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Microvascular anomaly conditions in psychiatric disease. Schizophrenia – angiogenesis connection

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Abstract

Schizophrenia (SZ) is a severe mental disorder with unknown etiology and elusive neuropathological and neurobiological features have been a focus of many theoretical hypotheses and empirical studies. Current genetic and neurobiology information relevant to SZ implicates neuronal developmental and synaptic plasticity abnormalities, and neurotransmitter, microglial and oligodendrocytes dysfunction. Several recent theories have highlighted the neurovascular unit as a potential contributor to the pathophysiology of SZ. We explored the biological plausibility of a link between SZ and the neurovascular system by examining insights gained from genetic, neuroimaging and postmortem studies, which include gene expression and neuropathology analyses. We also reviewed information from animal models of cerebral angiogenesis in order to understand better the complex interplay between angiogenic and neurotrophic factors in development, vascular endothelium/blood brain barrier remodeling and maintenance, all of which contribute to sustaining adequate regional blood flow and safeguarding normal brain function. Microvascular and hemodynamics alterations in SZ highlight the importance of further research and reveal the neurovascular unit as a potential therapeutic target in SZ.

Keywords

Schizophrenia; angiogenesis; gene expression; neurovascular unit; endothelial cells

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1. Background: Role of angiogenesis during neurodevelopment and CNS function

Schizophrenia (SZ) is a disabling psychiatric disorder that affects multiple brain functions, impairs real-world functioning and is only partially responsive to pharmacological treatments.

Although multiple pathological processes in the neurobiology of SZ have been identified to date, including neurotransmitter system dysfunctions (e.g., dopaminergic systems and the glutamatergic system), myelin, immune-response and infectious origins (Arion et al., 2007; Hakak et al., 2001; Harrison and Weinberger, 2004; Lewis et al., 1999; Meltzer, 1987; Wolf et al., 1993), none have accounted for heterogeneous array of clinical symptoms consistently, making pharmacological intervention difficult and only partially efficacious.

Vascular involvement in the pathophysiology of SZ (Bleuler, 1911) has gained some salience in recent years to explain and unify physiologic abnormalities seen in SZ (Hanson and Gottesman, 2005; Moises et al., 2015; Schmidt-Kastner et al., 2012). These hypotheses place impairment of the brain microvascular system as the central mechanism in the pathophysiology of SZ.

A fundamental physiological process in development and maintenance of the microvascular system is angiogenesis. Angiogenesis is the process of formation of new capillaries from existing vessels and it is the pivotal event in neovascularization during embryogenesis and throughout the life span. Angiogenesis is also engaged in response to brain injury, brain function demands and it is responsible for coupling the capillary endothelium with astroglial cells and neurons.

During brain development neurovascular coupling mechanisms assure simultaneous generation of neuroblasts and blood vessels. The primary perineuronal vascular network that surrounds the ventral neural tube is established very early during embryogenesis, in mice this occurs at approximately E7.5–E8.5 days (Risau, 1997). Vascularization of the neuroepithelium occurs via sprouting angiogenic endothelial progenitor cells from the pial surface to the periventricular areas by E11 and generates a vascular plexus (Risau, 1994). Formation of the blood brain barrier (BBB) is likely to begin at this time. Tracer-injection studies in embryos demonstrate permeability of brain endothelial cells in the first few days of vessel formation, but it becomes tightly restricted by E15.5 (Ben-Zvi et al., 2014) suggesting that the BBB has already formed. Diverse angiogenic factors including PDGF, IGF-1, TGFB, GDNF, BDNF, bFGF etc. secreted by neuroblasts, most notably VEGF are required to induce angiogenesis (Hellbach et al., 2014; Raab et al., 2004; Virgintino et al., 2003). Accumulated evidence from animal models also reveals that a preexisting vasculature is a necessary physical substrate for oligodendrocyte (OLG) precursor cells migration during early brain and spinal cord development. Interestingly, OLG precursor cells migration *in vivo* was disrupted in mice with defective vascular architecture, but was normal in mice lacking pericytes (Tsai et al., 2016). Thus, endothelial-OLG precursor cells interactions appeared to be critical for coordination of migration and differentiation of these precursors into OLG. Astrocytes migration, however, is different from OLG precursors during early

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development. Astrocytes progenitors migrate following radial glia without secondary tangential migration and, therefore, occupy restricted spatial domains related to their developmental site of origin (Tsai et al., 2012).

As brain circuitry matures during adolescence and attains adult levels of myelination-dependent saltatory action potential conduction, adequate access to blood supply and oxygen tension become central factors in the processes of the formation and compaction of myelin segments. Hypoxia inducible factors (HIF1/2a) stabilization in OLG progenitors as a result of oxygen strain inhibits their differentiation into OLGs. Remarkably, these undifferentiated OLG progenitors possess paracrine activity that induces robust postnatal angiogenesis *in vivo* and directly stimulate endothelial cell proliferation *in vitro* (Yuen et al., 2014). Given the strong evidence for the involvement of OLGs and myelin abnormalities in the pathophysiology of SZ (reviewed in (Haroutunian et al., 2014)), the functional impact of oligodendroglial by endothelial cell (EC) interactions may be fundamental to the pathophysiology of SZ and may explain, at least partially, the observed gene expression changes specific to OLG and ECs in SZ.

This review aims to assess the accumulated evidence for the involvement and role(s) of the brain microvasculature in SZ and answer the following questions: (1) is there evidence for regional brain hemodynamics changes in SZ? (2) What are the morphological abnormalities in BBB/microvascular structure in the brains of individuals with SZ? (3) Does microvascular/BBB gene expression signature associated with SZ show changes in angiogenesis/vascular remodeling and offer support for an anomalous BBB in SZ? (4) Could the genes involved in angiogenesis and associated with it signaling pathways be potential risk factors in SZ from the genomic and transcriptomic perspective? (5) Do animal models aimed at revealing the mechanism of cerebral angiogenesis demonstrate cognitive and socioemotional deficit similar to those observed in individuals with SZ?

2. Regional brain hemodynamic changes associated with SZ clinical symptoms

That the neurovascular system is involved in SZ has been repeatedly documented by neuroimaging studies. Extensive reviews of structural and functional neuroimaging in SZ (Buchsbaum and Hazlett, 1998; Lopes et al., 2015), including discordant monozygotic twins (Weinberger et al., 1992), have demonstrated that abnormalities in brain hemodynamics are generally consistent with historical concept of hypofrontality in individuals with SZ (Berman et al., 1986) and reflect functional deficit of frontal cortex (Davidson and Heinrichs, 2003; Taylor, 1996; Weinberger et al., 1986). Frontal hypoperfusion has been consistently documented in clinical cases involving first episode, neuroleptic-naïve (Andreasen et al., 1997; Catafau et al., 1994; Erkwoh et al., 1997; Rubin et al., 1994) and untreated individuals with chronic SZ (Kim et al., 2000) suggesting that this characteristic is independent of the length of disease, its treatment and duration. Other regions, where hypoperfusion in SZ has been extensively documented, include middle and anterior cingulate, temporal and parietal regions (Ojeda et al., 2002; Schultz et al., 2002; Yucel et al., 2002) and thalamus (Clark et al., 2001). More recently, MRI based investigation of

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alterations in the volume of small arterial (pial) and arteriolar vessels in brains of individuals with SZ suggested that microvascular abnormalities maybe more widespread across the entire brain (Hua et al., 2016). Reduced CBF has been also associated with psychotic symptoms. For example, it has been shown that reduced CBF was associated with greater severity of negative symptoms in frontal, cingulate and superior temporal cortices (Pinkham et al., 2011). In contrast, positive symptoms have been associated with increased CBF in temporal, cingulate and superior frontal gyri (McGuire et al., 1993; Pinkham et al., 2011) and in subfields of hippocampus (Schobel et al., 2013). Although most neuroimaging studies have made the underlying assumption that the SZ hypofunction in CBF reflects neuronal hypometabolism, a more direct association of SZ with cerebrovascular circulation and function is equally plausible.

