Pathologic sequelae of vascular cognitive impairment and dementia sheds light on potential targets for intervention

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ABSTRACT

Vascular contributions to cognitive impairment and dementia (VCID) is one of the leading causes of dementia along with Alzheimer's disease (AD) and, importantly, VCID often manifests as a comorbidity of AD(Vemuri and Knopman 2016; Schneider and Bennett 2010)(Vemuri and Knopman 2016; Schneider and Bennett 2010). Despite its common clinical manifestation, the mechanisms underlying VCID disease progression remains elusive. In this review, existing knowledge is used to propose a novel hypothesis linking well-established risk factors of VCID with the distinct neurodegenerative cascades of neuroinflammation and chronic hypoperfusion. It is hypothesized that these two synergistic signaling cascades coalesce to initiate aberrant angiogenesis and induce blood brain barrier breakdown trough a mechanism mediated by vascular growth factors and matrix metalloproteinases respectively. Finally, this review concludes by highlighting several potential therapeutic interventions along this neurodegenerative sequalae providing diverse opportunities for future translational study.

Introduction

Vascular contributions to cognitive impairment and dementia (VCID) is a complex syndrome that encompass a diverse array of pathologies resulting in disruptions of blood flow in the brain ([4]; T. [165]). It is becoming increasingly recognized that VCID is one of the leading causes of dementia along with Alzheimer's disease (AD) and is frequently found co-morbid with AD pathologies. Experts project a significant increase in the number of patients presenting with both cerebrovascular and neurodegenerative co-morbidities as the number of persons living into their 80 s and 90 s increases [114]. Recent studies have even demonstrated pathologic vascular changes precede the appearance of amyloid (Aβ) plaques and neurofibrillary tangles, characteristic proteinopathies associated with AD, further implicating cerebrovasculature pathologies as an important topic of study in the fields of dementia and neurodegeneration [[28], [59]].

The overlap between VCID and AD continues when considering significant risk factors associated with their disease progressions. Hypertension, diabetes, hyperhomocysteinemia (HHCy) and hyperlipidemia are risk factors for both AD and VCID all leading to a state of both chronic neuroinflammation and chronic cerebral hypoperfusion or hypoxia [[64], [121]]. Neuroinflammation is both an important instigator and consequence of cerebrovascular pathology making this an

important potential therapeutic target for impeding disease progression [[26], [114], [142]]. While there is currently no cure for VCID, several studies have been focused on mitigating the aforementioned risk factors leading to chronic hypoxia and inhibiting the subsequent neuroinflammatory sequelae [[48], [122]]

In the following review a brief overview of the current knowledge surrounding VCID will be provided with a focus on the basic mechanisms linking well-established risk factors with the distinct signaling cascades of neuroinflammation and chronic hypoperfusion. Then the coalescence of these two pathologic signaling cascades and their synergistic impact on the downstream activation of further neurodegenerative sequalae will be discussed. Finally, several potential therapeutic interventions to target specific aspects of the degenerative cascade leading to VCID progression will be highlighted.

Vascular contributions to cognitive impairment and dementia was first used as an umbrella term to update healthcare professionals on understanding the complexity and evolution of VCI [40]. It was subsequently coined VCID and after it was made a priority research area at the NIH, was further referred to as vascular dementia, vascular cognitive impairment and/or vascular contributions to dementia [[125], [149]]. There has been a variety of nomenclatures as relates to cerebrovascular insults impacting cognition. For the purposes of uniformity in this review and adoption of NIH defined nomenclature, the term VCID will be

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used, even if the original authors of the work used the term "vascular dementia (VaD)" or "vascular cognitive impairment (VCI)."

VCID

Given the diversity of vascular contributions that can culminate in cognitive impairment and dementia (microinfarcts, large vessel hemorrhages, white matter hyperintensities, cerebral amyloid angiopathy, etc.) the diagnostic criteria for VCID are not uniform. Two important consensus reports were recently released that provide clinicians and researchers alike with important diagnostic specifications for VCID [it is important to note that this paper uses the term VCI; vascular cognitive impairment] [[123], [124]]. Diagnostic criteria involved the assessment of 5 functional domains including executive function, attention, memory, language and visuospatial function. To be diagnosed with mild VCID, at least one domain has to be imparied but daily living must remain unimpaired. A diagnosis of major VCID must show deficits in at least one of these domains and independent living must also be impaired. As with other systemic vascular pathologies, risk factors such as diabetes, smoking, hypertension, hyperlipidaemia and hyperhomocysteinemia, contribute to VCID incidence(Van [[1], [52], [107], [147]]).

Hypoperfusion and VCID

VCID is characterized by the presence of systemic and cerebrovascular pathologies that can include peripheral perfusion injuries, cardiac pathologies, microinfarcts, microhemorrhages, white matter hyperintensities (WMH), large vessel strokes (ischemic or hemorrhagic), arteriolosclerosis and cerebral amyloid angiopathy (CAA) among others all leading to a state of chronic cerebral hypoperfusion(van [[35], [148]]). Initially, VCID was thought to be caused by arteriosclerotic lesions due to an early study on a cohort of patients with "unequivical evidence of dementia" as described in the original paper. 12% of the 50 cases that in this cohort showed arteriosclerotic lesions in the absence of any plaque formation leading the authors to document dementia of a purely vascular nature [140]. It was later appreciated that patients who experienced a number of large and small cerebral infarcts were more likely to develop dementia than patients with arteriosclerosis [44]. This report made in the early 1970s, led to a new theory claiming any instance of cerebral ischemia was a cause of cognitive impairment and arteriosclerosis contributed to said ischemia [44].

