Neurodevelopment, brain vasculature and schizophrenia

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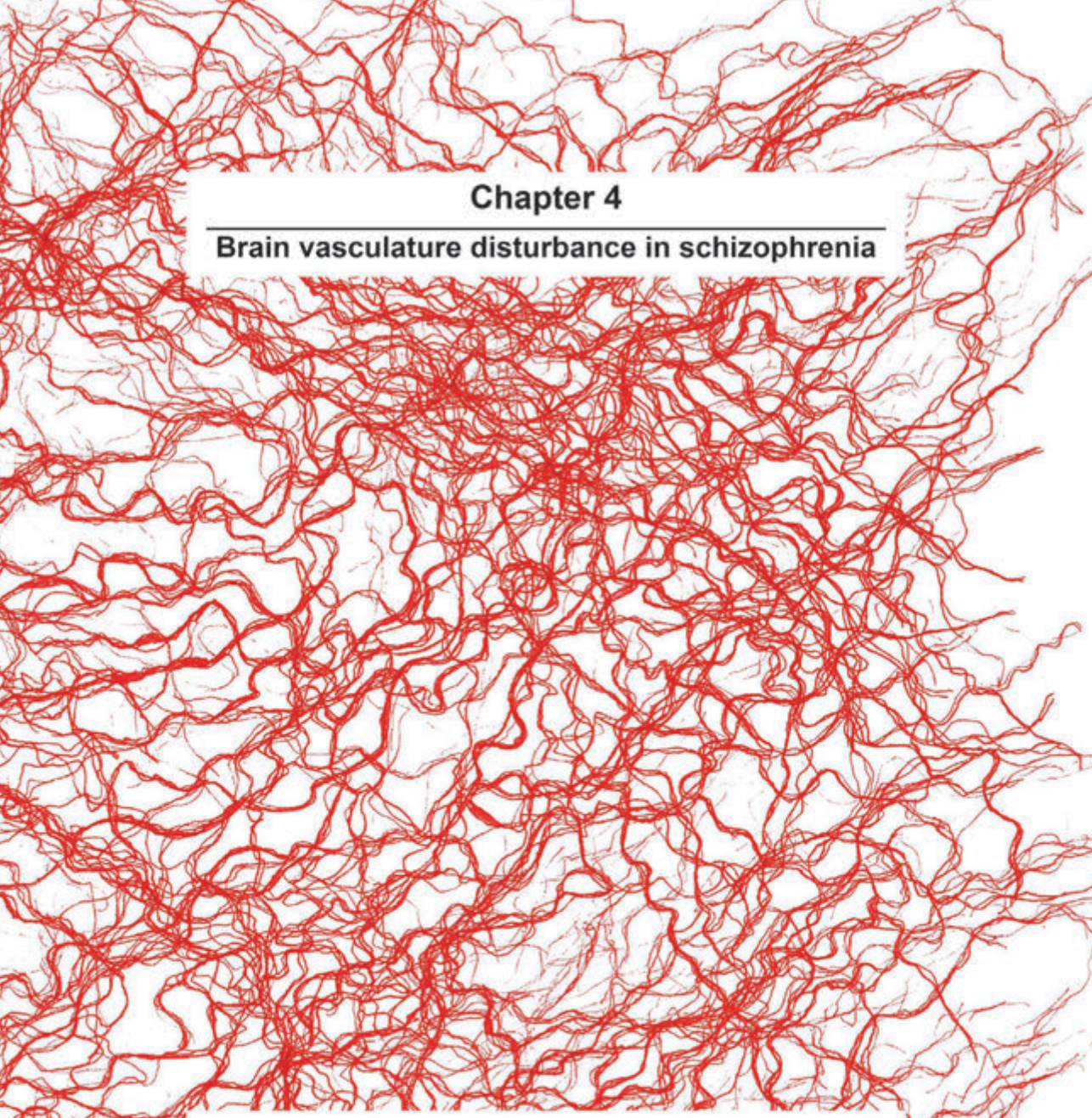
DOI:
10.33612/diss.582204366

Chapter 4

Brain vasculature disturbance in schizophrenia



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Current opinion in psychiatry, 2022

Abstract

Purpose of review The vascular hypothesis of schizophrenia postulates that brain endothelial dysfunction contributes to brain pathophysiology. This review discusses recent evidence for and against this hypothesis, including data related to blood–brain barrier (BBB), brain endothelium, and brain blood supply, to provide a critical weighed update. **Recent findings** Different studies report a consistent proportion of schizophrenia patients showing increased BBB permeability, reflected by higher levels of albumin in the cerebral spinal fluid. Of note, this was not a result of antipsychotic medication. The high inflammatory profile observed in some schizophrenia patients is strongly associated with increased BBB permeability to circulating immune cells, and with more severe cognitive deficiencies. Also, sex was found to interact with BBB integrity and permeability in schizophrenia. The strongest independent genetic association with schizophrenia has been identified in FZD1, a hypoxia-response gene that is 600-fold higher expressed in early development endothelium as compared to adult brain endothelium. Regarding brain blood supply, there is evidence to suggest alterations in proper brain perfusion in schizophrenia. Nonetheless, ex-vivo experiments suggested that widely used antipsychotics favor vasoconstriction; thus, alterations in cerebral perfusion might be related to the patients' medication. **Summary** In some patients with schizophrenia, a vulnerable brain endothelium may be interacting with environmental stressors, such as inflammation or hypoxia, converging into a more severe schizophrenia symptomatology. Gene expression and performance of human brain endothelium could vary along with development and the establishment of the BBB; therefore, we encourage to investigate its possible contribution to schizophrenia considering this dynamic context.