The evolutionary increase of cortical neuronal complexity, massive expansion of the cortical surface and cell number in humans depends on higher blood supply due to oxygen and nutrients requirements. Not surprisingly cerebral cortical vascularization is dramatically enhanced in humans relative to monkeys and cats (Lauwers et al., 2008). Moreover comparison of newborn and adult cerebral cortical depth in non-human primates using 3D-imaging has shown that relative cortical vascular volume nearly doubles in adults compared to newborns and that this increase occurred predominantly at the capillary level (Risser et al., 2009). Increase in capillary volume is sustained by the lengthening of pre-existing segments and by the formation of new segments, while the contribution of capillary diameter is minor and only marginal at the perforating vessel level. These findings indicate that structural adaptations of the cerebral vascular system induce multiple changes that include: vessel density and segment length, a decrease in inter-capillary distances and, to a lesser extent, vessel diameter increase. Therefore, postnatal cortical maturation typically described in terms of synaptogenesis, gliogenesis and interconnectivity is accompanied, and perhaps preceded, by an intensive remodeling of microvascular architecture.

Moreover, recent studies in mice using combination of high-throughput histology and computation models also suggest that the known changes in cortical blood flow (CBF) induced by changes in the neurotransmitter microenvironment are controlled at the level of microvessels (Blinder et al., 2013), likely through constriction or stiffening of contractile proteins in pericytes. The restricted lateral perfusion of cerebral cortical tissue is an additional distinctive parameter of brain hemodynamics. Although the microvasculature forms interconnected loops with a topology that follow organization of cortical columns, blood flow sourced by penetrating arterioles is effectively drained by penetrating venules limiting lateral perfusion. Experimental results for pathological condition such as local ischemia are also consistent with restricted lateral perfusion within the cerebral cortex, which prevents blood in neighboring penetrating vessels from entering the area previously sourced by the occluded vessel (Blinder et al., 2013), thus limiting damaging impact on neural cells proximal to supplying impaired vessel and sparing those in neighboring columns. Therefore, it is anticipated that reduced blood perfusion documented in individuals with SZ and possibility of associated with it, mild hypoxia will be restricted to cortical columns with reduced CBF.

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Astrocytes, which encase by their end-feet, almost the entire parenchymal arterioles and capillaries also play a pivotal role in regulating CBF. By contrast, the processes from neurons are rarely in direct contact with brain blood vessels (Koehler et al., 2009). Astrocytes sensing synaptic activity and the release of calcium and major neurotransmitters, such as glutamate, may additionally influence CBF by modulating the vascular smooth muscle tone around larger vessels, such as arterioles. Spill over glutamate elicits NMDA receptor signaling that engages the calcium syncytium by raising intracellular Ca2+ concentration in astrocytic end-feet modulating vascular tone via several pathways. The first involves the vasodilator-nitric oxide release by neurons, which activates smooth muscle guanylate cyclase. This activation results in a rise in cGMP levels causing dilatation of vessels. The second pathway involves phospholipase A_2 metabolism in both neurons and astrocytes, which results in prostaglandins release, which acts on vessels to dilate and induces arachidonic acid metabolism into 20-hydroxyeicosatetraeonic acid in smooth muscle, which constricts vessels (Attwell et al., 2010). A role of increased Ca^{2+}- sensitive K^+ current in astrocyte ends-foot on vasodilation and CBF has also been demonstrated (reviewed by (Koehler et al., 2009)). Given the accumulated evidence of regional abnormalities of amino acidergic (glutamatergic and GABAergic) and monoaminergic (dopaminergic and serotonergic) neurotransmission in SZ (Bunney and Bunney, 2000; Davis et al., 1991; Meyer-Lindenberg et al., 2002; Otsuki and Akiyama, 1997) and region-specific effect of ketamine (NMDA receptor antagonist) on CBF (Tamminga, 1999; Tamminga et al., 1995), the contribution of disturbed neuronal circuits to regional hemodynamic changes associated with clinical symptoms and cognitive performance in SZ appeared to be critical. Thus, there is ample evidence for strong interactions between the microvasculature and neurobiological processes known to be involved in SZ. On the one hand, the microvasculature influences the development and maintenance of brain circuitry, while at the same time changes in neurotransmitter and related systems influence CBF and potentially regulate and alter microvascular architecture dynamically.

Age is one of the main variables that need to be taken into account when assessing CBF changes in SZ patients. In general, there is consensus that CBF declines as a function of age. While negative correlations between age and CBF have been observed in the cingulate, frontal and parietal regions, it appears that individuals with SZ may experience unique and additional, age-independent, CBF impairments in frontal and parietal areas that are likely related to the expression of negative symptoms in late life (Schultz et al., 2002). In addition to age, antipsychotic treatment is a common variable that may have strong impact on regional CBF in SZ patients. The documented outcomes of antipsychotics treatment on CBF in SZ patients show conflicting results ranging from substantial to minor, or no efficacy of medication. Normalizing effect of antipsychotic treatment on regional CBF has been more evident in patients treated with atypical antipsychotics and show that increases in regional blood perfusion are positively correlated with cognitive performance improvement (Ertugrul et al., 2009; Lindenmayer et al., 2007). Examination of brains from antipsychotic-treated monkeys have shown that while the neuronal cell density has a tendency to increase in the parietal cortex of antipsychotic-exposed monkeys, glial cell densities and EC's densities remain unchanged (Konopaske et al., 2007). One possible outcome of antipsychotic-treatment is that increase of neuronal density may stabilize abnormal regional

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neurotransmission in SZ resulting in positive effects on local CBF modulated by contractile mechanisms in pericytes (Blinder et al., 2013). This evidence of antipsychotic-induced normalization, or partial normalization of CBF, suggests that hypoperfusion and reduced CBF may be more profound in untreated persons with SZ and suggest that hypoperfusion and reduced CBF may be components of the core pathophysiology of SZ.

3. Microvascular/ blood brain barrier abnormalities in SZ: evidence from postmortem studies

Given strong evidence for hypoperfusion in many cortical regions in SZ it is surprising that evidence of structural abnormalities in microvasculature are rare and inconsistent. This may be explained, at least in part, by differences in the brain regions studied, by methodological differences, and by the complexity of surveying the microvasculature in cerebral cortical tissue quantitatively. Early reports suggested abnormalities in the microvasculature in SZ, such as reduction of retinal vascular bed during progressive course of SZ (Cotton et al., 1940) and generally simplified angio-architecture with reduced arborization of the brain vessels in the orbito-frontal cortex of paranoid hallucinatory SZ individuals (Senitz and Winkelmann, 1991). Wide-spread small vessel disease in the cerebral white matter with small foci of demyelination has been shown in patients with SZ psychosis (Bruton et al., 1994). Morphometric analysis of electron microscopy images from the superficial layers (layers I-II) of prefrontal and visual cortices of individuals with SZ showed the presence of ultrastructural abnormalities of capillaries including thickening deformation of basal lamina and cytoplasmic vacuolation of endothelial cells, although the area of capillaries and their diameter did not show significant differences between SZ and controls (Uranova et al., 2010). Consistent with results in frontal cortex, mean capillary diameter was not significantly different in layers III and V of the anterior cingulate cortex between elderly antipsychotic-free SZ patients and age matching controls, irrespective of evident cortical thickness reduction in both layers in SZ (Sinka et al., 2012). Lack of differences in mean capillary length densities was also demonstrated in prefrontal and anterior cingulate cortices between control and SZ patients, although mean cortical volume and thickness was significantly reduced in SZ patients (Kreczmanski et al., 2005; Schmitz et al., 2005). Similar results were obtained in several subcortical regions including caudate, putamen, nucleus accumbens, mediodorsal thalamic nucleus and amygdala using several parameters of microvascular integrity (total capillary length, mean capillary density and length per neuron) (Kreczmanski et al., 2009). Noninvasive approach to assess cerebral vascular abnormalities in SZ by retinal imaging, since retinal and cerebral microvessels are structurally and functionally homologous, suggested that wider venules in SZ patients may represent a proxy marker of familial vulnerability to psychosis symptoms (Meier et al., 2013).