Careful studies performed since this conclusion have found the idea of ischemia related dementia to be more nuanced. One of the first studies to link cerebral hypoperfusion to cognitive impairment and risk of dementia was a report on the Rotterdam Study published almost two decades ago [115]. In a prospective study of adults over 55 years of age, cerebral blood flow velocity was measured using transcranial doppler imaging and cognitive decline was assessed using the Dutch version of the Mini-Mental State examination [115]. Investigators found that subjects with a greater cerebral blood flow velocity were less likely to experience cognitive decline and that low cerebral blood flow velocity was associated with a diagnosis of dementia. Of note, this study was not designed to determine if neurodegernation causes a reduction in cerebral blood flow velocity or if low cerebral blood flow velocity causes neurodegeneration [115]. More recent studies investigating the mechanisms connecting cerebral hypoperfusion to dimentia risk have explored the role of various vasoregulators in the context of different dementia diagnosis. One study conduced using human tissue from the South West Dementia Brain Bank at the University of Bristol (UK) found that endothelin 1 (ET1), an important vasoconstrictor was actually increased in the white matter in vascular dementia compared to controls [13]. This was an interesting finding as it was hypothesized that ET1 would be downregulated in vascular dementia to attempt to compensate for the previously reported white matter ischemia in vascular dementia [[13], [14]]. Finally, recent advances have allowed investigators to use

multifactorial data-driven analysis to analyze over 7000 imaging and fluid biomarkers in healthy and diseased samples to assess disease progression in late onset Alzheimer's disease [55]. This analysis revealed vascular dysfunction to be both the earliest and most impactful pathologic event in the progression of late onset Alzheimer's disease [55]. Further application of this multifactorial data-driven analysis is needed to better characterize the broad impact of vascuar dysfunction in diverse dementia diagnoses.

Now, with advances in brain imaging, small vessel pathologies were found to contribute significantly to VCID. Arteriolosclerosis, microinfarcts, microhemorrhages and white matter changes all were shown to contribute to a chronic state of cerebral hypoperfusion leading to VCID [[43], [44], [150]]. In more recent years a rare genetic mutation, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), was described and has been shown to lead to white matter lesions and early-onset VCID [62]. Additionally, in a study published in 2021, the authors use the TgNotch3^{R169C} mouse model of CADASIL that overexpresses the *Notch3* gene containing the same R169C mutation found in human CADASIL patients [111]. Using an optimized high pressure freezing and freeze substitution protocol, investigators were able to perform electron microscopy on samples with well-preserved myelin [111]. They found that the earliest CADASIL related white matter changes occurred at the level of the myelin sheath [111]. The authors propose a mechanism initiated by chronic hypoxia that terminates in their finding of myelin sheath destruction though further studies are needed to test this hypothesis [111]. Finally, CAA has been defined by its characteristic amyloid-β (Aβ) depositions within the cerebrovasculature. CAA has been shown to cause hypoperfusion and is associated with microhemorrhages, infarcts and white matter lesions, therefore contributing to VCID [8]. All of these distinct risk factors and pathologic halmarks of VCID contribute to a widespread state of chronic cerebal hypoperfusion.

Due to the vast array of pathologies that fall under the diagnosis of VCID, there are several distinct models that are used to provide evidence that hypoxia resulting from chronic cerebral hypoperfusion can cause white matter damage and are used to model various aspects of VCID. These models have been extensively characterized in two comprehensive reviews(Tuo, Zou, and Lei 2021; Y. [166]).

Most of the pathologies that are characteristic of VCID can induce hypoxia in the brain(T. [[38], [41], [57]]). Hypoxia is the condition that occurs when oxygen levels decrease below their normal physiologic state. During a hypoxic period, cells upregulate the transcription factor hypoxia-inducible factor 1 alpha (HIF-1α) resulting in a number of adaptations made in the surrounding tissue in an effort to restore perfusion and oxygenation [90]. This transcription factor can also be activated by reactive oxygen species following injury and by nitric oxide, an important vasodilator [78]. There are several downstream effects of HIF-1α upregulation that will be discussed later in this review.

Inflammation and VCID

Simultaneously, while the defined risk factors and pathologies underlying VCID are heterogeneous, inflammation is a common consequence of all of them. As the major resident immune cell of the brain, microglia are one of the first cells to respond to CNS injury. Generally speaking, in response to these stimuli, microglia transition through a number of phenotypic changes along a vast continuum of "activation states" including the secretion of important pro-inflammatory factors such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) [74]. Alternatively, microglia are capable of anti-inflammatory processes for homeostatic maintenance and repair characterized by the secretion of transforming growth factor-β1 (TGF-β1) and interleukin-10 (IL-10) [[23], [32], [100]].

In a study from our research group, a rodent model shown to produce vascular inflammation, microhemorrhages and cognitive decline similar to that seen in human VCID cases using a diet that induces

hyperhomocysteinemia, an important risk factor for VCID, showed increased microglial activation in both the frontal cortex and the hippocampus [130]. This microglial activation preceded increased microhemorrhages and was concurrent with transcription of important inflammatory mediators, TNF-α and IL-1β [130]. Interestingly, when the same dietary induced hyperhomocysteinemia model of VCID was given to APP/PS1 mice creating a VCID / amyloid pathology comorbidity model, microglial activation was transient and returned to below control levels after 18 weeks of VCID induction. However, this study did not specifically examine disease-associated and homeostatic microglial markers as has been described in recent literature [[65], [157]].

Another pre-clinical study conducted in a rodent model of CAA using rTg-DI rats identified interesting temporal differences in transcription levels of microglia mediated inflammatory markers. The authors observed increases in the more homeostatic or reparative marker TGF-β1 at both early and late-stage disease while the pro-inflammatory markers, TNF-α and IL-1β, were only increased in late-stage disease [173]. Additionally, a small study analyzed the cerebral spinal fluid of 17 patients with MRI confirmed cerebral small vessel disease (cSVD) and 26 healthy controls and found that TNF-α was significantly increased in patients with cSVD [137]. While the role of TNF-α in different neurodegenerative disorders is unknown this remains an important area of study and needs to be investigated further.

