse and human	Decreased in EAE mice and MS patients	122, 73	No	Unknown	

Table 1. (Continued).

Claudin type	Blood-brain barrier — Expression	Blood-brain barrier — Alteration	Reference	Gut-vascular barrier — Expression	Gut-vascular barrier — Alteration	Reference
Claudin-12	mRNA and protein detected in endothelial cells in mouse and human	Decreased in MCAO mice	15, 73, 75, 76, 112, 123	mRNA detected in colon vascular bed of mice	Unknown	15
	Protein detected in endothelial cells of mouse embryo (E18.5)	Unknown	59	Protein detected in rat intestinal microvascular endothelial cells	Decreased expression upon lipopolysaccharide treatment	138
Claudin-15	mRNA detected in endothelial cells of adult mouse	Unknown	64, 75, 76	mRNA detected in small intestine and colon vascular bed of mice	Unknown	15, 84
Claudin-17	mRNA detected in endothelial cells of adult mouse	Unknown	64	No	Unknown	
Claudin-19	mRNA detected in endothelial cells of adult mouse	Unknown	64	No	Unknown	
Claudin-20	mRNA detected in endothelial cells of adult mouse	Unknown	64	No	Unknown	
Claudin-22	mRNA detected in endothelial cells of adult mouse	Unknown	64	No	Unknown	
Claudin-23	mRNA detected in endothelial cells of adult mouse	Unknown	64	mRNA detected in small intestine vascular bed of mice	Unknown	84
Claudin-25	mRNA detected in endothelial cells of adult mouse and human	Unknown	73, 75, 76	No	Unknown	
Claudin-27	mRNA and protein detected in endothelial cells in human	Unknown	73	No	Unknown	
Claudin-34c1	mRNA detected in endothelial cells of adult mouse	Unknown	15	mRNA detected in colon vascular bed of mice	Unknown	15

Studies in animal models of AD have also shown contradictory data regarding BBB breakdown and claudins modulation, although this is due to the clinical manifestation of AD in the different animal models. In the Tg2576 AD mouse model, multiple studies have shown increased levels of albumin, low molecular weight tracers, IgG and fibrin in the brain, which were accompanied by decreased levels of tight junction proteins. This model shows over-expression of the human amyloid precursor protein (hAPP) with the Swedish mutation (K670N, M671).[116,117] Specifically, Winkler et al. described how deficiency of glucose transporter 1 (Glut-1) affects BBB integrity in humans with a family history of AD. They generated the double transgene AD/Glut-1–deficient mice and showed that at 6 months old, these showed significant down-regulation of tight junction claudin-5 and occludin, and increased plasma proteins in the brain parenchyma, including fibrin and IgG, compared to their control littermates.[117] In the same context, Hartz and colleagues described the toxic effects of Aβ on the BBB *in vivo* and *in vitro* using mice overexpressing hAPP (i.e., the Tg2576 mouse model). Isolated microvessels from these WT and hAPP mice showed down-regulation of the tight junction proteins claudin-1 (by 84%) and claudin-5 (by 43%), while the MMP-2 and MMP-9 proteins were up-regulated by 90% and 145%, respectively. Cultured brain endothelial cells from these hAPP mice showed a 1.5-fold increase in permeability to Texas red dye (641 Da), compared to WT endothelial cells. Finally, they showed a positive correlation for increasing concentrations of human Aβ isoform 1–40 (Aβ1-40) and greater loss of claudin-1 and claudin-5 in the WT mouse endothelial cells *in - vitro*.[118] Counter-intuitively, Keaney et al. suggested targeting claudin-5 and occludin for drug therapies in AD after showing a greater ratio of plasma:brain Aβ1-40 levels in the Tg2576 mice treated with siRNAs against claudin-5 and occludin, which thus revealed Aβ1-40 flux from brain to blood. Moreover, the systematic administration of claudin-5 and occludin siRNAs every 21 d over 9 months in Tg2576 mice improved their cognitive function as measured by T-maze assessment.[119] In contrast to studies on Tg2576 mice, experiments with AD mouse models (including SP2-APP, human Tau P301L, P301S) and mice lacking

apolipoprotein E or expressing the human protein have shown no claudin-5 decrease or BBB impairment as assessed by tracer leakage (range, 86 Da to 150 kDa).[120]