It is assumed that shear stress caused by blood flow is critical for vascular endothelial cell survival and continued vessel patency. Therefore, reduction of CBF in frontal cortex in SZ may directly impact shear stress and subsequently survival outcome of the vascular endothelium. Endothelial cell death is typically followed by collapse of the capillary walls, leaving only remnants of the extracellular matrix in the form of a thin string. Thus, the lack of gross pathological lesions of the microvasculature in SZ and the absence of thinning,

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reduced tortuosity, or string vessels in the cerebral cortex and subcortical structures, which are prominent features in Alzheimer's disease (Bailey et al., 2004; Hunter et al., 2012; Richard et al., 2010) arguing against lingering microvascular degeneration in SZ.

Given the strong association with cognitive and adaptive functioning decline at the onset of SZ, similar to that observed in normal aging, early-stage schizophrenia and normal aging might share common underpinnings (Tang et al., 2009). Studies of age-related changes in the cerebral microvasculature of cognitively normal individuals have led to varying conclusions. While some human studies have shown that various structural parameters in the cerebral cortical microvasculature network remain stable during aging (Hunziker et al., 1979; Meier-Ruge et al., 1980; Meier-Ruge et al., 1992; Schulz-Dazzi, 1986), others demonstrated significant decrease in density of vessels supplying the cerebral cortex and hippocampus compared to young controls accompanied by the increased diameter of the remaining vessels (Bell and Ball, 1981). Age-dependent increase in intercapillary distance, indicating reduction of vessel density, and increased diameter of vessels were also demonstrated in cortical regions and hippocampi of old rats compare to their adult counterparts (Amenta et al., 1995; Sonntag et al., 1997). Age related shrinkage of cortical thickness consequent to partial atrophy may have significant impact on morphometric parameters characterizing cerebral microvasculature in normal aging and could be the basis for reported inconsistencies.

The lack of unequivocal support for reduced cerebral capillary networks in SZ and normal aging suggests that non-morphometric parameters may be more informative to the understanding of the mechanisms contributing to regional hypoperfusion seen in SZ. Ultrastructural evaluation of BBB structural integrity and its associated permeability is one of these approaches.

The perivascular environment is highly susceptible to changes in ECs/BBB integrity and can therefore be used to gauge microvascular disturbances in SZ. Most notable abnormalities documented in the pericapillary environment in SZ include swelling of astrocytic end-feet and apoptotic pericapillary OLGs (Uranova et al., 2010). Assessment of the deeper layers (layer V) of the frontal cortex has indicated significant reduction of pericapillary OLGs in SZ (Vostrikov et al., 2008).

Based on the inflammatory-vascular theory of SZ (Hanson and Gottesman, 2005), brains of individuals with SZ are likely to display features of reactive astrogliosis and microglial infiltrations, due to concurrent inflammation associated microvascular/BBB damage and persistent hypoxia during the course of disease. Other studies have also suggested that activation of microglial cells maybe more relevant to the pathophysiology of psychosis (Bernstein et al., 2009; Steiner et al., 2008) and reflective of heterogenic pathophysiology of paranoid *vs.* residual SZ.

Overall, the data on neuroinflammation and astrogliosis in SZ (Arnold et al., 1996; Arnold et al., 1998; Bernstein et al., 2009; Casanova et al., 1990; Damadzic et al., 2001; Falke et al., 2000; Radewicz et al., 2000; Wierzba-Bobrowicz et al., 2004) are contradictory and inconclusive, which may be attributable to small sample sizes, and the inclusion of non-

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psychiatric controls, including elderly controls, without adequate assessment of common and uncommon neuropathologic lesions. (Schnieder and Dwork, 2011). Additionally, possible anti-inflammatory effects of antipsychotics on microglial activation (Kato et al., 2011) should be taken into account.

Stereological cell counting methods may shed additional light on protracted effect of vascular/BBB integrity impairment in SZ. Region specific glial cell loss (all types) was demonstrated in anterior cingulate cortex in SZ (Stark et al., 2004). Glial cell losses include significant reduction of GFAP-positive astrocytes restricted to layer V of the dorsolateral prefrontal cortex (Rajkowska et al., 2002) and astrocytes adjacent to blood vessels in gyral and underlying white matter (Webster et al., 2001; Webster et al., 2005), which is consistent with reported reduction of astrocytic markers in deep cortical layer of anterior cingulate cortex in SZ (Katsel et al., 2011; Steffek et al., 2008). In contrast, evaluation of several subcortical regions including internal capsule, putamen and thalamic nuclei in SZ showed increased expression of several astrocyte-specific markers (Barley et al., 2009), however, the contribution of potential confounding factors such as age, dementia and neuroleptic exposure (Arnold et al., 1996) cannot be ruled out.

4. Microvascular/blood brain barrier transcriptome signature associated with SZ

Reports of microarray-based genome-wide gene expression studies in SZ performed on crude brain tissue homogenates have not pinpointed abnormalities in the cerebral vasculature transcriptome. It has been argued (Harris et al., 2008) that the brain vascular endothelium volume represents only 1 – 3% of the brain volume (depending on different estimates (Lauwers et al., 2008)) crude brain tissue homogenates and thus, it is unlikely to contribute to overall transcriptional alteration in the disease state considerably. However, recent stereological analyses suggest that the numbers of vascular ECs and neurons maybe much closer to each other than previously thought. The estimated ratio of ECs to neurons in the human amygdala, a brain region associated with SZ, is estimated to be near 1:1 (Garcia-Amado and Prensa, 2012). In non-human primates parietal cortex, the ECs to neurons ratio is 1:3, where ECs constitute near 15% of total cells (Konopaske et al., 2007). Thus, while there are inconsistencies in reported cell-type counts in deferent brain regions, it is, nevertheless, irrefutable that EC-microvasculature is a noteworthy component of the total brain tissue transcriptome. A recent RNA-seq based approach on acutely purified different brain cells populations (Zhang et al., 2014) identified EC/BBB -specific markers and has provided tools for the interrogation of EC/BBB-specific genes in total brain transcriptome analyses.

We examined 1306 genes identified in the transcriptome of purified mouse cerebral microvascular ECs (pericyte-depleted and enriched in comparison to peripheral ECs) (Daneman et al., 2010) in a whole-tissue block homogenate-based microarray dataset from 15 cerebrocortical regions and the hippocampus of individuals with SZ (Katsel et al., 2005a; Katsel et al., 2005b). This subset of genes, therefore, represents transcripts specifically enriched in ECs and the BBB and identified BBB forming molecules and pathways related

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to angiogenesis (Daneman et al., 2010). Six hundred and fifty-seven of EC/BBB -specific transcripts appeared to be significantly changed in the 15 brain cortical regions and the hippocampus of individuals with SZ using microarray analysis tools, as previously described (Katsel et al., 2008; Katsel et al., 2009). Three hundred and eleven of these genes corresponded to a subset uniquely enriched genes in ECs (between 2 - 750 fold) compare to the other subsets of transcripts from acutely purified CNS cell types, including neurons, astrocytes, myelinating oligodendrocytes, oligodendrocyte progenitors and microglia (Zhang et al., 2014). Near 24% (~300) of EC/BBB -specific transcripts (Daneman et al., 2010) were differentially expressed in the brain regions examined in persons with SZ relative to controls.