The culmination of these studies suggest that there is a significant heterogeneity both between and within models of VCID temporally and spatially. These heterogenous findings further reinforce the utility of recent single cell approaches previously reported in the context of AD. In one such study authors isolated live microglia from cortical post-mortem samples collected by the Memory and Aging Project [98]. Investigators confirmed the populations of microglia to be heterogeneous in both healthy and AD samples though they were able to identify a subpopulation of microglia that were enriched for genes depleted in samples with AD thereby honing in on potential therapeutic targets for this disease state [98]. Additionally, important studies employing single cell analysis have also been conducted in other mouse models of neurodegenerative disease. One group used single cell sorting of immune cells isolated from 5XFAD mice to define a novel subset of microglia shown to associate with neurodegenerative disease coined DAM, or disease associated microglia [65]. These microglia seem to perform protective functions against Alzheimer's disease pathology [65]. Based on both the temporal and spatial heterogeneity seen within the glial cell population in various models of VCID, employment of single cell analysis techniques would likely close a significant knowledge gap in the field and should be prioritized moving forward. Due to this gap in knowledge, the contribution of microglial activation states to neurodegenerative pathology remains a topic of debate and intense study within the VCID and AD fields. There seems to be some consensus that the downstream effects of sustained pro-inflammatory signaling by microglia leads to pathologic neurodegeneration and toxicity, which will be the focus of the remainder of this review. Impo

Coalescent downstream effects

Blood brain barrier breakdown

While both HIF-1α and pro-inflammatory microglia are independently induced by hypoxic conditions in the CNS, their downstream signaling pathways coalesce with the upregulation of matrix metalloproteinases (MMPs) as shown in **Fig. 1** [11]. MMPs have been implicated in VCID via their role in the breakdown of the extracellular matrix and tight junctions of the blood brain barrier (BBB) leading to increased BBB permeability, neurovascular unit dysfunction, and microhemorrhages though ongoing studies to determine causal associations between these two endpoints remain an important focus of ongoing research [156]. Most MMPs including MMP2, MMP3, MMP9 and MMP14 are secreted as zymogens (proMMPx) indicating that they require an additional step before they can assume their active form. MMP9 and MMP2 are members of the gelatinase category of MMPs and have been shown to be expressed in the CNS [66]. MMP2 and MMP9 have been shown to both degrade and activate cytokines, degrade Aβ, impact axonal growth, induce synaptic plasticity, promote angiogenesis, as well as degrade basement membrane proteins and tight junction proteins ([[51], [66], [81], [92], [164], [167]]; B. Q. [171]). As previously mentioned, HIF-1α is upregulated during hypoxic conditions or conditions related to chronic hypoperfusion. HIF-1α in turn upregulates both furin, which enzymatically cleaves proMMP14 to active MMP14, and plasmin, which enzymatically cleaves proMMP3 to active MMP3 [[11], [45]]. MMP14 catalyzes proMMP2 to active MMP2 while MMP3 catalyzes proMMP9 to active MMP9 as shown in **Fig. 1** [[72], [120]]. Additionally, proMMP9 can also be converted into active MMP9 following S-nitrosylation and subsequent oxidation via nitric oxide [[17], [66]]. The expression of proMMP3 and proMMP9 are upregulated by inflammatory cytokines TNF-α and IL-1β via the NF-κβ transcription pathway, both of which are increased as part of the microglial pro-inflammatory response as discussed previously ([[39], [146], [156]]).

Several recent studies have investigated the cell specificity of MMP9 induction in the CNS. In pre-clinical models using two-photon microscopy, pericytes have been strongly associated with increases in MMP9
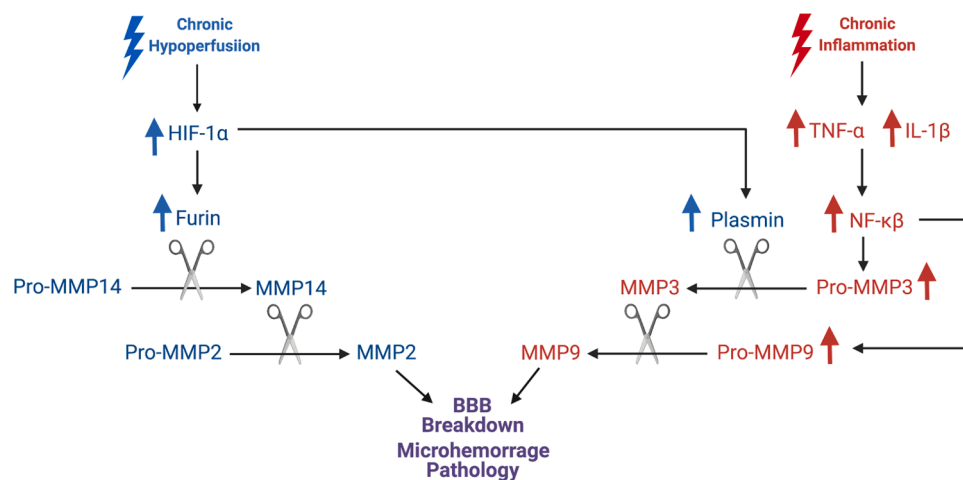

Fig. 1.. Schematic representing the induction and coalescence of the MMP2 and MMP9 systems. Scissors represent enzymatic cleavage and conversion of a substrate. Abbreviations in the figure are: HIF-1α, hypoxia inducible factor-1α; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NF-κβ, nuclear factor-κβ; BBB, blood brain barrier.

mediated BBB breakdown following ischemia [145] and pericytes are also associated with MMP9-related increases in Apolipoprotein-E4 (ApoE4)-dependent cognitive decline as defined by normalized z-scores from 10 neuropsychological tests including the Uniform Data Set battery (version 2.0 or 3.0) as recommended by the National Alzheimer's Coordinating Center's procedures [88] though the mechanism behind these associations remains undiscovered. Mechanistically focused experiments using previously characterized MMP9 knockout mice could be designed to study the causal relationships between these associations [151]. Additional studies in rodent and non-human primate pre-clinical models of both ischemic and hemorrhagic stroke have implicated astrocytes, endothelial cells, microglia and infiltrating neutrophils as additional sources of MMP9 (B. Q. [171]; Del [[129], [175]]).