Ischemic stroke

Following myocardial infarction, strokes are the second leading cause of death in humans worldwide, with ischemic stroke occurring more often than hemorrhagic stroke. The obstruction of a brain vessel by a forming or circulating clot leads to ischemic stroke.[121] Nutrient and oxygen deprivation cause rapid neuronal cell death in the area where the blood is not flowing. Late reperfusion and neuronal cell death can cause a cascade of adverse effects, including activation of glial cells and endothelial cells, tight junction dismantling, and disruption of the BBB due to the production of reactive oxygen species, MMPs, growth factors, cytokines and chemokines. These effects induce initial local inflammation and then immune cell infiltration into the brain parenchyma, which can later lead to further inflammation outspread and brain damage.[122] While some studies have proposed the increased BBB permeability in stroke to be an effect of increased vesicular trafficking or nonselective channels,[123–125] other studies have attributed this to tight junction protein expression and localization. The effects on expression and localization of tight junction proteins of blood reperfusion or reoxygenation over short and long periods of time have been widely described in animal models. Willis et al. (2010) studied the effects of hypoxia (a major component of stroke) and reoxygenation on tight junctions in a rat model of global hypoxia. When the rats were exposed to 6% oxygen for 30 min to 60 min they showed increased vascular permeability to 4 kDa and 10 kDa dyes and to endogenous IgG, with a more severe phenotype after 60 min, compared to normoxia-exposed rats. Moreover, claudin-5 staining showed a diffuse pattern, with decreased intensity in the microvessels of the hypoxic rats. Reoxygenation for 15 min increased claudin-5 localization to the tight junctions and its expression but not in the control rats. This study also provided evidence of increased expression and activation of the protein kinase Cθ and ζ isoforms, which disrupted the tight junctions.[126] In contrast, Bauer and colleagues exposed mice to 8% oxygen for 48 h

and reported decreased levels of occludin but not claudin-5 or ZO-1, in total brain lysates. Moreover, immunolabeling of the brains from these mice showed discontinuous and diffuse staining of ZO-1 and occludin, but not of claudin-5. They thus suggested that inhibition of vascular endothelial growth factor and MMP9 might reverse tight junction disassembly.[127] These studies by Willis et al. and Bauer et al. demonstrated that expression and localization of hypoxia-mediated tight junction proteins can vary according to the time under hypoxia and the mechanisms that are activated.

Moving to more representative rat stroke models, where middle cerebral artery occlusion (MCAO) has been applied, Jiao and colleagues demonstrated time-dependent changes in tight junctions, which included claudin-5.[128] They showed that in isolated brain microvessels, the highest mRNA and protein decreases for claudin-5 were seen in rats following 2 h MCAO and 3 h and 72 h of reperfusion, when the infarct sizes were mostly increased. Similarly, in a permanent MCAO rat model, it was shown that after 8 h and 120 h of reperfusion, the increased Evans blue extravasation was synchronized with decreased expression of claudin-5 and occludin in the ischemic penumbra brain tissues, thus demonstrating the biphasic mode of tight junction expression.[129] Although the earlier studies focused on claudin-5, in the recent study of Winkler et al. (2021), they showed that following 60 min of MCAO and 3 h reperfusion, claudin-1 and claudin-12 were decreased in claudin-3 knockout mice. The WT mice treated similarly showed claudin-1, claudin-3 and claudin-12 down-regulation.[95] Interestingly, in WT mice, 48 h of reperfusion after MCAO induced enlarged infarct volume and edema, but conserved tight junction formation, whereas in the claudin-3 knockout mice, the junctional localization of claudin-5 and the edema and infarction volume were reduced. They thus suggested a prophylactic role on tight junctions for claudin-3, along with correlation to a worse stroke phenotype.[130] A negative effect of claudin-1 in BBB recovery was demonstrated by Sladojevic and coworkers in post-stroke microvessels of human and mouse origin.[131] They showed that the rarely present claudin-1 in the brain capillaries was up-regulated after stroke at the mRNA and protein levels and was incorporated into the tight junctions, while claudin-5 was down-regulated. This claudin-1 competed with claudin-5 for the ZO-1 interaction, which led to BBB leakiness and decreased BBB recovery. Inhibition of claudin-1 returned the BBB permeability, and as a consequence, improved neurological recovery.[131]