Top differentially expressed in SZ genes that were distinctively enriched in EC/BBB are shown in Table 1 (p-val sorted). Core analyses for the pathways and networks that were represented in differentially expressed genes in SZ were performed with Ingenuity Pathway Analysis (IPA) and Metacore. Angiogenesis was among the top biological function with majority of genes exhibiting downregulation in SZ (p-val=3.65E-34; activation Z-score= −1.77). The other prominently downregulated EC/BBB-related functions in SZ include migration and proliferation of cells (p-val=1.8E-38; activation Z-score= −2.73), while functional categories representing apoptosis tended to be upregulated (p-val=3.7E-40; activation Z-score= 2.28). Pathway analysis performed by both IPA and Metacore platforms (Tables 2 and 3) indicate enrichment for well-established angiogenesis-related pathways, such as WNT-, VEGF-, IGF1-, oncostatin M-, angiopoietin-, ephrin receptor- signaling, in which the majority of pathway-associated genes demonstrated downregulation in the analyzed brain regions from individuals with SZ. These findings are in agreement with the overall transcription pattern of laser capture microdissected cerebral vascular ECs from the prefrontal cortex of individuals with SZ in which majority of pathway and biological processes enriched in ECs were directionally associated with downregulation suggesting functional impairment of cerebral vasculature and BBB (Harris et al., 2008), and further supported by dysregulated VEGF signaling in dorsolateral prefrontal cortex (Fulzele and Pillai, 2009) and thalamus (Chu et al., 2009) in SZ. Interestingly, study of systemic blood factors in antipsychotic-naïve young relatives at familial high risk for psychosis and first-episode SZ patients showed that an anti-angiogenic factor - soluble fms-like tyrosine kinase (sFLT1) was significantly increased in the plasma of familial high risk for SZ individuals compared to controls (Lizano et al., 2016). Upregulation of sFLT1, the factor that binds and reduces circulation of pro-angiogenic factors, including VEGF, and blunts their beneficial effect on vascular endothelium homeostasis, suggest that the vascular dysfunction may presage the onset of psychosis in young relatives of persons at familiar high risk for SZ.

4.1 Receptor tyrosine kinase family

Notably, IGF1-, VEGF-, ephrin receptors belong to the receptor tyrosine kinase family, which are known to trigger multiple signaling cascades, including PI3K-AKT-mTOR and MAPK signaling. Both cascades have been shown to be dysregulated in thalamic nuclei from individuals with SZ, but not from individuals with bipolar disorder or major depression (Chu et al., 2009). Detected abnormalities are corroborated by earlier reports of reduced AKT-GSK3b (Emamian et al., 2004; Kozlovsky et al., 2001, 2002; Zhao et al., 2006) and

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insulin receptor content and autophosphorylation levels (Zhao et al., 2006), suggesting that aberrant insulin receptor signaling in SZ may be linked to reduced glucose metabolism in cerebral cortical regions of the brains of SZ patients (Buchsbaum et al., 2007). Growth factors, PDGFβ and TGFβ are involved in the recruitment of pericytes and maturation of the BBB (reviewed by (Blanchette and Daneman, 2015)), signifying the involvement of receptor tyrosine kinase signaling pathway in the development and maintenance of the cerebrovascular system.

4.2 Proangiogenic signals

Differentially expressed in SZ EC's angiopoietins (ANGPT2 and ANGPTL4, Table 1) are the signaling molecules secreted in the neurovascular unit and involved in maintenance of the vascular plexus and BBB integrity. Proangiogenic signal, VEGF-A secreted by astrocytes, also promotes endothelial sprouting from existing vasculature by stimulating motility, filopodia extension and proliferation, and, together with Notch signaling, controls whether specific endothelial cells become lead tip cells, or trailing stalk cells(Eilken and Adams, 2010). In contrast to elevated circulating blood VEGF levels in patients with SZ (Pillai et al., 2015), reduced cerebral VEGF gene expression was observed in the current study and reported in the prefrontal cortex of individuals with SZ (Fulzele and Pillai, 2009).

4.3 Notch signaling

While early genetic studies linked polymorphisms in genes involved in the Notch pathway to SZ (Passos et al., 2006; Schmidt-Kastner et al., 2006; Tiwari et al., 2010), very few hypothesis-free gene expression changes in the Notch pathway have been reported in SZ. However, our hypothesis-driven analyses based on expression patterns of EC/BBB-related transcripts showed that consistent with earlier findings NOTCH −3, −4 (Sinkus et al., 2013) and JAG1(Kerns et al., 2010) were affected in the brain regions of the persons with SZ. NOTCH4 and JAG1 are distinctly enriched in vascular ECs, while NOTCH3 and HES5, the downstream NOTCH signaling effector, that are significantly affected in SZ (Kerns et al., 2010) are highly expressed in astrocytes compare to other neural cell-types (Zhang et al., 2014) suggesting cross-talk between perivascular astrocytes and the vascular endothelium.

Motility and filopodia extension of endothelial tip cells share many structural and functional features with axonal growth cones. While significantly affected in SZ (Table 2; Axonal guidance signaling) along with ephrin receptor signaling, semaphoring / plexin, netrin and slit / roundabout receptors appeared to be guidance cues in endothelial sprouting and possibly coordinate the formation of growing vessels and neural cells. These pathways show a tendency to be downregulated in analyzed brain regions in individuals with SZ (Table 2). While changes in the expression of genes involved in axonal guidance signaling prefrontal cortex (Arion et al., 2010) and cerebellum (Eastwood et al., 2003) in SZ have been ascribed, predominantly, to neural function, including synaptic plasticity and neural progenitors migration during development, the above analysis suggests that guidance and maturation of the microvasculature is another plausible feature of these guidance signaling systems.

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4.4 WNT/β-catenin signaling

Recent evidence suggests that a unique brain-specific angiogenic mechanism integrates a WNT/β-catenin signaling pathway that is elicited by WNT ligands released by neuroblasts (Daneman et al., 2009). In addition, activation of WNT signaling initiates maturation of the BBB (reviewed by (Blanchette and Daneman, 2015)). Genetic disruption of WNT signaling leads to severe CNS angiogenesis defects (reduction in vessel number, loss of capillary beds, and the formation of hemorrhagic vascular malformations) with loss of BBB integrity, indicating that both processes are tightly linked (Daneman et al., 2009; Liebner et al., 2008; Stenman et al., 2008). Earlier studies indicated that WNT-1 immunoreactivity is increased, while β-catenin is reduced in the CA3 and CA4 pyramidal cell layers of the hippocampus (Cotter et al., 1998; Miyaoka et al., 1999) and is associated with low protein levels of GSK3B in prefrontal cortex from individuals with SZ (Kozlovsky et al., 2000). However, other studies have shown conflicting results; the initial reduction of GSK3B protein levels in prefrontal cortex of SZ (Beasley et al., 2001) was not confirmed in the same region using samples from a different SZ cohort (Beasley et al., 2002). These differences may be due to vagaries of crude brain homogenate studies since a recent evaluation of WNT signaling using a cell-type specific approach showed elevated mRNA levels of WNT7B and frizzled-1, while downstream mediators of WNT signaling, DAAM1 and LEF1 were decreased in LCM isolated parvalbumin-positive neurons from individuals with SZ (Pietersen et al., 2014). An RNA- seq pilot study of human induced pluripotent stem cell derived into forebrain neural progenitor cells from patients with SZ, additionally, show perturbation in canonical WNT signaling related to SZ (Brennand et al., 2011; Topol et al., 2015). These findings suggested that disturbed Wnt signaling in SZ is likely more common and not limited to only neuronal phenotypes. Moreover, a recent genetic study found polymorphisms in the CTNNB1 and WNT7B genes to be associated with SZ (Levchenko et al., 2015).

WNT signals are transduced by at least two distinct pathways: the canonical WNT/β-catenin pathway and the β-catenin independent diverse non-canonical WNT pathways (Wnt/PCP and Wnt/$Ca2^{+}$). During neovascularization, angiogenesis can be envisioned as the coordinated growth and migration of EC sheets towards an angiogenic signal. ECs polarization is likely responsible for the pattern formation, which further organized into the complex vascular networks. Planar cell polarity signaling (WNT/PCP, Table 2) is a central mechanism, which impacts extracellular polarity cues and has important implications for vascular remodeling and in coordinated assembly of ECs into vascular structures (Cirone et al., 2008). WNT/PCP signaling is regulated at multiple levels: downstream, or upstream of the dishevelled homolog, DVL. For example, one of the WNT/PCP signaling branches involves the asymmetrical localization of frizzled by the protocadherin CELSR receptor and VANGL protein by recruiting PRICKLE, a putative nuclear translocation receptor to the plasma membrane, which subsequently binds DVL and inhibits PCP signaling. Both, vascular endothelium enriched VANGL1 and PRICKLE1 genes (Zhang et al., 2014) were among three hundred eleven differentially expressed genes of the EC/BBB ensemble (please see above) and showed upregulation in the 15 cortical regions studied in SZ (Tables 2 and 3; Wnt/PCP signaling). Negative effect on WNT signaling in SZ further supported by the upregulation of secreted frizzled-related proteins, SFRP2 and, particularly SFRP4, which is

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a potent angiogenesis inhibitor (Muley et al., 2010), in neural progenitor cells derived from SZ patients (Topol et al., 2015).