Interestingly, while MMP9 and MMP2 have primarily been studied in the context of ischemia, they have also recently been associated with AD and CAA pathologies in humans and a murine model of CAA using hAPP transgenic mice though again, no causal relationships have been reported ([50]; J. M. [[47], [71]]). Additionally, in the post-mortem brains of subjects with pathologically confirmed CAA MMP2-positive reactive astrocytes were discovered surrounding Aβ deposits in both grade 3 and grade 4 CAA lesions [50]. Another important study investigated the cerebral spinal fluid of cognitively normal patients and showed that a patient's ApoE allele impacts the magnitude of BBB breakdown with ApoE4 showing increased proinflammatory cytokines, increased levels of MMP9 and increased markers of BBB breakdown [46]. It is also worth noting, however, that ApoE4 individuals with AD present with significantly more CAA at autopsy(P. T. [95]).

In a mouse model of experimental autoimmune encephalomyelitis, a widely accepted model of central nervous system inflammation, MMP9 has been shown to cleave β-dystroglycan, a protein found on the end-feet of perivascular astrocytes [2]. β-dystroglycan on astrocytes binds to its counterpart α-dystroglycan, which is primarily localized on endothelial cells but is also found bound to laminin within the basement membrane, anchoring the astrocytic end-foot to the cerebrovasculature [[138], [162]]. Physiologically, the cleavage of β-dystroglycan is important during development to facilitate the basement membrane remodeling that occurs during the maturation of the vasculature [168]. Due to the localization of the substrates of MMP9, increases in MMP9 transcription resulting from hypoxia and / or inflammation could lead to the detachment of astrocytic end-feet from their associated vessels via the cleavage of β-dystroglycan though additional studies confirming this mechanism are ongoing [105]. Perivascular astrocytes play an essential role in regulating cerebral blood flow and maintaining osmotic and ionic homeostasis in the brain. Changes in cerebral blood flow have been shown to play an important role in the progression of both VCID and AD. Additionally, perivascular astrocytes are important for regulation of local cerebral perfusion and maintenance of autoregulation of systemic blood pressure [54]. The inability to autoregulate changes in cerebral blood flow due to changes in peripheral blood pressure significantly impacts glucose metabolism, ionic homeostasis, and waste clearance including the clearance of Aβ [64].

Astrocytes also play an important role in neurovascular remodeling following injury. Following an insult, astrocytes secrete ApoE which can induce expression of transporters such as adenosine triphosphate-binding cassette subfamily B1 (ABCB1) on endothelial cells [49]. Increased expression of ABCB1 helps restore the BBB, stabilizing the microenvironment and mitigating future injuries [49]. Additionally, astrocytes downregulate the secretion of growth restricting factors following injury to promote an environment conducive for both neural and vascular repair and restoration [49]. As expected, all of these injury repair mechanisms in astrocytes following injury are attenuated with age [49]. Furthermore, in a mouse model of experimental autoimmune encephalomyelitis, when perivascular astrocytes were subject to MMP2 and / or MMP9, BBB integrity was decreased as evidenced by increased leukocyte infiltration [2].

The BBB serves several important functional roles including regulation of ion concentrations, transport of important energy / metabolism components and prevention of neurotoxic components from entering the central nervous system (CNS). The endothelial tight junctions and the astrocytic end-feet are considered components of this BBB. There are several ways to measure changes in the BBB integrity in living humans and animal models including measuring the cerebral spinal fluid/blood albumin ratio ($Q_{albumin}$) or assessing blood derived protein level in the CNS tissue and using various imaging techniques including MRI and PET [[89], [131], [139]]. Recently, it has been reported that BBB integrity as assessed by $Q_{albumin}$, was significantly different between patients diagnosed with AD, Parkinson's Disease, VCID and frontal temporal dementia, compared to control subjects [58]. In this same patent cohort, researchers found that increased BBB breakdown was associated with increases in cerebrospinal fluid (CSF) markers of endothelial dysfunction and increases in proteins associated with angiogenesis [58]. Finally, in our research group, two unique hierarchical clustering analyses were able to use plasma levels of vascular endothelial growth factor (VEGF), MMP1, MMP3, MMP9, and inflammatory cytokines to identify a subset of individuals with VCID from control samples in a cohort of adult research volunteers enrolled in a study for mild cognitive impairment and cerebrovascular disease [161]. These findings suggest that understanding this neurodegenerative sequalae terminating in BBB breakdown and progression to VCID pathology may yield targetable mechanisms alongside important biomarkers used for diagnostic purposes though pre-clinical models need to be conducted to investigate the specific causal relationships leading to these associations.

Aberrant angiogenesis

Angiogenesis is an essential component of recovery following neurologic insult. It helps stabilize cerebral blood pressure, restore neurotrophic support via growth factor secretion to limit neuron loss and promote restoration, and removes waste [49]. Unfortunately, it has been shown that this proliferation of the cerebrovasculature in patients following a stroke is diminished with age [[102], [132]]. Induction of angiogenesis has been shown to occur via multiple pathways including both the chronic hypoperfusion induced HIF-1α pathway and the pro-inflammatory NF-κβ signaling pathway as shown in Fig. 2 [[91], [174]].

As previously discussed, sustained neuroinflammation has been associated with several different downstream pathologies associated with VCID. Neuropathological studies using human autopsy tissue from the Religious Orders Study showed increased microglial activation (as
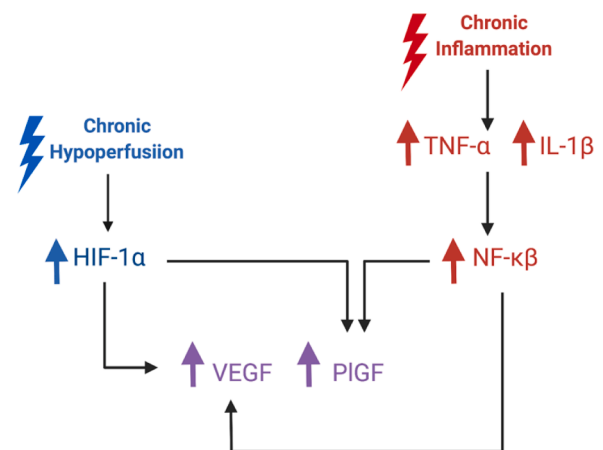

Fig. 2.. Schematic representing the induction of angiogenic factors VEGF and PlGF. Abbreviations in the figure are: HIF-1α, hypoxia inducible factor-1α; VEGF, vascular endothelial growth factor; PlGF, placental growth factor; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NF-κβ, nuclear factor-κβ.

evidenced by IHC staining) in the midfrontal cortex, the substantia nigra and the locus ceruleus but not the hippocampus [33]. Increased angiogenesis was observed in all of these aforementioned areas, however, increased angiogenesis and increased vascular density were also seen in the hippocampus despite a lack of microglial activation in that specific brain region [33]. In this same patient cohort, TNF-α and IL-1β have also been correlated with the induction of angiogenesis and have been shown to increase expression of VEGF, another important angiogenic factor though their direct impact on angiogenesis has yet to be investigated in pre-clinical studies [33].