Key Points

- A group of the patients with schizophrenia presents blood-brain barrier (BBB) leakage.
- A high inflammatory profile in schizophrenia is strongly associated with increased BBB permeability to circulating immune cells, and to severe cognitive deficiencies.
- A vulnerable brain endothelium to environmental insults, such as inflammation or hypoxia, may be present in some of the patients with schizophrenia.
- The contribution of the different brain cell types to schizophrenia pathophysiology should be studied along human development.

Introduction

Schizophrenia (schizophrenia) is a heterogeneous psychiatric disorder, affecting around 24 million people worldwide [1]. Although early death by suicide reduces life expectancy in schizophrenia patients, the largest reduction comes from cardiovascular morbidity [2], illustrating the importance of the vasculature for them. Genome-wide association studies (GWAS) evidenced a polygenic architecture underlying schizophrenia, with a few rare variants with large effects and hundreds of common genetic variants with small effect (reviewed in [3]). In addition, it has been described that genetic risk is better for predicting schizophrenia in the presence of early life complications [4]. Therefore, an adverse environment, such as intrauterine hypoxia or obstetric complications [5], amongst others, interacting with multiple susceptible genes could trigger the disease during adulthood [6]. Accordingly, the neurodevelopmental hypothesis of schizophrenia has been extended to a developmental risk factor model [7], and schizophrenia is considered a multifactorial disease.

The brain is a high-energy- and oxygen-demanding organ. To meet these needs, it is highly vascularized and this vascular network develops in synchrony with the nervous system [8]. The blood vessels of the brain are structured within a multicellular niche called the neurovascular unit (NVU), composed of brain endothelial cells (BECs), smooth muscle cells, fibroblasts, pericytes, astrocytes, neurons, and microglia [9]. NVU cells secrete growth factors with neurotrophic, neuromodulator, and angiogenic effects [10] such as Vascular Endothelial Growth Factor (VEGF), Brain-Derived Neurotrophic Factor (BDNF) [11] and Nerve Growth factor (NGF) [12], among many others. In conjunction with other cellular components of the NVU, the brain endothelium contributes to regulating local blood flow based on neuronal activity changes; a phenomenon known as neurovascular coupling [13, 14].

At the first stages of central nervous system (CNS) development, molecular signals from neural progenitor cells and neurons induce BECs to express a barrier phenotype. This blood–brain barrier (BBB) maintains a homeostatic CNS microenvironment and promotes neuronal activity by responding dynamically to the ongoing physiological demands [15]. Adherent (cadherins) and tight junction proteins (e.g., Claudins (CLDN) and Occludins (OCLN)) confer paracellular tightness between adjacent BECs, contributing to the barrier integrity and restricting the migration of cells and molecules across it [16]. ATP-driven transmembrane efflux pumps, such as ABC transporters,

clear toxic material by removing it from the brain. Fetal astrocytes are also involved in BBB formation, through retinoic acid signaling [17, 18], and contribute to the barrier maintenance throughout adulthood [13, 19, 20].

Alterations in BBB integrity due to down-regulation of tight or adherent junction proteins can trigger the loss of barrier function by enhancing general permeability (leakage) across brain endothelium. Some proteolytic enzymes, such as Matrix Metalloproteinase 9 (MMP9), can degrade blood vessels basement membrane and tight junction proteins. Therefore, MMP9 up-regulation can impair BBB integrity and increase its permeability [21, 22]. Elevated albumin in cerebrospinal fluid (CSF), the most abundant protein in blood, is an indicator of increased BBB permeability to macromolecules [23]. Also, increased blood levels of brain-derived neuronal growth factors, such as S100 Calcium-Binding Protein (S100B), are potential biomarkers of BBB leakage.

BECs activation by systemic inflammatory challenges increases BBB selective permeability to circulating immune cells, through the up-regulation of Vascular and Intercellular Cell Adhesion molecules (VCAM1 and ICAM1, respectively), P-selectin and E-selectin [24-26]. Based on the aforementioned, a BBB function more sensitive to environmental stressors due to intrinsic alterations in BECs performance in schizophrenia, could lead to neuroinflammation by allowing ingression of harmful material and infiltration of peripheral immune cells into the CNS. On the other hand, increased systemic inflammation may be a causal factor of increased BBB permeability in schizophrenia patients. To study endothelial intrinsic mechanisms underlying schizophrenia, human induced pluripotent stem cells (hiPSC)-derived BBB models have been employed [27, 28]. hiPSC are obtained from schizophrenia and control subjects' somatic cells and differentiated to brain endothelium. Thereby, hiPSC-derived BBB models account for the patients' whole genetic information [27, 29-32] and allow to assess the performance of BBB isolated from other factors that might be present in schizophrenia, such as inflammation and use of medication.