4.5 Endothelial basement membrane

Ultrastructural analysis of capillaries in prefrontal and visual cortices in SZ showed thickening and deformation of the basal lamina (Uranova et al., 2010). Collagen IV is a type of collagen found primarily in the basal lamina, a structural component of the basement membrane and known for its role in angiogenesis and differentiation of EC. Six genes, COL4A1-6 are known to encode type IV collagen subunits. At least two of them, COL4A1-2 showed significant decreases in the cingulate, temporal and parietal cortices in SZ (Table 1) and downregulated COL4A1 mRNA levels were shown in LCM isolated ECs from the prefrontal cortex of individuals with SZ (Harris et al., 2008). Two other essential components of vascular basement membrane are laminin and SPARC (osteonectin) (both shown in Table 1). SPARC is one of the most downregulated EC-specific genes in SZ and it is additionally involved in pericytes recruitment to the microvessels, vascular remodeling, modulation of the mitogenic activity of VEGF on ECs, and reduces the association of VEGF with its cell-surface receptors (Kupprion et al., 1998).

4.6 Vascular inflammatory responses

Identified biological process networks related to adhesion, transport and vascular remodeling shown in Table 3 are integral processes in angiogenesis and the maintenance of the vascular endothelium. Gene expression changes associated with these processes are indicative of functional impairment of cerebral vasculature and BBB integrity (Harris et al., 2008). A recent vascular transcriptome study showed that the inflammatory response is an intrinsic component in VEGF elicited signaling in human ECs (Schweighofer et al., 2009). Specifically, VEGF and IL1 treatment of human umbilical vein EC elicits expression changes in genes involved in the regulation of vascular permeability, EC lineage specialization, epithelial to mesenchymal transition, which is characteristic to vascular remodeling, and genes, previously implicated in axon guidance (Schweighofer et al., 2009). Once again, these observations highlight a parallelism between axon growth guidance and vascular sprouting.

Transcriptome data from postmortem brain studies in SZ suggest “hypo-inflammatory” signature associated with SZ supported by two independent microarray studies (Harris et al., 2008; Katsel et al., 2005b; Roussos et al., 2012), which were confirmed in large coexpression dataset from seven independent transcriptome studies of SZ (Mistry et al., 2013). Overall, decrease clustering and reduced number of genes participating in immune-response modules suggests functional dysregulation of brain immune related processes in SZ. Reports of expression patterns of immune response loci genetically associated with SZ represented by the major histocompatibility complex genes (Kano et al., 2011; Morgan et al., 2016; Sinkus et al., 2013), which may be influenced by prolong smoking are generally agree that the upregulation pattern of MHC genes, which is normally associated with infection and systemic inflammation fails to occur in individuals with SZ.

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Recently, increased expression of structural variants of complement component 4 (Sekar et al., 2016), which is part of classical complement cascade activation have been linked to excessive complement activity in the development of SZ and associated with reduced synaptic densities in SZ brains. Role of the complement system is also increasingly recognized as contributor to the brain endothelial activation (Wu et al., 2016). Moreover, the critical contribution of microglia to the repair of damaged BBB, which involves mechanisms elicited by purinergic receptor signaling, has been demonstrated recently (Lou et al., 2016). Additional factors, including age, duration of illness, cognitive and functional deterioration, regional susceptibility, can argue for (Arion et al., 2007; Najjar and Pearlman, 2015; Narayan et al., 2008; Saetre et al., 2007; Shao and Vawter, 2008), or against inflammation in SZ (Arnold et al., 1998). Despite these discordant observations, whether perivascular inflammation influences angiogenesis and the integrity of the neurovascular unit remains an important question.

4.7 HIF1A signaling

During embryonic and early postnatal development increasing demands for oxygen, supply of nutrients and low levels of oxygenation are prerequisites for angiogenesis and formation of mature vascular plexus in the brain. This process could be disrupted by fetal hypoxia, which have all been proposed as a common environmental risk factor for SZ (Hanson and Gottesman, 2005; Schmidt-Kastner et al., 2006; Tsuang, 2000). Hypoxia-inducible factor 1 alpha (HIF1A) and vascular-specific endothelial PAS domain protein 1 (EPAS1, HIF2A) are among the main transcription factors that mediate the effect of hypoxia on the expression of genes involved in angiogenesis (Tian et al., 1997). Activated HIF1A/EPAS1 initiates transcription of multiple target paracrine signaling genes involved in angiogenesis, vascular remodeling and tone regulation. As we mentioned above, the majority of genes involved in these processes appeared to be downregulated in analyzed cortical regions from individuals with SZ compare to controls (Tables 1–3). In agreement with the directionality of changes in angiogenesis/vascular remodeling in analyzed brain regions from individuals with SZ, EC-specific EPAS1 (Table 1) and HIF1A (not shown) transcription factors showed modest but significant reduction of mRNA levels in the same regions in SZ.

A concise summary of the plausible mechanisms of postnatal angiogenesis and their effects in the brains of healthy and SZ individuals are shown in Figure One.

The analysis undertaken in this review does not include transcriptional changes specific to astrocytes, which play a pivotal role in angiogenesis, maintenance of endothelial junctions in BBB, transport across BBB and dynamic regulation of cerebral circulation (reviewed in (Koehler et al., 2009)). Astrocytes- secreted angiotensinogen, a precursor for angiotensin II - a powerful vasoconstrictor of arterioles and larger vessels (Yang et al., 1999) can also regulates permeability of BBB. As has been shown before, angiotensin II binding to type 1 angiotensin receptors expressed by ECs of BBB elicits downstream signaling that restricts passage of molecular tracers across human BBB (Wosik et al., 2007). Both, astrocytes and ECs secrete extracellular matrix (ECM) proteins to generate and maintain the basement membranes of vascular endothelium. The composition of the ECM is altered at the sites of BBB damage and the basement membrane disruption. For example, it has been shown that

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activated astrocytes at the sites of BBB damage by the leaked fibrinogen increase deposition of the chondroitin sulfate proteoglycan -neurocan and induced glial scar formation (Schachtrup et al., 2010). In SZ, strong increases in the chondroitin sulfate proteoglycan -positive glial cells were detected in the deep amygdala nuclei and entorhinal cortex compare to the normal controls, but not in bipolar disorder subjects (Pantazopoulos et al., 2010) demonstrating increased production of ECM proteins as an distinctive aspect of the pathophysiology of SZ.

In this review we have not discussed pericytes transcriptional changes relevant to SZ because many of the pericytes genes overlap with ECs (Daneman et al., 2010) making it difficult to draw distinctions from transcription-based data sets derived from mixed cell populations.