The process of angiogenesis is complex and has to be carefully orchestrated to be successful. HIF-1α influences a number of downstream signaling pathways including pathways involved in glucose transport, glycolysis, red blood cell production and angiogenesis [174]. Using mice with a targeted deletion of HIF-1α in macrophages, investigators showed that HIF-1α increases transcription of TNF-α, IL-6, IL-1α, IL-1β, and IL-12 in a lipopolysaccharide induced mouse model of sepsis [103]. As previously mentioned, TNF-α and IL-1β are important regulators of the NF-Kβ mediated pro-inflammatory response while in a human cohort of MRI confirmed cSVD, IL-6 and IL-1α have been found to be significant predictors of recurrent lacunar stroke, vascular Parkinsonism, or vascular dementia [127].

Several studies have shown that HIF-1α participates in almost every step of angiogenesis [174]. Studies on tumor angiogenesis support the idea that HIF-1α is essential in the formation of a tumor's blood supply, either via hypoxic conditions present at the tumor site or via genetic manipulation by the cancer cells to induce HIF-1α expression [152], suggesting this transcription factor may play a role in aberrant angiogenesis in the periphery. During episodes of ischemia, HIF-1α accumulates and translocates to the nucleus which stimulates production of vascular endothelial growth factor-A (VEGF-A) [[34], [174]]. Upregulation of VEGF-A is simultaneously induced by TNF-α mediated activation of the transcription factor NF-κβ as shown in **Fig. 2** [135]. VEGF-A increases vascular permeability allowing several plasma proteins to form a scaffold for new endothelial cells [174]' [[7], [106]]. MMPs, which are also upregulated by both the NF-κβ and HIF-1α transcription pathways, degrade existing basement membrane proteins to further enhance angiogenesis(A. R. [94]). Chemotactic molecules direct the new endothelial cells and scaffolding proteins toward new small vessels to complete the lumen [174]. Pericytes surround the new capillaries to reform the BBB and increase stability [174]. Placental growth factor (PlGF), another protein upregulated by both the inflammatory and hypoperfusion signaling pathways as shown in **Fig. 2**, assists in the maturation and differentiation of newly formed vessels and the lumen diameter expands forming a complete vessel [[30], [113]]' [174].

It has long been established that proteins in the VEGF family are the primary mediators of neovascularization though the diversity of this family of proteins is still expanding [[31], [36], [79], [96], [133], [134]]. Currently, seven different ligands and five different receptors (three primary receptors and two co-receptors) have been defined as members of the VEGF family [163]. Each protein has several isoforms and ligands bind selectively to particular receptors with specific affinities contributing to the diverse array of signaling outcomes that this family of proteins are responsible for [163]. One important member of the VEGF family is the aforementioned VEGF-A. This protein is produced in endothelial cells and its expression is potentiated following both hypoxic and hypoglycemic conditions [163]. VEGF-A is a known inducer of angiogenesis [163] and most isoforms of VEGF-A bind VEGF receptor 1 (VEGFR1) and VEGFR2, though VEGF-A binds to VEGFR1 with a significantly higher affinity [[84], [109]]. The various VEGF isoforms have different binding partners and affinities and identifying specific isoforms present may be essential in isolating particular functions. The angiogenic effects of VEGF-A are primarily mediated by VEGFR2 signaling while VEGFR1 has been characterized as a "decoy" receptor that sequesters VEGF-A and inhibits its angiogenic activity [163]. This may be an important process to exploit as researchers seek to ameliorate VEGF-A mediated angiogenesis. PlGF is another member of the VEGF family that also has several isoforms and, upon creating a homodimer, is also specific for VEGFR1 [163]. PlGF alone has little proliferative effects but when forming a heterodimer with VEGF-A signals through VEGFR2, amplifying angiogenesis because of its ability to induce migration of endothelial cells [163] .

For many groups studying ischemia, the angiogenic properties of VEGF-A and PlGF are important physiologic mechanisms to promote in an effort to restore perfusion. Some researchers in the stroke field hypothesize that the many homeostatic functions the angiogenic response may provide, such as increased secretion of neurotrophic factors, increased waste removal and the reinforcement of the BBB, will promote recovery post-stroke [33]. While many of these functions are essential, like most restorative mechanisms, angiogenesis can become pathologic [33]. In a model of stroke examining unilateral infarcts in young and aged rats, most angiogenic functionalities are reduced in aged animals compared to young [[24], [49]]. In mice with hyperlipidemia, an important vascular risk factor, growth factor mediated angiogenesis following injury was attenuated, resulting in reduced cerebral blood flow, a breakdown of homeostatic cerebral energy metabolism and increased secondary brain injury compared to control mice [[49], [153], [170]]. Many studies in both pre-clinical animal models of AD and in patients with AD show angiogenic vessels in the diseased brain are vastly different from patent vessels in structure [[63], [82], [93]]. Angiogenic vessels show weakened integrity, aberrant branching patterns, increased fenestrae, and abnormal basement membranes [33]. These structural differences may contribute to functional discrepancies including reduced cerebral blood flow and decreased BBB integrity, even when the angiogenic process is active.