This review aims to summarize and discuss the latest findings related to impaired brain vascular function in schizophrenia. As the brain vasculature is involved in the functioning of the BBB and in the regulation of local brain perfusion, the present review is divided into these two main topics. Data on structural and functional alterations of the cerebral vasculature, and how these features could be interacting with other altered

phenotypes described in schizophrenia, such as inflammation, will be covered. We will focus on the most recent literature.

The blood–brain barrier in schizophrenia

The BBB mediates communication between the CNS and the periphery. Thus, its integrity and performance are crucial to maintain a proper brain environment. Recent data, obtained through different techniques, have suggested impairments in BBB integrity and increased permeability in schizophrenia. These will be detailed in the following paragraphs.

Evidence from peripheral and cerebrospinal fluid markers

A meta-analysis found increased blood levels of S100B and MMP9, and increased CSF albumin (24-2 studies; 1107–69 schizophrenia and 873–92 control subjects), suggesting BBB damage and increased barrier permeability in schizophrenia [33]. A subsequent study also reported increased CSF albumin, as compared to reference control levels, but only in 15% of the patients with schizophrenia (40 schizophrenia) [34].

Genetic variation in *CLDN5* has been weakly associated with schizophrenia in Chinese [35-37] and Iranian populations [38]. *CLDN5* codes for a tight junction protein crucial to maintain BECs paracellular tightness and thus BBB integrity [39]. On the contrary, Zonulin secretion enhances barrier permeability by reducing epithelial paracellular tightness [40, 41]. In 2021, decreased CLDN5 and increased zonulin serum levels were observed in schizophrenia (50 schizophrenia and 50 controls) [42].

Evidence from postmortem studies

Immunohistochemical staining of postmortem brain samples (15 schizophrenia and 15 control samples) revealed a significant CLDN5 decrease in the hippocampus of schizophrenia patients, whereas no difference was observed in the orbitofrontal cortex [43]. This might suggest that alterations in BBB integrity in schizophrenia could be regional rather than widely distributed throughout the brain.

Evidence from in-vitro experiments

Deletion at the 22q11.2 chromosomal region (22qDS) is a relatively common chromosomal anomaly, which is associated with a 20-fold increase in schizophrenia

risk [44]. Interestingly, *CLDN5* is located in this chromosomal region. In a matched-pair experimental design, hiPSC-derived BBB from 22qDS-schizophrenia patients (5 schizophrenia and 5 control cell lines) exhibited a disorganized CLDN5 expression pattern and higher expression of ICAM1, as compared to their matched controls. Furthermore, 22qDS-schizophrenia-derived endothelium showed impaired barrier function, with reduced transendothelial electrical resistance and increased permissiveness to monocyte transmigration [27]. These findings suggest intrinsic BECs dysfunction in 22qDS-schizophrenia patients.

SEP-363856 is a Serotonin 1A receptor (5-HT1A) agonist and has been recently proposed as a promising potential drug for schizophrenia treatment [45]. In 2021, Sugimoto et al. demonstrated that 5-HT1A is also expressed in human brain endothelium. Of note, serotonin signaling through 5-HT1A binding increased CLDN5 in cultured human BECs [46]. Thus, SEP-363856 beneficial effects may be partially related to BBB protection.

Inflammation and blood–brain barrier performance in schizophrenia

According to the neuroinflammatory hypothesis of schizophrenia, brain vasculature may be affected by chronic inflammation. Increased expression of pro-inflammatory genes in the brain is associated with schizophrenia [47], as has been described for the complement pathway (discussed in Woo et al., (2020) [48]). Peripheral blood levels of pro-inflammatory cytokines, such as IL-6, IL-1, TNF, and IFN are also often reported to be increased [49]. Different studies suggested an association between BBB permeability and the inflammatory status of the patients; these are described below.

Evidence from peripheral and cerebrospinal fluid markers

The major histocompatibility complex locus, a chromosomal region coding for hundreds of genes and including the complement C4, carries a strong genetic association with schizophrenia [50]. Increased plasma level of C4-anaphylatoxin (C4-ana), a fragment of C4 released upon its activation, was reported in schizophrenia (15 schizophrenia and 14 controls). The authors evidenced that C4-ana increases BBB permeability to macromolecules in primary cultures of BECs [51].

A group of first-episode schizophrenia patients presented slightly increased systemic inflammation (41.4%, 116/280 schizophrenia subjects), reflected by increased C-

reactive protein level in the blood, whereas signs of BBB leak were reported solely in 15.8% of the first-episode schizophrenia patients (49/310 schizophrenia), as reflected in higher CSF albumin [52]. In a cohort of 456 schizophrenia patients, only 7% showed signs of neuroinflammation, although 19% of the schizophrenia patients reported BBB leakage. Antipsychotic medication did not affect the albumin quotients [53]. Summarizing, BBB leakage seems to be present in a consistent proportion of schizophrenia cases, but its relationship with systemic or neuroinflammation is not yet clear.