5. Evidence for vascular component association with SZ from the existing genetic data

SZ is a disorder with estimated heritability ranging from 64 to 90% (Lichtenstein et al., 2009; O'Donovan et al., 2003; Sullivan et al., 2003). SZ is thought to be a highly polygenic disorder where each genetic variant has a very small risk effect. Recently, it has been proposed that the genetic risk for SZ may operate at the pathway level (Sullivan, 2012) and hypothesize that multiple polygenetic variations alter a more limited set of biological pathways in SZ. Intra- and inter-cellular pathways related to energy supply to neurons and their protection from ischemia have been implicated by pathway enrichment analyses (Moises et al., 2015). More specifically, Moises et al. established a candidate pathway related to energy supply to synapses and protection from ischemia based on genes discovered in family association studies. The enrichment of that pathway with SZ-associated variants was confirmed using the PGC-SCZ genome-wide association study (GWAS) results, the largest GWAS analysis, conducted by the Psychiatric Genomics Consortium-Schizophrenia Workgroup. PGC-SZ GWAS comprises a sample set of 36,989 SZ cases and 113,075 controls and identified 108 common variants that show statistical associations with SZ (Schizophrenia Working Group of the Psychiatric Genomics, 2014). In addition, this interpretation of functional genomics data was supported by the significant overrepresentation of the genes involved in post-ischemic repair in postmortem gene expression studies. The constructed functional genomics data candidate pathways affected in SZ suggested strong representation of genes associated with PI3K/AKT signaling pathway, which exert an influence on vasoconstriction via reuptake of serotonin, dopamine and norepinephrine and vasodilation by modulating nitric oxide production. Taken together these data suggest that SZ may be a mild adult vascular-ischemic disorder (Moises et al., 2015). To further explore this hypothesis, we tested whether SZ-candidate genes, defined as genes that localize within PGC-SZ associated regions, that are also EC/BBB -specific markers (as described in previous section) show transcriptional changes in SZ. By leveraging the microarray dataset from 15 brain cortical regions and the hippocampus of individuals with SZ (Katsel et al., 2005a; Katsel et al., 2005b), we identified 11 out of 18 genes that were dysregulated in SZ (Table 4). This set of 18 genes are significantly enriched for differential expressed genes (Chi square = 7.43, df = 1, p = 0.0064) when compared to the overall

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significant differential expressed gene probes (11,600 out of 44,928 gene probes), indicating a significant dysregulation of gene expression for SZ-candidate genes that participate in EC/BBB related functions.

6. Neurobehavioral sequelae of mutations of the endothelial cells/ microvascular genes

Few studies have evaluated neurobehavioral consequences of manipulation of EC genes. Here, we will review the pertinent reports and will propose the future directions to advance the field.

6.1 SPARC, secreted protein acidic and cysteine rich

Campolongo and colleagues(Campolongo et al., 2012) assessed the behavioral abnormalities in adult mice with constitutive deletion of the *Sparc* gene (the association of *Sparc* with EC was discussed above and it is one of the most downregulated EC-specific genes in SZ). In an open field test, both $Sparc^{+/-}$ and $Sparc^{-/-}$ mice showed no significant changes in horizontal locomotor activity; however, mice of both genotypes exhibited more anxiety-related responses. $Sparc^{-/-}$ mice demonstrated decreased time spent and distance travelled in the center of open field, and mice of both genotypes displayed less rearing events. In the light dark box test, both $Sparc^{+/-}$ and $Sparc^{-/-}$ mice spent less time in the lit compartment of the box. No effects of deletion were seen in elevated plus maze. Taken together, these behavioral changes suggest elevated anxiety-related behaviors in *Sparc* mutants. Somewhat unexpectedly, increased anxiety of *Sparc* knockout mice was associated with decreased depression-like behaviors. Both *Sparc* mutant mice showed less immobility in forced swim test that is widely used to evaluate efficacy of anti-depressants rather than depression-related behaviors(Nestler and Hyman, 2010). More physiologically relevant behavioral tests will be needed to confirm this phenotype. In addition, the authors evaluated the effects of deletion on the response to spatial novelty and to a novel object in the novel object recognition and novel object exploration tests. Compared to wild-type (WT) mice, $Sparc^{-/-}$ mice demonstrated less exploration of novel object without alteration in general exploratory activity. In order to uncover the underlying mechanisms, neuronal activity and adult neurogenesis in the dentate gyrus (DG) were assessed. Compared to WT mice, $Sparc^{-/-}$ mice showed higher levels of neuronal activity assessed by footshock-induced c-Fos expression in the DG and decreased cell proliferation but not maturation or survival in the subgranular zone of the DG. While Sparc is most abundantly expressed in EC, recent studies from the Barres lab have also demonstrated that astrocytes secrete SPARC to regulate synaptogenesis and glutamate receptor levels(Clarke and Barres, 2013). As this model does not include cell type-specific deletion, it is impossible to link the behavioral changes and brain pathology in $Sparc^{-/-}$ mice to altered functions of Sparc in EC.

6.2 CLDN5, claudin 5

Claudin 5 is a tight junction protein and has a key role in the formation of paracellular pores that function in mediating selective ion permeability at the BBB (Anderson, 2001; Nitta et al., 2003). Campbell and colleagues (Campbell et al., 2012) demonstrated that administration of short interfering RNA against Cldn5 significantly improved neurological

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severity scores in mice with focal oedema. These effects were observed as early as 8 h post-injury and lasted for 1 week. The treatment led to decreased cell death in the CA1 region of the hippocampus and improved performance in the T-maze. Kanoski et all (Kanoski et al., 2010) studied the effects of a high-energy diet on hippocampal function and BBB integrity in the rat. Consumption of the high-energy diet impaired the hippocampus-dependent but not hippocampus-independent discrimination task in the T-maze. Deficient hippocampal memory was associated with decreased mRNA expression of tight junction proteins, including Claudin-5 and −12, in the choroid plexus and the BBB. Consistent with impairment of hippocampus-dependent memory, an increased permeability of BBB was observed in the hippocampus, but not in the striatum and prefrontal cortex in rats exposed to high-energy diet. De Senna and associates (de Senna et al., 2015) assessed the effects of treadmill training on cognitive and motor behavior in diabetic rats, and associated expression of BBB proteins in the hippocampus and striatum, including Cldn5 and aquaporin 4. Diabetes decreased Cldn5 expression in the hippocampus and striatum and AQP4 in the hippocampus. Exercise restored levels of Cldn5 in the striatum, but not in the hippocampus of diabetic rats. Reduced expression of aquaporin 4 was not influenced by exercise. The data suggest exercise can improve memory retention and enhance motor skills, possibly by restoring expression of the key BBB factors. Although attributing the effects of these genetic and environmental manipulations to EC/BBB disturbances alone is not warranted, the findings are consistent with hypotheses that link the behavioral consequences of down-regulation of *Sparc* and *claudin 5* to EC/BBB and to behaviors reminiscent of aspects of SZ.

6.3 FLT1, fms-related tyrosine kinase 1 (VEGF receptor 1)

As a receptor for VEGFA, VEGFB and PGF, this tyrosine-protein kinase plays a critical role in the development of vasculature during embryogenesis and regulates endothelial cell proliferation and survival in adulthood (Seetharam et al., 1995; Sela et al., 2008). An indirect indication of the possible role of *FLT1* in behavior comes from two studies that assessed effects of enriched environment, or spatial training in the Morris water maze test on expression of VEGF in the hippocampus or over-expression of VEGF in this brain region (Cao et al., 2004; During and Cao, 2006). Both enriched environment and training increased hippocampal expression of VEGF. Consistent with this effect, over-expression of VEGF in the hippocampus increased neurogenesis and improved cognition in adult animals. In contrast, placental growth factor, which signals through *Flt1*, decreased neurogenesis and learning, but increased endothelial cell proliferation. New animal models of this receptor are needed to directly evaluate its role in possible neurobehavioral abnormalities related to symptoms of SZ.

6.4 FN1, Fibronectin 1

Fibronectin is a major element of the ECM in the brain and is involved in the molecular mechanisms of neuronal development, cell adhesion and migration (Humphries et al., 2015; Schwarzbauer and DeSimone, 2011). Alternative splicing at three conserved genomic regions gives rise to multiple FN polypeptides (Kornblihtt et al., 1996), which play specific roles in FN dimer secretion and adhesion. In order to assess the role of the FN's alternative exons splicing *in vivo*, Chauhan and associates generated two constitutive mouse models (Chauhan et al., 2005) devoid of extra domain A (EDA) exon regulated splicing in the FN

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gene that constitutively include ($EDA^{+/+}$), or exclude the same exon ($EDA^{-/-}$). $EDA^{-/-}$ mice exhibited decreased motor-coordination skills on accelerated rotarod and rearing activity in open field, while $EDA^{+/+}$ mice showed diminished ambulation in the open field test. No group differences were found in anxiety- or depression-related behaviors. Although the data implicate the *FN1* gene in the brain functions, limited brain and behavioral evaluation provides little if any insight into a possible link of *FN1* to SZ in humans. As with all other genes being reviewed here, the biological mechanisms whereby abnormal expression of *FN1* may be responsible for aspects of SZ remain poorly understood and await the development of relevant experimental models to link this gene to the disorder.