Interestingly, increases in VEGF expression has been implicated in pericyte disfunction and decreased BBB integrity in post-mortem human AD brains, in pre-clinical rodent models of stroke and in vitro studies. [[10], [19], [42], [85]]. However, there has been mixed data regarding changes in VEGF expression and how it relates to dementia and cognitive decline [[29], [136], [137]]. One reason for this discrepancy in the data could be that VEGF plays a role in a diverse array of biological pathways, so increases in VEGF may be neuroprotective because of its role in one discrete pathway while simultaneously, increases in VEGF may be pathologic as a result of completely separate signaling cascade. Another factor contributing to these conflicting results is inconsistent methodology. Changes in VEGF expression have been investigated by quantifying protein levels, analyzing mRNA levels, determining percent positive area using immunohistochemical staining and by looking at association with the vasculature with a multi-photon scope. Additionally, due to the vast array of VEGF proteins and isotopes, the specificity of each quantitative method varies significantly. Each unique methodology answers a different question and, as such, the results need to be interpreted with specific context in mind.

The contribution of angiogenesis to the progression of VCID needs to be further investigated. While many groups in the field of cerebral ischemia have shown that restoring cerebral perfusion following vascular injury is an essential component of functional recovery and longer survival in both human and murine models [[60], [67], [68]] other groups have argued that aberrant angiogenesis may play a role in the progression of various neurodegenerative diseases including both vascular dementia and Alzheimer's disease [[25], [73], [108]]. Importantly, many of these studies have been correlational and further study in pre-clinical models investigating the causal associations behind these findings is needed. One pivotal study examines non-productive angiogenesis (NPA), a term initially described by the cancer field to describe pathologic angiogenesis in the context of tumor metastasis [[5], [97]]. Investigators examined human control and pathologically confirmed AD post-mortem brains from the Banco de Tejidos CIEN in Madrid, Spain and APP/PSEN1 mouse brains and found that though angiogenesis was increased surrounding Aβ plaques, important markers of NPA were colocalized with these vessels suggesting an increase in aberrant

angiogenesis [5]. The authors also suggested that phagocytic microglia may play a role in this pathology [5]. A number of antiangiogenic agents that have been developed to study other conditions such as cancer and macular degeneration could be used in pre-clinical models of cerebrovascular injury to further elucidate these mechanisms ([3]; Y. [172]; R. K. [56]).

Treatment approaches to mitigate progression of VCID

Identifying treatments for VCID is essential because of the high impact this disease has, both on its own and as a significant co-morbidity with other progressive neurodegenerative conditions. Recent studies have shown that targeting AD pathology alone is often insufficient in preventing progression of dementia related diagnosis and even more interestingly, previous pre-clinical studies conducted in an VCID-amyloid comorbidity mouse model suggest that untreated VCID may contribute to inefficacies recently reported in promising AD therapeutics ([[20], [104], [110], [155], [158]]; Stephen [118]; S. [117]). In this model, investigators placed APP/PS1 mice on either control diet or a diet that induced an important risk factor for VCID, hyperhomocysteinemia as previously described. The investigators administered 36D, an anti-Aβ antibody, and saw reduced levels of Aβ in both the APP/PS1 mice on control diet and in the APP/PS1 mice on hyperhomocysteinemia inducing diet [155]. The investigators found that 36D administration in the APP/PS1 mice on control diet significantly reduced the number of errors on the radial arm water maze learning and memory test, making them indistinguishable from WT animals on control diet while administration of hyperhomocysteinemia inducing diet ameliorated this effect [155].

Due to the cascading nature of VCID progression, several targetable checkpoints present unique opportunities for intervention. Chronic hypoperfusion and pathologic neuroinflammation represent two of the earliest pathologies observed in VCID progression and both of these conditions represent two likely mechanisms underlying the association between risk factors and pathology as shown in Fig. 3. Preventative approaches focus on targeting the risk factors themselves like hypertension and hypercholesterolemia that lead to cerebral hypoperfusion and inflammation. More direct treatment strategies focus on targeting chronic cerebral hypoperfusion and neuroinflammation simultaneously, individually or targeting the coalescent downstream effects of chronic cerebral hypoperfusion and inflammation like angiogenic mediators, MMPs, or BBB dysfunction.

Treatment of risk factors

As with many disease states, mitigation of risk factors remains the most promising method of disease prevention, however, patient compliance to long-term lifestyle interventions is sub-optimal. As discussed previously, important risk factors for VCID include diabetes, smoking, hypertension, hyperlipidaemia, heart disease and hyperhomocysteinemia as illustrated in Fig. 3. (Van [[1], [52], [107], [147]]). While lifestyle interventions remain the most difficult to sustain the importance of these interventions should not be diminished. In a preclinical comorbidity mouse model of metabolic disease using induction of high fat diet and VCID using unilateral common corotid artery occlusion researchers reported deficits across multiple cognitive domains as determined by a battery of behavior tests including novel object recognition testing, the Morris water maze, nest building and open field tests [116]. Additionally, in another pre-clinical study, rats were fed a high fat diet to induce atherosclerosis then subjected to a physical exercise routiine of swimming for 60 min a day during 5 days a week for 8 weeks(J. [70]). Investigators reported that compared to mice on the same high fat diet that did not undergo a physical exercise routine the mice who exercised had significantly lower levels of TNF-α, IL-6 and NF-κβ(J. [70]).