Aside from its possible involvement in BBB leakage, systemic inflammation in schizophrenia could facilitate immune cells infiltration into the CNS through the up-regulation of cell adhesion molecules, such as ICAM1, VCAM1 and P-selectin, in BECs [24-26, 54]. The soluble forms of these adhesion molecules serve as indicators of endothelial activation [55]. In 2020, a meta-analysis reported increased blood levels of soluble P-selectin in schizophrenia (3 studies; 98 schizophrenia and 72 control subjects). However, no differences were observed in blood sICAM1 or sVCAM1, nor in the CSF, between schizophrenia and controls (2–12 studies; 56–833 schizophrenia and 50–941 control subjects). Worth mentioning, heterogeneity was high across all these meta-analytic results (> 80%, except for CSF sICAM1) [33]. Radu et al., (2020) reported lower sICAM1 and higher sVCAM1 plasma levels in first-episode schizophrenia patients (50 schizophrenia, 50 controls). sVCAM1 correlated positively with the severity of symptoms, suggesting an association between increased BBB permeability and worsening of the disease [56].

Plasminogen activator inhibitor-1 (PAI-1) is a pro-coagulant protein and its expression is enhanced in a pro-inflammatory environment [57], facilitating leukocyte migration across the endothelium [58]. Meixensberger et al., (2021) quantified CSF levels of endothelial cell adhesion molecules in schizophrenia (40) and unipolar depression patients (39), and reported increased sICAM1, sVCAM1, and PAI-1 in schizophrenia compared to the depressed patients. In schizophrenia, CSF sICAM1 was also higher than reference control level. Levels of these proteins were similar among first-episode and chronic schizophrenia patients, suggesting that alterations were not a consequence of a degenerative process, nor of cumulative antipsychotic use [34]. Elevated serum levels of PAI-1 and other pro-inflammatory cytokines were also reported in another study with large samples, and levels were even higher in

schizophrenia patients that had experienced Acute Ischemic Stroke (schizophrenia-AIS) (150 schizophrenia, 150 schizophrenia-AIS, and 150 controls) [59]. The latter indicates a gradually increased inflammatory profile in schizophrenia, exacerbated even further in schizophrenia-AIS, along with the same trend in BBB permeability to circulating immune cells.

A cohort of 78 schizophrenia patients and 73 controls was annotated into high and low inflammation groups based on circulating inflammatory cytokine transcripts. Plasma sICAM1 was elevated in the schizophrenia-high-inflammation group compared to the high-inflammation control group (number of high-inflamed subjects not reported). Leukocytes can also produce ICAM1; however, authors reported no differences in white blood cells *ICAM1* expression between diagnostic, nor between inflammatory subgroups. Thus, suggesting that the source of increased sICAM1 is from the endothelium. Of note, high sICAM1 was associated with worse verbal memory [60]. Klaus et al. also reported increased ICAM, together with an increase in pro-inflammatory cytokines in blood of schizophrenia patients (20 schizophrenia and 20 controls) [61].

Evidence from postmortem studies

In 2020, Purves-Tyson et al. grouped schizophrenia postmortem cases into ‘schizophrenia-high-inflammation’ and ‘schizophrenia-low-inflammation’, based on transcript measurements of inflammatory cytokines in brain tissue. Midbrain *ICAM* and the macrophage marker *CD163* expression was substantially increased in the schizophrenia-high-inflammation group, but no difference was observed in the schizophrenia-low-inflammation compared to controls (13 schizophrenia-high-inflammation, 15 schizophrenia-low-inflammation, 28 controls) [62]. The same scientific group also measured different brain endothelial transcripts in the dorsolateral prefrontal cortex (DLPFC). Considering the previously described ‘high’ and ‘low inflammation’ schizophrenia biotypes, the authors found altered expression of structural and functional BECs markers such as increased *CDH5* and *OCLN*, and decreased *ABCG2* in the DLPFC of the schizophrenia-high-inflammation group, as compared to controls (13 schizophrenia-high-inflammation, 21 schizophrenia-low-inflammation, 37 controls) [63]. In addition, schizophrenia-high-inflammation samples presented higher *ICAM1*, along with an increased number of $CD163^{+}$ macrophages [63]. Thus, neuroinflammation, endothelial dysfunction, increased BBB permeability

and more peripheral macrophages in cerebro are linked with the high inflammatory biotype in schizophrenia patients. Expression of *ICAM* and classic BBB genes in cultured BECs was independent of therapeutic doses of different antipsychotics [63].