To conclude, our brief overview of the available models clearly demonstrated how little is known regarding the relationship of the dysregulated EC/BBB genes to behavioral outcomes relevant to schizophrenia; however, the studies mentioned above provide evidence to suggest that further exploration of these relationships could provide valuable insights. The major roadblock in the field is scarcity of animal models with conditional deletion or over-expression of the specific EC factors in a time, region-, and cell type-specific manner. The composition of BBB is very complex (Blanchette and Daneman, 2015). Sorting out convergent and divergent contributions of the different components of BBB will be necessary to understand their specific roles in relation to neurobehavioral consequences. Combining new genetic, imaging and circuitry approaches will help address these difficult questions.

7. Perspective: Vascular remodeling and hypoxia signaling as risk factors in SZ

Active angiogenic mechanisms elicited by growth factors and tissue remodeling proteins released by neuronal and glial cells promote vascularization and distribution of adequate blood supply during neuro- and glia- genesis and provide for a well-developed and plastic vascular plexus that maintains healthy brain function throughout the lifespan.

While morphometric data on area of coverage, total length, mean density and diameters of cerebral vascular endothelium in individuals with SZ are sparse, conflicting and inconclusive, the cerebral vascular transcriptome changes and functional genomics data reinforced by the ultrastructural abnormalities of capillaries and pericapillary cellular environment and regional CBF deficit are supportive of cortical vascular/BBB dysfunction in SZ. The evaluation of the vascular component of the multiregional cortical transcriptome described above, additionally, supports a hypothesis of a defective cerebral vasculature in SZ characterized by aberrant transcriptional changes within the multiple pathways involved in the maintenance and activation of angiogenesis, such as receptor tyrosine kinase-, Notch-, WNT- and hypoxia-signaling; genes associated with structural integrity of vascular basal membrane and ECM/BBB. The hypothesis is strengthened further by independent gene-set studies of SZ-associated variants and confirmation of previous analyses (Moises et al., 2015; Schmidt-Kastner et al., 2012) showing strong representation of vascular specific markers among SZ candidate genes.

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Defective cerebral angiogenesis linked to an immature vascular plexus may create conditions influencing a wide-range of neural processes, including BBB permeability, myelination, formation of perineuronal nets-ECM and neuronal connectivity. This hypothesis can be tested in multiple ways, including (i) pharmacological intervention targeting vascular endothelium remodeling, similar to the one initiated by Ehrenreich, et al (Ehrenreich et al., 2007) using recombinant human erythropoietin, which improves ischemic tissue recovery by stimulating angiogenesis (Adelibieke et al., 2013; Chai et al., 2016; Mengozzi et al., 2012), and showed a significant improvement in cognitive function even in chronic SZ patients; (ii) development of sensitive neuroimaging in live subjects such as delayed contrast extravasation (Israeli et al., 2010) and molecular techniques for BBB integrity assessment in postmortem brain tissue. (iii) Animal models of defective angiogenesis and cerebral hypoperfusion can unveil the consequences of immature vascular plexus, permeable BBB and mild ischemia on neurobiology and behavioral traits, testing plausible mechanisms and potential treatments. Inarguably, examination of gene-environment interaction between SZ susceptibility genes and angiogenesis will shed light on the effect of prenatal or perinatal hypoxia, like it has been initiated in mice haploinsufficient for reelin (Howell and Pillai, 2014).

Vulnerability of OLGs and related myelin deficits, neuronal mitochondrial dysfunction and impaired energy metabolism, synaptic response to both negative and positive symptoms of SZ, are all features that can stem from persistent mild cerebral ischemia (Moises et al., 2015), including oxygenation and perfusion compromises caused by cerebral microvascular dysfunction. No single factor such as angiogenesis or vascular inflammation is likely to be responsible for the molecular changes, including repair capacity in vascular endothelium, and behavioral manifestations seen in SZ, however, the evidence reviewed here suggests that multiple events affecting coupling in the neurovascular unit are likely to play a significant role in the etiopathology of SZ. The pathways and mechanisms highlighted in this review provide a rational, evidence-based, basis for hypotheses testing in future research.

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HIGHLIGHTS

- Angiogenic processes guide development and maintenance cerebral vascular endothelium, which guarantees adequate regional blood flow and safeguard normal brain function.
- Genetic, neuroimaging, postmortem gene expression and morphological studies implicate cerebral microvasculature as a potential contributor to the pathophysiology of schizophrenia.
- Microvascular gene expression signature show changes in angiogenesis/vascular remodeling and offer support for an anomalous blood brain barrier function in schizophrenia.
- Neurobehavioral consequences and limitations of manipulating the mechanisms of cerebral angiogenesis in animal models relevant to schizophrenia are discussed.

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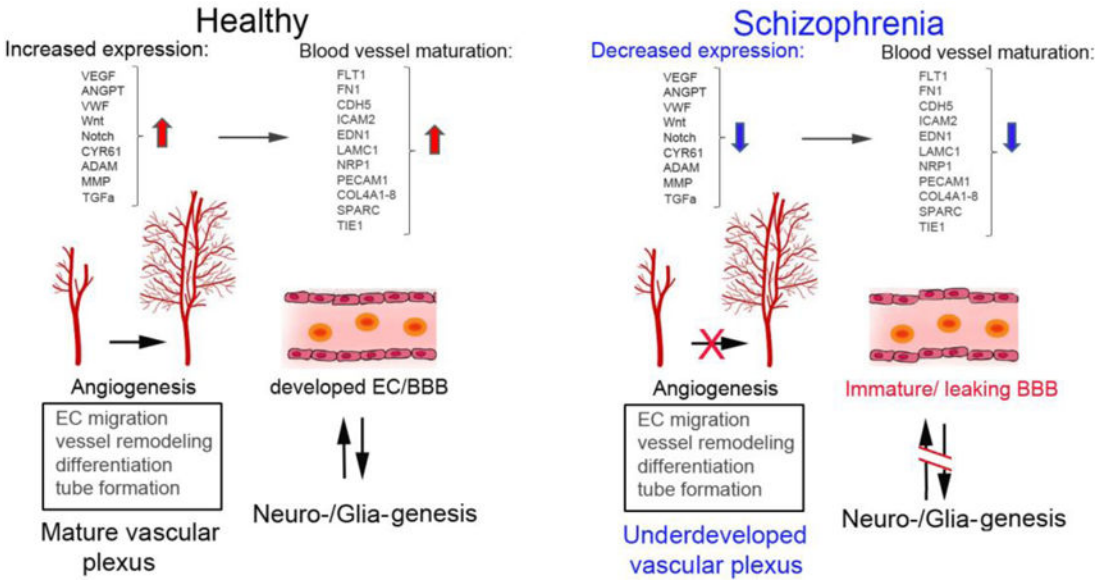

Figure 1.

Plausible mechanisms (including participating genes) that take place in postnatal angiogenesis and their general effects in healthy and schizophrenia brains.

Active angiogenic mechanisms aiming development of adequate vascular supply take place during early development (neurogenesis) and after birth, during myelination and synaptic plasticity, the processes that entail extraordinary metabolic demands. Growth factors, signaling angiogenic pathways and tissue remodeling proteins are released from neural cells, leading to the building of well-developed vascular plexus that maintain healthy brain function. In schizophrenia reduced gene expression of angiogenesis signals and remodeling proteins suggests reduced vessel sprouting/microvascular length, leading to an overall reduced blood flow and oxygenation of brain tissue. At the cellular level, the ECs begin to reduce the expression of growth factor receptors affecting the survival capacity of vascular endothelium and formation of endothelial junction in BBB. Many of these factors combine may create conditions for permeable/underdeveloped BBB, which result in spill over systemic plasma proteins into the brain tissue affecting wide-range of neural processes, including myelination, formation perineuronal nets-ECM and ultimately neuronal connectivity.