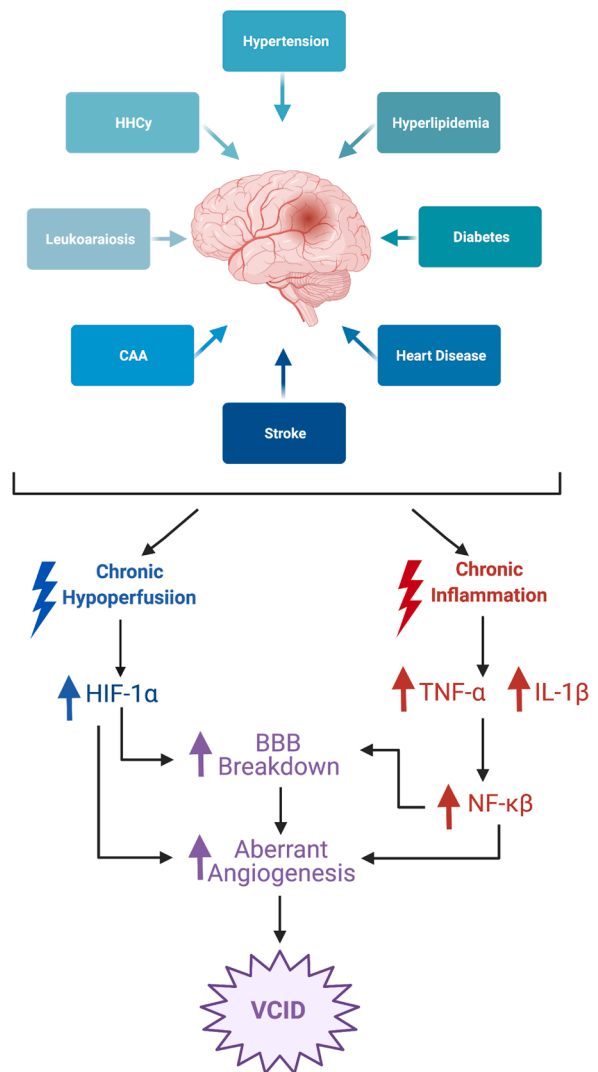Fortunately, there have also been several pharmacologic

Fig. 3.. Working hypothesis for the mechanism relating known VCID risk factors with the development of VCID pathology. Abbreviations in the figure are: HHCy, hyperhomocysteinemia; CAA, cerebral amyloid angiopathy; HIF-1α, hypoxia inducible factor-1α; BBB, blood brain barrier; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; NF-κβ, nuclear factor-κβ.

interventions that ameliorate certain risk factors, and that have also been shown to reduce cerebrovascular pathology. In one study, a high cholesterol diet was given to WT mice and to a mouse model of vascular disease (TGF-β overexpression) at 6 and 12 months of age. Simultaneously, simvastatin, an HMG CoA reductase inhibitor that lowers cholesterol, was administered. The results indicate that the mice predisposed to vascular pathology had worse outcomes on high cholesterol diet compared to WT mice but the cognitive and pathological consequences were significantly countered in mice receiving simvastatin [141]. More clinically relevant are findings from the SPRINT-MIND study, in which intensive lowering of blood pressure to levels below 120 mmHg slowed progression toward mild cognitive impairment (MCI) as defined by a battery of cognitive tests including the Montreal Cognitive Assessment, the Wechsler memory Scale and the Wechsler Adult Intelligence Scale in randomized trial involving adult participants diagnosed with hypertension [159]. This suggests that targeting and treating risk factors of VCID is a promising approach deserving of further study.

Targeting inflammation

Targeting inflammation remains difficult because it is appreciated among most experts that acute inflammation is essential in the CNS healing and repair process in response to many injuries. However, recent genome wide association study (GWAS) data has underscored the important role pathologic inflammation plays in white matter hyperintensities. One study used three sample sets including the Cohorts for Heart and Aging Research in Genomic Epidemiology, the UK Biobank and the White Matter Hyperintensity in Stroke Study as described previously [[101], [144]]. Investigators performed genome wide association studies on three MRI markers of cSVD including white matter hyperintensities, fractional anisotropy and mean diffusivity. They found 66 genes associated with cSVD pathology and many of these genes were involved in inflammatory process further highlighting the link between cSVD and inflammation. In light of these findings, targeting essential components of the inflammatory response are promising therapeutic targets.

The selective TNF-α inhibitor, XPro1595 has been evaluated in the context of Parkinson's Disease, another neurodegenerative disease strongly associated with aberrant neuroinflammation, and AD ([[15], [61], [76]]; De Sousa [126]). Cytokines like TNF-α act in a feedforward mechanism to propagate an inflammatory signal to surrounding microglia, while simultaneously communicating with nearby astrocytes to signal the presence of an inflammatory stimulus [74]. XPro1595 is unique in that it selectively binds to soluble TNF-α, creating TNF-α heterotrimers that no longer have affinity for the TNF-α receptor [128]. This allows transmembrane TNF-α to continue to bind normally to its receptor and prevents any impairment to the innate immune response [169]. XPro1595 is able to penetrate the BBB in therapeutically relevant concentrations and following its administration, XPro1595 is able to attenuate microglia and astrocyte activation in the pre-clinical 6-hydroxydopamine (6-OHDA) hemiparkinsonian rat model. This attenuation of both microglia and astrocyte activation is followed by decreased neuronal death and significantly improves motor function as evidenced by the cylinder test [15]. TNF-α is an attractive therapeutic target for VCID given the previously described association with MMP activation and microglial activation, and the mechanism of action of XPro1595 retains the neuroprotective effects of TNF-α while restricting its inflammatory activity. Further understanding of the temporal and spatial nature of inflammatory signaling will be necessary to determine when and how to target inflammation in VCID.

Targeting cerebral hypoperfusion

Targeting chronic cerebral hypoperfusion may be challenging due to the diverse pathologies that can lead to this condition. Mitigation of these risk factors remains the most promising strategy in preventing VCID. Ongoing trials are currently being conducted to increase vascular integrity following lacunar strokes, a common manifestation of cSVD. Incidence of lacunar strokes has been linked to further progression cSVD, cognitive decline and ultimately diagnosis of VCID. The LACI-1 and LACI-2 trials are investigating the use of isosorbide mononitrate (ISMN) and cilostazol in patients with prior lacunar stroke to prevent recurrent vascular events and to improve functional outcomes. ISMN has been shown to increase nitric oxide, a compound shown to improve BBB integrity, increase perfusion as a vasodilator, and reduce neuroinflammation [[16], [160]]. Cilostazol is a phosphodiesterase 3′ inhibitor that has also been shown to increase BBB integrity, reduce inflammation and increase perfusion by acting as a vasodilator [[16], [86]]. Currently, all feasibility, recruitment, compliance and tolerability components have been met and investigators are currently collecting data to power a phase III trial [[21], [22], [154]]. While these therapeutics remain promising, once a state of chronic cerebral hypoperfusion is initiated, HIF-1α becomes the most important, targetable signaling mediator that has been shown to propagate the downstream