SERPINA3 can exert antiangiogenic and anti-inflammatory effects on the endothelium [64]. Its expression was increased in postmortem cerebral cortex of schizophrenia subjects [65]. In 2020, high expression of *SERPINA3* in schizophrenia was replicated and was even higher in schizophrenia-high-inflammation cases (14 schizophrenia-high-inflammation, 23 schizophrenia-low-inflammation, and 33 low-inflammation controls). Immunostainings indicated that blood-vessel-associated astrocytes are the major source of SERPINA3 increment in the schizophrenia-high-inflammation group [66]. SERPINA3 upregulation in schizophrenia could be a compensatory response to chronic inflammation [65]. However, SERPINA3 inhibits the catalytic activity of leukocyte elastase [67], a protease that cleaves ICAM1 which in turn favors attachment of leukocytes to endothelium [68]. Hence, elevated SERPINA3 in blood-vessel-associated astrocytes could be cooperating with $CD163^{+}$ CNS infiltration in the schizophrenia-high-inflammation group [63].

Brain endothelial cells- and blood–brain barrier-related growth factors in schizophrenia

Growth factors secreted by the cellular components of the NVU modulate different aspects of BECs performance, including growth, proliferation, and BBB establishment [10]. Recent data about BECs-related growth factors in schizophrenia are described below.

Evidence from peripheral and cerebrospinal fluid markers

Monocyte Chemoattractant Protein-1 (MCP1) is produced by different cells of the NVU, including BECs. It is involved in monocyte migration across endothelium [69] and can modulate BBB integrity [70]. In 2020, a meta-analysis reported unaltered MCP1 blood levels among drug-naive first-episode schizophrenia (3 studies; 57 schizophrenia and 127 controls) [71]. In contrast, Klaus et al., (2021) found increased blood levels of MCP1 in schizophrenia patients (20 schizophrenia and 20 controls). Also, schizophrenia subjects with particularly high MCP1 presented reduced cognitive flexibility [61].

Glial Cell-derived Neurotrophic Factor (GDNF) is involved in BBB establishment [72, 73]. Amyloid precursor protein (APP) is highly expressed in murine endothelium during the first stages of embryogenesis [74] and *in-vitro* studies indicated a positive effect of APP on endothelial cells migration, proliferation [75] and nitric oxide production [76]. CSF measurements of 11 different growth factors associated with neuro-plasticity indicated lower levels of APP and GDNF in schizophrenia (94 schizophrenia and 118 controls) [77]. Of note, Human Umbilical Vein Endothelial Cells (HUVEC) lacking APP presented four times increase in permeability to FITC-dextran in the presence of an IL-1b stimulus compared to control HUVEC, suggesting a more sensitive endothelial barrier function to inflammation in the absence of APP [75].

NGF, VEGF, Insulin (INS), Insulin-like Growth Factor (IGF), Insulin-like Growth Factor 1 Receptor (IGF1R) and Insulin Receptor (INSR), are growth factors and receptors exerting powerful activities on BECs functioning. VEGF and NGF promote proliferation [12], whereas INS, IGF1R and INSR modulate nitric oxide production [78]. Interestingly, increased antibody reactivity for all these molecules was found in the CSF of schizophrenia patients (17 schizophrenia and 12 controls) [79]. Cakici et al. meta-analysis revealed decreased blood BDNF and NGF levels in drug-naive first-episode schizophrenia patients (24, 4 studies; 962, 145 schizophrenia and 1,193, 227 control subjects, respectively) [71]. Also, pro-inflammatory cytokines were increased in schizophrenia at disease onset [71], associating inflammation with more severe pathology. Accordingly, NGF decrease [71] may be related to the reported NGF reactivity [79], at least in the patients with an increased pro-inflammatory signature. On the other hand, in a small cohort of only drug-resistant schizophrenia patients, BDNF and VEGF blood levels were similar to control levels (31 schizophrenia and 19 controls) [80]. Two meta-analyses conducted in 2020 reported unaltered VEGF blood levels in schizophrenia as compared to controls (5, 17 studies; 117 drug-naive first-episode, 986 schizophrenia and 225, 438 controls, respectively) [71, 81]. However, the heterogeneity of the pooled result was 79% and 83.4% [71, 81], respectively. In contrast with these findings, a study published in 2020 and not included in either of the two mentioned meta-analyses reported increased blood VEGF in schizophrenia (79 schizophrenia and 60 controls) [82].

Evidence from postmortem studies

Based on the heterogeneity of causes and symptomatology in schizophrenia, Huang et al. (2020) applied a statistical methodology to identify genes with higher expression variance in schizophrenia (212 schizophrenia and 214 control postmortem samples). The authors describe a general over dispersion in gene expression among schizophrenia brains and identified *VEGFA* as the most differentially variable expressed gene in schizophrenia as compared to controls [83]. This finding allows reconciling different studies that point to such diverse results regarding the participation of VEGF in schizophrenia. Variation in *VEGF* expression among patients may be related to a differential role in distinct stages of the disease.

Astrocytes mediated blood–brain barrier dysfunction in schizophrenia

Astrocytes are involved in BBB formation [17, 18] and maintenance [13, 17-20]. Nonetheless, astrocytes contribute with pro-inflammatory signals in schizophrenia patients with neuroinflammation [66]. Data indicating involvement of astrocytes in BBB dysfunction in schizophrenia are described in the following lines.