Multiple sclerosis

Multiple sclerosis is a chronic autoimmune CNS disorder that is characterized by progressive loss of neuronal myelin, with inflammation, scar tissue and lesions in the brain and the spinal cord.[132] As confirmed by gadolinium-diethylenetriamine penta-acetic acid enhancement, BBB impairment can precede MS lesion development and the pathological symptoms of this disease.[133] In humans, MS lesions, serum protein leakage, and tight junction abnormalities have been shown in brain endothelial cells.[134–136] Although imperfect, the experimental autoimmune encephalomyelitis (EAE) mouse model has been used to study immune cell extravasation and MS pathology in humans. Early immunolabeling studies on EAE mouse brains showed loss of claudin-3, but not claudin-5, in tight junctions of venules surrounded by inflammatory cuffs.[94] In contrast, Errede et al. (2012) showed that mice with mild EAE have linear, but discontinuous, claudin-5 localization, while mice with severe EAE lose claudin-5 linearity in the microvessels close to or far from demyelinated neurons.[137] In addition, Wang et al. (2016) reported decreased claudin-5 protein levels in whole EAE mouse brain tissue lysates, and increased Evans blue leakage.[138] Treatment of these mice with resveratrol, an anti-inflammatory and antioxidative with neuroprotective properties, ameliorated the EAE-induced loss of claudin-5, and decreased Evans blue extravasation. Similar data to these resveratrol effects on claudin-5 and Evans blue were shown for a Kv1.3 (voltage-gated K$^+$ channel) blocker in EAE rats.[139] Finally, in 2019, Uchida et al. analyzed the involvement of claudin-11 in the disruption of the BBB in MS using the brains of postmortem human MS patients and EAE mice. They showed significant down-regulation of claudin-11 in the microcapillaries in the brain and spinal cord from both the humans and mice. Moreover, claudin-11 knockdown *in vitro* in human brain capillary

endothelial cells increased the monolayer permeability to fluorescein isothiocyanate–dextran.[140] Discrepancies in the expression of claudins or other tight junction proteins in these studies might be due to differences in the EAE models (e.g., disease progression) and brain areas analyzed.

Schizophrenia

Schizophrenia is a chronic and severe mental disorder that affects over 20 million people worldwide. Common symptoms include distortions in thinking, perception, emotions, language and self-awareness. Several studies have shown that BBB-associated tight junction disruption is a hallmark of schizophrenia.[141] Indeed, transcriptomic studies of the prefrontal cortex have revealed that 12 out of 21 tight-junction-related genes are down-regulated in schizophrenia patients compared to controls.[142] Likewise, genetic studies have shown that individuals with the chromosomal abnormality 22q11 deletion syndrome (22q11DS) have a 30-fold increased lifetime risk of developing schizophrenia, due to the microdeletions in the chromosomal region 22q11.21.[143–145]

Claudin-5 is located within this deleted region in 22q11DS, which means that these individuals are haplo-insufficient for *claudin-5*, and therefore they only produce 50% of the claudin-5 compared to the normal population. Campbell and colleagues showed that there is a significant association between the rs10314 allele and a diagnosis of schizophrenia in 22q11DS patients, which suggested that these individuals are producing only about 25% as much *claudin-5* compared to the normal population, and therefore they might have greater BBB permeability.[146] Loss of claudin-5 in the hippocampus and medial frontal cortex is associated with schizophrenia-related behaviors in rodents, where treatments with antipsychotic drugs directly increase claudin-5 expression.[146] Similarly, in postmortem human brain tissue from schizophrenia patients, the levels of claudin-5 are decreased in the vessels of the hippocampus, while the mRNA and protein levels of claudins-5 and −12 and ZO-1 are associated with the age of onset and duration of schizophrenia.[141] In addition, in postmortem specimens from individuals with schizophrenia, claudin-5 levels were decreased in the frontal cortex, and this reduction was associated with protein kinase A signaling.[147]

Finally, deficit schizophrenia is characterized by a breakdown of both the tight junctions and adherens junctions of the paracellular pathways in the gut and BBB. It has been shown that the IgA response to paracellular proteins, E-cadherin, claudin-5 and β-catenin were significantly increased and positively associated with deficit schizophrenia *versus* non-deficit schizophrenia and controls.[148]

