A Vascular-Centric Approach to Autism Spectrum Disorders

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ABSTRACT: Brain development and function are highly reliant on adequate establishment and maintenance of vascular networks. Early impairments in vascular health can impact brain maturation and energy metabolism, which may lead to neurodevelopmental anomalies. Our recent work not only provides novel insights into the development of cerebrovascular networks but also emphasizes the importance of their well-being for proper brain maturation. In particular, we have demonstrated that endothelial dysfunction in autism spectrum disorders (ASD) mouse models is causally related to altered behavior and brain metabolism. In the prenatal human brain, vascular cells change metabolic states in the second trimester. Such findings highlight the need to identify new cellular and molecular players in neurodevelopmental disorders, raising awareness about the importance of a healthy vasculature for brain development. It is thus essential to shift the mostly neuronal point of view in research on ASD and other neurodevelopmental disorders to also include vascular and metabolic features.

KEYWORDS: Autism, cerebrovascular, metabolism, neurodevelopment, angiogenesis, endothelium

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Brain Health Relies on Neurovascular Interactions

The brain is a relatively small organ, approximately 2% of body mass, yet it consumes close to one-quarter of the total body energy available at rest. In other words, about 25% of the cardiac output is dedicated to the brain's energy needs for sustaining function of neural circuits.[1] The elevated energy demands, and low capacity to store energy fuel, together imply that the maturing brain becomes reliant on an uninterrupted supply of oxygen and nutrients from the bloodstream and in turn must efficiently use energy substrates as well as remove waste products. The ensemble of biological processes put in place to regulate delivery, intake, and utilization of energy sources is known as metabolism. During brain development, the formation and maturation of neurons, blood vessels, and glial cells are precisely orchestrated to ensure proper blood supply to withstand healthy brain growth. These "neuro-vascular" interactions actively regulate energy allocation for daily brain functioning, orchestrating a tight balance known as metabolic homeostasis. The synergistic function of vascular endothelial cells (lining the blood vessel wall), glial cells (eg, astrocytes and microglia), smooth muscle cells, pericytes, and neurons is required to uphold brain metabolism. The anatomical substrate of these multicellular interactions is known as the neurovascular unit (NVU), or neuro-glio-vascular unit, which regulates energy import and utilization. Healthy brain growth and function rely not only on the establishment of neuronal networks, but also on key vascular features: (i) The establishment of dense vascular networks (through neovascularization and angiogenesis) for effective brain blood perfusion; (ii) The formation and function of a tight blood-brain barrier to maintain a controlled environment, providing suitable conditions for cellular health; and (iii) The regulation of cerebral blood flow to match the demands of neural cells through the phenomenon of neurovascular coupling (whereby increases in neural network activity lead to increased regional cerebral blood flow). However, neurovascular coupling is not functional until approximately 3 weeks of age in rodents and at full term birth in humans.[2] It is hypothesized that the developing brain exists in a state of relative hypoxia, where metabolic demands are not satisfied by a corresponding increase in blood flow.[2] Hence, it must rely on alternative mechanisms to support demands imposed by its rapid expansion. In both humans and mice, developing brain vessels also arise from veins and venules.[3,4] Overall, genetic programs and hypoxia must act as major drivers for vascular

growth during embryonic development, and neuronal activity promotes brain angiogenesis early after birth.[1,5]

Consequently, common rules apply for guiding neuronal and vascular development, and molecules secreted by endothelial cells modulate the genesis and fate of neuronal and glial progenitors.[4,6] In this context, it becomes intuitive that early abnormalities in the establishment and function of the NVU will affect brain function in the long term. This emphasizes the need of investigating non-neuronal cells in the context of neurodevelopmental disorders, and the importance of shifting the neuronal point of view when studying the brain.

Neurodevelopmental Disorders Are Not Just About Neurons

Several inherited or acquired neurological disorders can originate from, or be perpetuated by, a defective brain vasculature. For instance, CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) and *COL4A1* mutations, small vessel diseases, lead to cerebrovascular dysregulation and in turn to cognitive impairment.[7] In addition, onset and/or progression of various neurological disorders have been associated with anomalies in cerebrovascular networks, including schizophrenia, dementia, and neurodegenerative disorders like multiple sclerosis or Alzheimer's disease.[8] Moreover, as reviewed recently,[8] neurodevelopmental and neurodegenerative diseases share similar vascular abnormalities despite emerging at different stages of life. However, the links between cerebrovascular deficits and neurodevelopmental disorders are only starting to emerge.