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Table 1

Differentially expressed EC/microvascular genes in SZ across 15 cortical regions (p-Val sorted) (as described in (Katsel et al., 2005a)).

Symbol	Entrez Gene Name	Affymetrix	p-value (corr)*	Fold change SZ*	t-scores*	Fold enriched in EC†
ICAM2 only EC	intercellular adhesion molecule 2	213620_s_at	1.09E-10	−1.48	−7.98	730.3
SPARC	secreted protein, acidic, cysteine-rich (osteonectin)	212667_at	7.55E-09	−1.77	−7.51	34.1
CEBPD	CCAAT/enhancer binding protein delta	203973_s_at	6.09E-08	−1.65	−7.46	4.1
CLDN5	claudin 5	204482_at	1.30E-07	−1.38	−7.01	676.9
IFITM1	interferon induced transmembrane protein 1	201601_x_at	6.53E-07	−1.61	−7.37	50.2
CDH5	cadherin 5	204677_at	6.84E-07	−1.30	−5.69	306.7
IL13RA1	interleukin 13 receptor, alpha 1	201887_at	1.22E-06	−1.35	−5.23	3.2
IL6ST	interleukin 6 signal transducer (GP130, oncostatin M receptor)	212195_at	1.22E-06	−1.53	−6.19	18.7
IFITM2	interferon induced transmembrane protein 2	201315_x_at	1.23E-06	−1.59	−6.81	4.4
EMP1	epithelial membrane protein 1	201324_at	1.37E-06	−1.81	−6.31	6.4
STOM	stomatin	201061_s_at	1.66E-06	−1.38	−5.36	5.8
PECAM1	platelet/endothelial cell adhesion molecule 1	208982_at	1.88E-06	−1.38	−5.34	291.1
MAFF	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F	36711_at	3.88E-06	−1.64	−7.02	4.1
ELTD1	EGF, latrophilin and seven transmembrane domain 1	219134_at	4.65E-06	−1.15	−3.75	584.4
SDPR	serum deprivation response	222717_at	6.26E-06	−1.13	−3.65	69.4
ANGPTL4	angiopoietin-like 4	223333_s_at	6.39E-06	−1.25	−4.22	7.2
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1	222162_s_at	2.06E-05	−1.39	−6.21	5.2
LAMC1	laminin, gamma 1	200771_at	3.11E-05	−1.21	−3.01	7.9
VWF	von Willebrand factor	202112_at	3.36E-05	−1.39	−4.65	43.1
ANGPT2	angiopoietin 2	205572_at	5.80E-05	−1.24	−3.98	183.5
FLT1	fms-related tyrosine kinase 1, VEGF receptor 1	226498_at	7.63E-05	−1.36	−4.57	411.2
FN1	fibronectin 1	212464_s_at	1.01E-04	−1.27	−4.29	137.1
MSN	moesin	200600_at	1.65E-04	−1.39	−4.62	23.4
EDN1	endothelin 1	222802_at	1.72E-03	−1.17	−3.21	106.8
COL4A1	collagen, type IV, alpha 1	211980_at	2.41E-04	−1.36	−5.44	44.7
TAGLN	transgelin	205547_s_at	3.77E-03	−1.92	−3.19	3.1
SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1	201250_s_at	4.65E-03	−1.13	−0.98	35.5
TIE1	tyrosine kinase with immunoglobulin-like and EGF-like domains 1	204468_s_at	8.81E-03	−1.20	−3.91	388.2

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Symbol	Entrez Gene Name	Affymetrix	p-value (corr)*	Fold change SZ*	t-scores*	Fold enriched in EC†
COL4A2	collagen, type IV, alpha 2	211964_at	9.64E-03	−1.32	−2.42	52.7
ADM	adrenomedullin	202912_at	1.11E-02	−1.33	−2.05	31.2
EPAS1	endothelial PAS domain protein 1	200878_at	1.51E-02	−1.15	−1.73	5.1

*p-Val (corr) Benjamini-Hochberg corrected (Benjamini and Hochberg, 1995), one-way ANOVA; Fold change in SZ compare to controls, shown for hippocampus; t-scores, contrast analysis 15 brain regions (all $p<0.001$).

†Fold enriched in EC compare to neurons, astrocytes, OPCs, oligodendrocytes (excluding microglia), based on an RNA-Seq transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex (Zhang et al., 2014) (http://web.stanford.edu/group/barres_lab/brain_maseq.html).

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Table 2

Ingenuity Canonical Pathways enriched in vascular endothelium differentially expressed genes in SZ.

Pathways	p-val	z-score	Total genes	In Data (genes)
Interferon Signaling	3.72E-11	−3.05	36	13
Tight Junction Signaling	1.57E-09	−2	140	21
Integrin Signaling	3.81E-07	−1.53	191	23
Cardiac Hypertrophy Signaling	2.17E-07	−0.43	223	24
Ephrin Receptor Signaling	5.34E-06	−1.29	163	18
Axonal Guidance Signaling	1.46E-05	NA	442	31
VEGF Signaling	3.55E-05	−1.90	92	12
IGF1 Signaling	7.48E-05	−1	91	11
Oncostatin M Signaling	8.28E-05	−2.45	28	6
Wnt/PCP pathway	8.49E-04	−0.38	63	8
Angiopoietin Signaling	1.63E-03	−1.41	66	8

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Table 3

Metacore process networks enriched in vascular endothelium differentially expressed genes in SZ.

Process Networks	p-val	Total genes	In Data (genes)
WNT signaling	7.27E-07	177	32
Regulation of angiogenesis	8.02E-07	222	36
Cell junctions	1.65E-06	162	29
Integrin-mediated cell-matrix adhesion	5.86E-06	214	33
Regulation of epithelial-to-mesenchymal transition	1.55E-05	225	33
Actin filaments	1.85E-05	176	28
Anti-Apoptosis mediated by external signals via MAPK and JAK/STAT	2.25E-05	179	28
Wnt, beta-catenin, Notch, VEGF, IP3 and integrin signaling	7.93E-05	151	24

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Table 4

Gene expression changes for identified vascular endothelium specific GWAS SZ genes.

Symbol	Gene Name	Affymetrix	Entrez	t-score
MANA2A	mannosidase, alpha, class 2A, member 1	226538_at	4124	−6.33
NCK1	NCK adaptor protein 1	211063_s_at	4690	−3.94
CTNND1	catenin delta 1	208862_s_at	1500	−3.12
SLC39A8	solute carrier family 39 (zinc transporter), member 8	219869_s_at	64116	−3.08
NFATC3	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	210555_s_at	4775	−2.87
STAG1	stromal antigen 1	202294_at	10274	−2.66
BAG5	BCL2 associated athanogene 5	202985_s_at	9529	−2.18
DPP4	dipeptidyl-peptidase 4 (CD26, adenosine deaminase complexing protein 2)	203717_at	1803	−2.16
STAB1	stabilin 1	38487_at	23166	−1.81
SRPK2	SRSF protein kinase 2	203181_x_at	6733	3.06
DGKZ	diacylglycerol kinase zeta	207556_s_at	8525	4.99
RRAS	related RAS viral (r-ras) oncogene homolog	212647_at	6237	ns
PSMB10	proteasome subunit beta 10	202659_at	5699	ns
STAT6	signal transducer and activator of transcription 6, IL-4 induced	201331_s_at	6778	ns
GATAD2A	GATA zinc finger domain containing 2A	238324_at	54815	ns
EGR1	early growth response 1	201694_s_at	1958	ns
PPP1R13B	protein phosphatase 1 regulatory subunit 13B	216347_s_at	23368	ns
VSIG2	V-set and immunoglobulin domain containing 2	228232_s_at	23584	ns

† ns - non significant