Diseases related to the gut–vascular barrier

The maintenance of GVB is fundamental to the control of translocation of antigens into the bloodstream and protection from entry of the microbiota. Independent of the intestinal epithelial barrier, endothelial barrier modifications can lead to some pathological conditions, such as inflammatory bowel disease,[149] irritable bowel syndrome, deficit schizophrenia,[148] and liver disease.[150,151] Altered endothelial barrier function has often been linked to pathogens and immune activation, and is closely associated with changes in tight junctions, such as for ZO-1 and occludin. Although the vascular contribution of claudins in these diseases is currently poorly understood, the regulation of claudin expression in the intestinal epithelial barrier also suggests a role for claudins in intestinal diseases (for review, see[42]). It has been shown that in patients with chronic intestinal pseudo-obstruction, the gut epithelial and vascular barriers are impaired due to the loss of the tight junction proteins to form oligomers, and the up-regulation of claudin-4, which in turn induces experimental glial cell activation.[152] Liu and colleagues established an *in-vitro* intestinal endothelial barrier model composed of rat intestinal microvascular endothelial cells, and they exposed this model to TNF-α, a cytokine that is involved in many intestinal diseases and that can induce altered barrier function and increased vascular permeability. TNF-αdecreased the expression of the tight junction proteins, including ZO-1, occludin and claudin-1, and increased claudin-2 expression. Claudin-2 is a mediator of the leaky gut barrier during intestinal inflammation,[153] and has been

reported to be up-regulated in the progression of inflammatory bowel disease.[149] This will contribute to the regulation of intestinal inter-endothelial tight junctions and the maintenance of the endothelial barrier during inflammatory stimulation.

Naringin is known for its anti-inflammatory activities in experimental ulcerative colitis and intestinal tumors,[154] and it has been shown to increase the expression of claudin-1 and reduce that of claudin-2 in TNF-α–challenged rat intestinal microvascular endothelial cells. On this basis, Liu and colleagues proposed the application of naringin as a potential agent in intestinal disorders. In addition, rat intestinal microvascular endothelial cells exposed to lipopolysaccharide (as a model of sepsis) not only dramatically increased endothelial permeability to fluorescein isothiocyanate–dextran but also decreased trans-endothelial electrical resistance and showed reduced expression of claudin-12, β-catenin and VE-cadherin, compared to control cells. These effects of lipopolysaccharide were antagonized by berberine, which rescued the expression of claudin-12[155].

In addition, and regardless of the involvement of claudins, GVB impairment has been detected in patients with celiac disease,[9] ankylosing spondylitis[156] and colorectal cancer with liver metastasis,[157] and in mouse models of cirrhosis[150] and nonalcoholic steatohepatitis.[151–158] These complex diseases involve the intestine and the liver, and they have been associated with increased vascular permeability, and linked to increased expression of plasmalemma vesicle-associated proteins in intestinal vessels.

various junctions of diverse tissues. Indeed, endothelial barriers can be more or less stringent, depending on the organ that they protect. The well-studied BBB defends the brain from the entry of serum proteins, inflammatory cells, pathogens and other substances, to thus maintain CNS homeostasis. Less stringent barriers, such as the GVB, still help to avoid dissemination of unwanted molecules throughout the organism.

In this review, we have described the claudin composition in the vasculature of the BBB and GVB. While for the BBB the roles of the different claudins and their regulation in homeostatic and pathological scenarios have been widely reported, detailed evidence is missing for the recently identified GVB. The overall information is summarized in Table 1.

Claudins-1 and −12 are expressed at similar levels in the brain and gut endothelium. Conversely, claudin-5 is highly enriched in the vascular bed of the brain, compared to the intestine, which thus suggests that claudin-5 might be critical in the regulation of the stringency of both of these barriers. Regardless of the expression levels of the different claudins, their distinctive polymerization through heteromeric and heterotypic claudin–claudin interactions might explain the unique endothelial barrier properties of both brain and gut. Further studies are needed to explore the involvement of claudins in the mechanisms that control vascular barriers.

In conclusion, as claudins have an essential role in vascular homeostasis and disease, targeting claudin-related signaling pathways might provide new therapeutic strategies for the treatment of a broad spectrum of human diseases in the near future.

Conclusion and perspectives

The composition of the tight junction components closely correlates with barrier function. Claudins are key components of tight junctions that regulate paracellular permeability. Although much effort has been devoted to an understanding of the structural and functional aspects, and the regulation and physiology of the different members of the claudin family, the question as to how claudins regulate the selective permeability has not been answered yet. Some claudins serve as barriers, while others serve as pores in the epithelium, thus generating the heterogeneity observed in the tightness of the

Notes on contributor

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Conflicts of interest

The authors declare that they have no potential conflicts of interest to disclose.

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