Evidence from genetics

ALDH1A2 codes for aldehyde dehydrogenase that catalyzes a key step in retinoic acid synthesis by astrocytes, and retinoic acid is involved in the establishment of the BBB [17, 18] and may prevent inflammation-induced increase in BBB permeability [84]. Methylome-wide association studies (MWAS) investigate correlations between a particular trait and the methylation state across different sites throughout the genome [85]. Interestingly, one of the most variably methylated site associated with schizophrenia is located in the region of *ALDH1A2* (744 schizophrenia and 704 control subjects) [86].

The AQP4 is expressed in brain perivascular astrocytic processes. It is a water channel protein involved in brain homeostasis and in BBB development and function [87]. In 2020, three different SNPs in *AQP4* were associated with schizophrenia risk (100 schizophrenia and 100 control subjects) [88].

Sex differences in blood–brain barrier integrity, permeability and brain endothelial cells functioning in schizophrenia

Sex differences in prevalence and symptomatology are evident in schizophrenia [89]. There are sex differences in the immune system, as sex hormones have opposite effects in particular on Th cells, leading to stronger (adaptive) immune activation in females [90], which might be contributing to the sex differences observed in the development of schizophrenia. Additionally, recent data suggest interactions between sex and the performance of BECs in schizophrenia. These findings are detailed below.

Evidence from genetics

In 2021, a GWAS assessed interactions between sex and schizophrenia genetic risk (33,403 schizophrenia and 109,946 control subjects). The strongest gene-sex interaction for schizophrenia was found in a locus harboring the gene *MOCOS*, a gene predominantly expressed in BECs [91].

As mentioned previously, *CLDN5* is located at 22q11.2 chromosomal region and deletion of this region carries the largest genetic risk factor for schizophrenia [44]. The *CLDN5* rs10314 variant was associated with a 50% reduction in CLDN5 protein expression when assessed *in-vitro* [92]. Therefore, a genetic background combining 22qDS with *CLDN5* rs10314 is expected to converge in even stronger reductions of CLDN5 protein expression. Whole-genome sequencing of 490 subjects with 22qDS indicated an increase in schizophrenia frequency for females carrying rs10314. The association was absent in males carrying the variant [93]; thus, suggesting possible interactions between sex and protection of BBB function in males.

Evidence from postmortem studies

Angiogenesis Inhibitor 3 (BAI3) is involved in angiogenesis [94, 95]. A recent postmortem study evidenced an exclusive reduction of hippocampal *BAI3* expression in male schizophrenia patients (104 schizophrenia and 174 control samples) [96].

Evidence from mice models

As part of the renin-angiotensin system, angiotensinogen (AGT) is hydrolyzed into angiotensin I (ANG1), which is then converted into ANGII [97]. ANGII increases BBB permeability through its binding to AT1R, expressed in BECs [98, 99]. Increased serum AGT was reported in schizophrenia (111 schizophrenia and 109 controls) [100].

Vasconcelos et al. (2021), studied the effect of peri-pubertal administration of an AT1R blocker, candesartan, in a two-hit mice model of schizophrenia that combines a neonatal immune challenge with stressors during puberty. Low doses of candesartan prevented schizophrenia-like behavior in female and male mice. But only males induced with the two-hits had an increase in pro-inflammatory cytokines in the hippocampus that could be significantly reduced to control levels with the peri-pubertal administration of candesartan [101]. These results add novel evidence for differential sex contribution in schizophrenia neurobiology, with different pathways possibly contributing to BBB susceptibility. Moreover, the administration of an AT1R blocker could help to reduce schizophrenia risk, indirectly implying that protection of BBB could prevent stress-triggering schizophrenia.

Brain blood supply in schizophrenia

Besides the relevance of BECs to the BBB, they are also involved in the regulation of local brain blood supply. Astrocytes, pericytes, and BECs sense changes in neuronal activity and modulate the diameter of the vessels, modifying the blood flow according to the metabolic and oxygen demands resulting from increased brain activity [102]. Oxygen diffuses freely from the blood to the brain across the BBB, as does carbon dioxide in the opposite direction [103], and diverse glucose transporters expressed in BECs ensure a continuous delivery of glucose to the brain [104]. Therefore, under physiological conditions, regional cerebral blood flow (CBF) is firmly coupled to cerebral glucose metabolism, and to brain activity [105, 106]. Although the vasculature density, the morphology of blood vessels, and their ability to dilate in response to brain activity are variables that may modulate the strength of this coupling and thus brain blood supply [107]. In the following lines, recent studies intended to evaluate brain blood perfusion in schizophrenia will be detailed.

Evidence from different imaging techniques

Deviations in the brain vascular network structure may interfere with proper blood supply. Microtomographic images of the anterior cingulate cortex and the superior temporal gyrus showed strong correlations between neurite and capillaries curvatures in both schizophrenia and controls (4 schizophrenia and 4 control samples) [108], but higher neurite curvatures in the schizophrenia group [109]. Capillary diameters did not

differ between groups [108], whereas slices from schizophrenia patients showed thinner neurites [109], leading to a volumetric mismatch between neurons and vessels.