Considering the tightly regulated metabolism of proper brain development, disruption of energy supply and/or utilization during critical developmental periods can irreversibly impact brain maturation. The young brain is indeed particularly vulnerable to shortfalls in blood supply or defects in energy metabolism, and early metabolic disturbances may lead to atypical brain maturation and altered cognitive development, such as in autism spectrum disorders (ASD). ASD represent a set of complex neurodevelopmental disorders that impact attention, memory, motor coordination, language, speech, and social interactions. While ASD have environmental and genetic origins, the underlying causes remain largely unknown. The ASD brain has been characterized with exaggerated rates of growth in the first year of life, altered neuronal patterning, increased prevalence of seizures, and enhanced expression of neurotransmitter receptors. The development of genetically engineered rodent models have made it possible to characterize mutations linked to ASD such as in *Shank3, Neurexin1, Syngap1*, or *Mecp2* genes known to normally control synaptic transmission, neuronal morphology, and excitability, as well as animal behavior. These mouse models of ASD have demonstrated abnormal corticogenesis during postnatal development, cortical neuronal asynchrony, spontaneous seizures, and synaptic defects. While these models enable the clarification of neuronal underpinnings of ASD symptoms, the contribution of vascular and metabolic features to ASD pathophysiology have been largely overlooked. This critical knowledge gap now represents a growing focus in neuroscience.

Recent studies have obtained strong evidence demonstrating structural and functional cerebrovascular deficits in ASD patients and mouse models. In humans, evaluations by functional magnetic resonance imaging, positron emission tomography, or arterial spin labeling revealed cerebral hypoperfusion in approximately 75% of children with ASD compared with healthy individuals.[8] In a mouse model, we revealed a surprising contribution of vascular deficits to the pathogenesis of 16p11.2 deletion syndrome,[9] one of the most common genetic causes of human ASD. Copy number variations associated with psychiatric conditions are found at a higher frequency in the human 16p11.2 locus. The 16p11.2 deletion (heterozygous) comprises loss of approximately 30 highly conserved genes, and carriers display intellectual disabilities, learning and communication deficits, large head size, an increased prevalence of seizures, as well as metabolic problems. Among the conserved genes found in the human 16p11.2 locus, several genes are involved in maintaining cell function and metabolism. For instance, the genes *Taok2* and *Kctd13* regulate neuronal differentiation and maturation, while *Doc2A* and *Prrt2* control neuronal excitability. *Mapk3* regulates endothelial cell proliferation and migration, *Tmem219* and *Coro1A* negatively modulate endothelial cell apoptosis, and *Maz1* potentiates vascular growth. In addition, *AldoA* regulates glycolysis and gluconeogenesis. Working with the widely accepted *16p11.2*$^{df/+}$ mouse model[10] of 16p11.2 deletion syndrome, we revealed a causal relationship between cerebrovascular endothelial deficits and neuronal/behavioral abnormalities.[9] Neurovascular coupling was attenuated in adult *16p11.2*$^{df/+}$ male mice, which was attributed to endothelial dysfunction following quantification of cerebral artery responses to vasomodulators. Although adult vessel density was unchanged in *16p11.2*$^{df/+}$ mice, two-week old *16p11.2*$^{df/+}$ male mice displayed impaired brain angiogenesis compared with control littermates, which was confirmed in vitro as an endothelial cell autonomous deficit. Deep RNA-sequencing analysis of the *16p11.2*$^{df/+}$ mouse brain endothelial transcriptome revealed important changes in the expression of genes involved in the regulation of angiogenesis such as *Angpt2* and *Apln*. These results altogether demonstrated altered endothelial function caused by 16p11.2 haploinsufficiency. From this, we suggested that vascular factors may participate in the onset of ASD, at least in mice. To test this, we generated a mouse model with an endothelial-specific 16p11.2 haploinsufficiency. Notably, brain endothelial cells have essential roles in brain health including regulating vessel tone, chemical exchange with astrocytes and neurons, protecting the brain via the blood brain barrier, as well as releasing vesicles as Weibel-Palade bodies to regulate hemostasis, inflammation and angiogenesis through trophic factors. The endothelial-specific

16p11.2 deletion model displayed ASD-related behaviors,[9] highlighting the necessity of functional endothelial cells for maintaining proper brain function.