effects of chronic cerebral hypoperfusion. Inhibition of HIF-1α has been well studied as a potential therapeutic target in other diseases such as endometriosis and cancer [174]. Several modes of targeting HIF-1α have been proposed including inhibiting its transcription or translation, blocking dimerization, stimulating protease induced degradation, and interfering with its DNA binding ability [174]. The food and drug administration (FDA) has approved bortezomib and amphotericin B for multiple myeloma and cryptococcal meningitis respectively which inhibit HIF-1α downstream signaling [174]. Silibinin has been shown to inhibit HIF-1α transcriptional activity [174]. SAHA and FK228 promote HIF-1α degradation [174]. Several clinical trials using flavopiridol (Alvocidib) an inhibitor of HIF-1α transcription among other genes involved in the cell cycle are currently underway in the context of diseases such as diffuse large B-cell lymphoma, acute myeloid leukemia and chronic lymphocytic leukemia [[9], [75], [83], [174]]. HIF-1α inhibitors have yet to be investigated in the context of VCID progression but may be promising in cases of chronic cerebral hypoperfusion.

Targeting downstream sequelae of vascular pathologies

Inhibition of MMP9 represents another promising target for preventing progression of VCID pathologies. The cancer field has been investigating MMP9 inhibition for a number of years since its upregulation was correlated with metastasis in many different cancer subtypes. The mechanism of action of MMP9 in the context of tumor metastasis remains undefined but it is hypothesized that it induces invasion and migration through its interaction with angiogenic agents [69]. In 2017, a humanized monoclonal antibody inhibitor of MMP9, GS-5745, was shown to decrease tumor growth and metastasis in a rat model of colorectal cancer [[6], [77]]. Investigators showed that GS-5745 was able to inhibit both pro-MMP9 by binding to its cleavage site preventing activation as well as inhibit active MMP9 by binding to its catalytic site [6]. In 2020, GS-5745, now Andecaliximaub, underwent a Phase I clinical study for patients with advanced pancreatic cancer. The safety profile is favorable, and this drug is currently undergoing Phase II studies [18]. While this drug is tolerated in the periphery, no pre-clinical studies have been conducted to study its ability to cross the BBB or the affect it has on the CNS; however, the need for CNS penetrance in VCID may be lower than diseases like AD as the BBB breakdown in VCID may permit the transit of IgG molecules into the brain. Interestingly, one drug that has been shown to directly impact the CNS is the anti-fungal agent, miconazole. This drug has been shown to be well tolerated in human patients, even at therapeutic levels high enough to penetrate the BBB [99]. In pre-clinical models of hemorrhagic stroke in both zebrafish and in rats, administration of miconazole was shown to decrease hemorrhagic events by inhibiting MMP9-mediated vessel rupture [112]. Further investigation regarding the mechanism of miconazole dependent MMP9 depletion is necessary to fully understand the potential this well-tolerated drug may have in the future. A significant challenge of developing small molecule inhibitors of MMP9 that would have brain penetrance is the homology among the members of the MMP family, limiting the potential for a truly specific MMP9 or MMP2 inhibitor.

In the previously described LACI-1 and LACI-2 patient cohort, GWAS and transcriptome-wide association studies (TWAS) have been conducted [143]. 12 loci were associated with either white matter hyperintensities or lacunar stroke using GWAS and TWAS identified associations between 6 genes and lacunar stroke [143].Following pathway analysis of these genes disruption of the vascular extracellular matrix was a prominent mechanism in disease progression highlighting both the potential for therapeutic targets and the need for continued study in this area [143].

Finally, targeting VEGF remains a promising therapeutic strategy to prevent the effects of aberrant angiogenesis from contributing to VCID progression. Fortunately, several groups in the cancer and macular degeneration fields have pioneered VEGF as a therapeutic target because both conditions are associated with such robust, pathologic

angiogenesis. Bevacizumab is a recombinant humanized monoclonal antibody currently approved to target VEGF-A and prevent angiogenesis in metastatic colorectal cancer [87]. Generally, this drug is well tolerated by patients and has few contraindications [87]. Of note, there is an increased incidence of thromboembolic events [87]. In one study, patients with primary and locally advanced adenocarcinoma of the rectum were given an intravenous treatment protocol of bevacizumab and showed reduced blood vessel density and reduced vascular permeability as evidenced by macroscopic and histologic analysis following surgical excision of the tumor and surrounding microvasculature [[37], [80]]. In clinical trials Bevacizumab significantly improved progression-free survival. Additional anti-VEGF agents (Ranimizuab, Aflibercept, Bevacizumab) have been approved for various diseases including retinal vein occlusion, retinopathy of prematurity, diabetic macular edema and neovascular glaucoma with varying degrees of success and tolerance. It was noted that the optimal delivery method and dosing paradigm had yet to be determined [[12], [27], [53], [119]].

Conclusion

VCID has been well characterized in terms of cognitive domains affected and neuropathologic features. It is now widely recognized that VCID is one of the leading causes of dementia along with AD [4]. Recent studies have even demonstrated that pathologic vascular changes precede the appearance of Aβ plaques and neurofibrillary tangles in early-onset familial AD mutation carriers, further implicating cerebrovascular changes and hypoxia in the progression of AD [[28], [59]]. The compilation of several decades of pivotal research has allowed us to better understand VCID and its complex diagnostic criteria. Current research focuses on both the basic mechanisms of VCID progression and the characterization of novel, sensitive biomarkers to track disease progression; all key research necessary to fuel therapeutic development for VCID. By enhancing our understanding of the mechanisms underlying VCID, scientists can better comprehend how these important inflammatory and vascular changes may affect the efficacy of promising dementia therapeutics and ultimately, researchers will be able to systematically target various aspects of the neurodegenerative sequelae to prevent the progression of VCID.

Acknowledgments

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