Schizophrenia patients showed a deviant hemodynamic profile during cognitive challenges compared to control subjects, with a delay in mean flow velocity evolution in the middle cerebral artery (30 schizophrenia and 15 controls). The delay was longer in patients with increased symptoms severity [110]. Frontal and temporal CBF variations have been associated with schizophrenia [111]. Three months of treatment with long-acting injectable aripiprazole in 51 patients with first-episode of schizophrenia significantly increased regional CBF at the temporal and frontal right side of the brain. These improvements were accompanied by an enhancement in patients' cognitive functioning, suggesting a correlation between cognitive performance and CBF in schizophrenia [112].

Positron emission tomography (PET) and Arterial spin labeling (ASL) are imaging techniques that allow evaluation of regional CBF and cerebral glucose metabolism, respectively. In a recent bimodal neuroimaging meta-analysis combining data from whole brain PET and ASL, authors analyzed 'coupled' and 'uncoupled' deviations of these two parameters in schizophrenia (557 schizophrenia and 584 healthy controls). Authors reported 'coupled' reductions and 'coupled' increases of CBF and cerebral glucose metabolism across different brain regions in schizophrenia respect to controls. Also, 'Uncoupled' changes were reported in the superior frontal gyrus and cerebellum of schizophrenia patients [113].

Evidence from animal experiments

Recent ex-vivo findings indicate inhibition of K^+ rectifier currents in coronary arterial smooth muscle cells by olanzapine [114], ziprasidone [115], and risperidone [116]; widely used antipsychotic treatments for schizophrenia. This leads to cell membrane depolarization and can promote vasoconstriction. Accordingly, brain perfusion in patients with schizophrenia might be influenced by their treatment.

Retinal vasculature in schizophrenia

The retina and optic nerve are derived from the diencephalon during embryonic development and are components of the CNS [117]. Correspondingly, assessment of the retinal vasculature may serve as a reliable window to approach the cerebral

vasculature of schizophrenia patients. Recent studies that assessed the density and the morphology of retinal blood vessels in schizophrenia are mentioned below.

Evidence from different imaging techniques

Fundus photography is a widely used method to obtain a two-dimensional representation of the retina and its vasculature [118]. Two studies using fundus photography indicated greater venular and narrower arterial diameters in schizophrenia compared to control retinas (98, 34 schizophrenia and 92, 45 controls, respectively) [119, 120]. Furthermore, retinal venular diameters correlated with lower performance on working memory tests, whereas retinal arterial diameters correlated with better performance [120]. Another study using fundus imaging reported greater vein diameters in schizophrenia, but also increased arterial diameters (39 schizophrenia and 32 controls) [121]. Korann, et al., (2021) combined fundus photography with brain magnetic resonance imaging to investigate the relationship between the diameter of retinal blood vessels and brain structure in schizophrenia (20 schizophrenia and 17 controls). They found a negative correlation between average vein diameters and mean cortical thickness; the correlation was absent in the control group [122]. These results relate the decreased cerebral cortex thickness in schizophrenia [123] with recent hypotheses implicating vasculature as an important factor in schizophrenia pathophysiology.

Optical coherence tomography angiography (OCTA) is a more modern optical method that allows volumetric mapping of the retina. Four studies conducted during 2020 and 2021 provided evidence for decreased density of retinal blood vessels in schizophrenia (22–39 patients and 15–37 control subjects), in at least one region of the retina (macular or peripapillary area) [124-127]. In contrast, one recent study found a higher density of blood vessels in the retina of schizophrenia patients (26 schizophrenia and 21 control subjects) [128].

A possible declined response to hypoxia in schizophrenia and the role of early development endothelium

When cells are faced with limited oxygen levels, hypoxia-induced signaling pathways are activated to overcome these adverse conditions. BECs are sensitive to hypoxia and are relevant contributors to these adaptation processes [129]. Early life complications, including pre- and peri-natal hypoxia, are risk factors for schizophrenia

(discussed in [130]), and become stronger predictors when co-occurring with a high genetic risk for this disease [4]. Accordingly, a diminished or altered hypoxia-induced signaling might be contributing to schizophrenia. Recent data related to hypoxia-induced response in schizophrenia are described below.

Evidence from genetics

Hypoxia inducible factors (HIFs) up-regulate transcriptional cascades that induce adaptative responses to protect the hypoxic tissue [131]. In 2020, an analysis was conducted to better understand the biological relevance of 145 schizophrenia-related loci [132], previously reported by a schizophrenia GWAS [133], and 33 of the schizophrenia-associated genes were identified as HIFs-related genes. Also, schizophrenia-mutation intolerant genes (112 genes) were enriched for three different gene sets related to hypoxia-response [132]. Comparisons of whole-genome transcriptomic data from mice embryonic periventricular endothelial cells (PVECs) and adult BECs showed that thousands of genes are differentially expressed. Early development brain endothelium indicated an increased sensitivity to hypoxia as compared to adult brain endothelium, reflected by the up-regulation of HIF1 target genes in PVECs [134]. Strikingly, the expression of WNT frizzled class receptor 1 (FZD1), an hypoxia-response gene [135-137] that carries the strongest independent genetic association with schizophrenia (429 schizophrenia and 255 control samples) [138], was 600-fold higher in the early development brain endothelium. Taken together, these results suggest an altered response to hypoxia in schizophrenia, with a contribution from BECs possibly during early development.