Brain Metabolism in ASD

Glucose transporter-1 deficiency syndrome (GLUT-1 DS) is an infantile-onset neurodevelopmental disorder characterized by pediatric epileptic encephalopathy and a movement disorder comprised of ataxia and spasticity. Brain hypometabolism in GLUT-1 DS has been compared to the Alzheimer's disease brain, with the latter also showing signs of reduced GLUT-1 function.[11] Intriguingly, *16p11.2$^{df/+}$* mice displayed striking similarities with mouse models of GLUT-1 DS, including reduced brain angiogenesis, enhanced evoked neuronal activity, and altered behaviors with hyperactivity.[9,11] Anomalies in brain metabolism have been identified in Alzheimer's disease, schizophrenia, and ASD[8] but the underlying mechanisms in ASD remain to be defined. As neuronal, vascular, and glial cells are metabolically interdependent to ensure proper brain maturation, it is expected that constitutive genetic mutations associated with ASD lead to perturbed cellular machinery and to brain metabolic imbalance. While we have identified that *16p11.2$^{df/+}$* mice display striking sex-specific differences in body composition, energy expenditure, and concentrations of circulating plasma metabolites related to mitochondrial function, suggesting that constitutive 16p11.2 haploinsufficiency also affects whole-body metabolism,[12] we have recently demonstrated brain metabolism alterations in adult *16p11.2$^{df/+}$* males. These include increased glucose uptake, changes in glucose and lactate utilization, as well as altered cortical metabolite profiles.[13]

Often, metabolic discrepancies in ASD rodent models are associated with concurrent mitochondrial dysfunction. In endothelial cells from adult *16p11.2$^{df/+}$* males, we revealed a reduced number of mitochondria and a lack of transcription factor NT-PGC-1α, a truncated variant of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial biogenesis.[13] In mice with endothelial-specific 16p11.2 haploinsufficiency, brain glucose metabolism alterations were also observed, suggesting that these alterations are secondary to (or compensatory to) endothelial dysfunction. Nevertheless, the cellular and molecular mechanisms of these alterations remain to be fully elucidated. These findings open new questions in ASD brain metabolism research field: How are metabolites utilized during the activation of these compensatory mechanisms? When can these changes be detected during brain development? What is the long-term impact of metabolic shifts on brain function? Can these metabolic changes be targeted for future therapeutic solutions? With such metabolic link to ASD in mind, existence of vascular and metabolic players must be taken into consideration when investigating ASD risk factors.[13]

Prematurity and Perinatal Hypoxia as Risk Factors for ASD

Separate from genetic etiologies, prematurity is an independent risk factor for ASD.[14] Germinal matrix hemorrhage (GMH), a feared complication of extremely premature birth, can cause hydrocephalus and stroke immediately, cerebral palsy, intellectual disability, ASD, and/or psychiatric disorders in the long-term.[15] While the exact cause of GMH remains elusive, it is hypothesized to occur due to a combination of disturbed oxygen delivery and vascular immaturity in this particular brain region.[16] With emerging evidence, another way to frame GMH may reflect metabolic failure in neurovascular coupling. For example, brain vascular cells were shown to be exquisitely sensitive to changes in oxygen tension during development in a mouse model of GMH.[17] Importantly, periventricular blood vessels were the last to mature. Typically, brain endothelial cells are thought to primarily use glycolysis as an energy source.[11] In human brain development, endothelial cells may switch from oxidative phosphorylation to glycolysis during the second trimester.[4] These differential metabolic states may underlie this vulnerable window for GMH and the subsequent ASD. Future investigations will need to map out the temporal and spatial metabolic programs of neurovascular dependence in the human brain to address germinal matrix hemorrhage.

Perinatal hypoxia is another environmental condition significantly contributing to dysfunction in the central nervous system and leading to neurodevelopmental disorders.[18] It is characterized by insufficient oxygen delivery to the fetus before, during, or after birth. Chronic intermittent hypoxia (CIH), more prevalent than continuous hypoxia in early postnatal life (often stemming from sleep-disordered breathing), emerges as a notable contributor to disability in neonates. This impact is particularly noteworthy in premature infants, especially those with extremely low birth weight. Evidence indicates that CIH is associated with heightened neuroinflammation and oxidative stress, impacting the integrity of both gray and white matter and contributing to cognitive impairment. While the specific effects of CIH on cerebrovascular development remain poorly understood, CIH may induce enduring alterations in cerebrovascular structure and function, possibly by disrupting the finely regulated process of developmental angiogenesis.[19]

Concluding Remarks

Worldwide, 1 in 160 children has been diagnosed with ASD (World Health Organization [WHO] fact sheet on ASD, 2021). As ASD incidences are on the rise, exploring novel avenues for care represents an urgent clinical and economic need. Outcomes of innovative ASD research will promote the identification of new players in its pathogenesis, which is an essential prerequisite for developing the novel diagnostics and therapeutics that are desperately needed for patients, their relatives, and caregivers. Since most studies have focused on neuronal aspects of ASD, looking at neurodevelopmental disorders through the

vascular lens is a much-needed paradigm shift. Insight from such game-changing studies will allow scientists to gain novel understanding into how the vasculature control critical features of brain organization and function.

Author Contributions

B.L. and J.O. conceived the idea. All authors participated in drafting and editing the manuscript.

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