Conclusion

This review summarized many recent findings reflecting structure and function of the brain vasculature in schizophrenia in general and that of the BBB in particular. Some findings were only reported in one or two small samples. Meta-analyses showed high heterogeneity among included studies. This is a clear indication that this field is still in its infancy and few conclusions can be drawn at this stage. Nevertheless, some findings derived either from large studies or replicated are discussed below.

A meta-analysis indicated BBB leak in schizophrenia, reflected by higher levels of CSF albumin [33]. Three subsequent studies corroborated this result and indicated BBB leakage across a relatively similar percentage of the patients (15.8% [52], 19% [53],

15% [34]). Reductions in the tight junction protein CLDN5 were reported in the hippocampus [43] and serum of the patients [42]. Also, aberrant CLDN5 expression pattern was described in 22qDS-schizophrenia-derived BECs [27]. Accordingly, BBB integrity may be disrupted in some of the patients with schizophrenia, causing BBB leakage. Additionally, the imbalances observed in BECs-related growth factors in schizophrenia [61, 71, 77, 79, 82, 83] might be translated into an altered BBB integrity and function after stressful events [75].

Multiple works indicate the presence of a schizophrenia group associated with a pro-inflammatory immune status and increased BBB permeability to peripheral immune cells, reflected in higher brain or blood levels of ICAM [60-62]. Furthermore, two postmortem studies demonstrated increased number of peripheral macrophages in the CNS of schizophrenia patients with high inflammatory profile [62, 63]. Whether inflammation in schizophrenia increases BBB permeability or if it is a cause of intrinsic endothelial dysfunction remains an open question.

No recent studies using PET to assess BBB function in schizophrenia have been reported. Clinical studies using novel hydrophilic radioactive tracers, unidirectionally transported from the blood to the brain and with low baseline brain uptake [139-141], would contribute with highly-sensitive and quantitative *in-vivo* measurements of BBB integrity in schizophrenia patients.

Considering that BECs are also involved in the regulation of regional brain blood supply, we reviewed evidence related to CBF and blood vessels morphology in schizophrenia. Reductions in brain perfusion and activity are jointly present in schizophrenia [110, 112, 113], but it is not clear whether there is a common origin for both alterations and how they might be influencing each other. Regarding the morphology of the blood vessels, greater retinal venular diameters were described in schizophrenia [119-121], suggesting deviations of the local vascular network structure. It is important to consider that widely used antipsychotics for schizophrenia treatment promoted depolarization of arterial smooth muscle cells [114-116]; thus, may favor vasoconstriction. Hence, we cannot rule out that perfusion anomalies in schizophrenia are related to its treatment. Brain irrigation measurements in drug-naive schizophrenia patients would help to better understand brain perfusion in schizophrenia.

In summary, while the field is not very consistent and the number of methods applied are large, there is converging evidence that some of the patients with schizophrenia have increased BBB permeability (potentially those with a pro-inflammatory signature) and altered brain perfusion. Astrocytes may be contributing to the pro-inflammatory signature [66] and to BBB dysfunction in some of the patients [86, 88]. It is important to have in mind that gene expression along development can vary drastically within the same brain cell type [134]. Accordingly, schizophrenia genetic background may be differentially manifested along with the establishment of the BBB [134, 138]. Overlaying schizophrenia-associated genes with the transcriptomic profile of different cellular components of the NVU at distinct neurodevelopmental stages could shed lights on particular cells, cellular states, and molecular pathways being altered during schizophrenia etiology. Additionally, the sex of the patients should also be taken into account when evaluating BBB performance, due to potential sex interactions with brain barrier integrity and permeability in schizophrenia [91, 93, 96, 101].

Acknowledgements

The authors would like to thank Prof. Dr Bart Eggen and BSc. Vera Maksaev for critical reading, and MSc Bote Smid for language editing of the review.

Financial support and sponsorship

This work is supported by a grant to I.E.C.S. from ZonMw mental health (GGZ) (ZonMw, project code: 63631 001 0).

V.P. is recipient of a Fondecyt grant (# 1190083). S.P is a recipient of 'Graduated School of Medical Sciences, University of Groningen', and 'Agencia Nacional de Investigación y Desarrollo de Chile' fellowships for PhD studies (#21181102).

Conflicts of interest

I.E.C.S. was a consultant to Gabather, received research support from Janssen Pharmaceuticals Inc. and Sunovion Pharmaceuticals Inc. The rest of the authors do not have any conflicts of interest.

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