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*Small Blood Vessel Disease in the Brain Theme Issue*

# REVIEW

# Overlapping Protein Accumulation Profiles of CADASIL and CAA

## *Is There a Common Mechanism Driving Cerebral Small-Vessel Disease?*

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and cerebral amyloid angiopathy (CAA) are two distinct vascular angiopathies that share several similarities in clinical presentation and vascular pathology. Given the clinical and pathologic overlap, the molecular overlap between CADASIL and CAA was explored. CADASIL and CAA protein profiles from recently published proteomics-based and immuno-based studies were compared to investigate the potential for shared disease mechanisms. A comparison of affected proteins in each disease highlighted 19 proteins that are regulated in both CADASIL and CAA. Functional analysis of the shared proteins predicts significant interaction between them and suggests that most enriched proteins play roles in extracellular matrix structure and remodeling. Proposed models to explain the observed enrichment of extracellular matrix proteins include both increased protein secretion and decreased protein turnover by sequestration of chaperones and proteases or formation of stable protein complexes. Single-cell RNA sequencing of vascular cells in mice suggested that the vast majority of the genes accounting for the overlapped proteins between CADASIL and CAA are expressed by fibroblasts. Thus, our current understanding of the molecular profiles of CADASIL and CAA appears to support potential for common mechanisms underlying the two disorders. *(Am J Pathol 2021, 191: 1871–1887; https://doi.org/10.1016/j.ajpath.2020.11.015)*

Cerebrovascular diseases affect a significant portion of our aging population and are major contributors to cognitive impairment and dementia.[1,2] Improved understanding of the molecular basis of cerebrovascular diseases is imperative for the development of novel and effective clinical therapeutics. The purpose of this review is to compare two seemingly distinct vascular disease processes with small-vessel disease (SVD) burden to identify potentially shared properties and/or disease pathways. In particular, increasing evidence from two vascular angiopathies, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and cerebral amyloid angiopathy (CAA) is discussed, that supports molecular overlap in cerebrovascular disease mechanisms.

To highlight the extent of overlap between these diseases, first the clinical overviews, genetics, histopathology, protein processing, protein aggregation, and protein accumulation involved in CADASIL and CAA were reviewed.

This article is part of a review series on small blood vessel disease in the brain, addressing current knowledge, new mechanisms, biomarkers, and therapeutic approaches.

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## Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

### Clinical Overview of CADASIL

Cerebral SVD is a prevalent vascular disorder of the brain and a major contributor to neurologic deterioration in our elderly population. Recent estimates report that sporadic SVD affects over half of the population aged >65 years and significantly elevates the risk of stroke, vascular dementia, and progression of neurologic diseases.[1,2] In addition to sporadic SVD, monogenic causes of SVD have also been described, which may shed light on pathomechanisms. The most common type of SVD is CADASIL, caused primarily by stereotypical cysteine mutations in the *NOTCH3* gene that result in altered cysteine number.[3]

Clinically, patients with CADASIL present with an accelerated and often more severe clinical course compared with sporadic SVD. Although there can be variability in disease presentations, patients with CADASIL typically present with migraine with aura, subcortical ischemic events, mood disturbances, apathy, and cognitive impairment.[4] In most patients, migraine with aura is the first observed symptom, often occurring roughly 15 years earlier than ischemia.[4]

### Genetics of CADASIL

CADASIL was first described in 1993 when several unrelated families presented with a mendelian syndrome that caused recurrent strokes.[5] Initially, these syndromes were reported under different names until the affected gene was mapped to chromosome 19q12.[5] Subsequent linkage analysis of additional families led to the mapping of the CADASIL critical region to the *NOTCH3* gene.[3] Although the prevalence of genetically proven CADASIL is estimated to be roughly 2 per 100,000 adults, the actual prevalence is thought to be higher when taking into account sporadic mutations.[5]

*NOTCH3* gene encodes a vascular smooth muscle transmembrane protein involved in smooth muscle cell differentiation and vascular development.[6] NOTCH3 contains 34 extracellular epidermal growth factor–like repeats, and each epidermal growth factor–like repeat contains six cysteines that are predicted to form three disulfide bonds important for protein structure.[7] Virtually all CADASIL-causing mutations in *NOTCH3* affect cysteines, leading to a change in cysteine number.[8] This provides support for the idea that aberrant NOTCH3 undergoes abnormal disulfide bonding that is important for disease pathogenesis. In addition, three-dimensional modeling shows that at least a subset of known *NOTCH3* mutations leads to domain misfolding, supporting the notion of misfolded proteins driving CADASIL.[9] It is thus postulated that cysteine mutants of NOTCH3 are associated with abnormalities in protein folding and could possess neomorphic properties responsible for CADASIL pathology. Recently, there have also been reports of CADASIL in patients without stereotypical cysteine mutations in NOTCH3.[10] Many of the noncysteine involving NOTCH3 mutations are also thought to disrupt NOTCH3 protein structure, and *in vitro* examination of cysteine-sparing NOTCH3 mutants shows that they also form aggregates similar to those of typical cysteine mutants.[11]

As of now, no clear genotype-phenotype correlation has been identified, although studies have emerged that suggest a relationship between the position of mutations and disease severity. For example, Rutten et al[12] provides evidence to suggest that C-terminal mutants/polymorphisms could contribute to delayed onset small-vessel disease that is less severe compared with the classic CADASIL phenotype. In addition, a recent review of 224 CADASIL case reports suggests that the pathogenicity of CADASIL mutations is related to the location of the mutation.[10] Currently, it is thought that both genetic and environmental factors play a role in disease presentation.

### CADASIL Diagnostic Testing

Presently, the gold standard for diagnosis of CADASIL is genetic testing.[13] Screening 23 exons of *NOTCH3* for mutations has a specificity and sensitivity close to 100%.[13] Another useful diagnostic tool is skin biopsy. An ultrastructural hallmark of CADASIL is the deposition of granular osmiophilic material (GOM) within vessels.[13] GOMs are electron-dense extracellular deposits typically found between neighboring vascular smooth muscle cells and most readily visualized by electron microscopy.[13] In addition to being found in the cerebrovasculature, GOMs can also be detected in extracerebral vasculature, such as that of the skin.[13] Thus, skin biopsy can be used to diagnose CADASIL with a specificity of 100% and a sensitivity that ranges from 45% to 100%.[14] Currently, the exact GOM composition remains unclear. However, NOTCH3 ectodomain, N-terminal fragment of NOTCH3 (NTF), metalloproteinase inhibitor 3 (TIMP3), vitronectin (VTN), latent transforming growth factor-β 1 (LTBP1), amyloid P (SAP), annexin 2, and periostin have been identified to be components, suggesting that GOMs consist of abnormal protein aggregates.[15–19] Finally, presence of subcortical infarcts and leukoencephalopathy is a feature of CADASIL best detected by magnetic resonance imaging (MRI).[20] MRI lesions become apparent at a mean age of 30 years and are found in virtually all patients with CADASIL aged >35 years.[21] Typically, MRI changes precede development of other CADASIL symptoms and worsen with age and disease progression.[20,21] In particular, MRI involvement of the anterior temporal pole has approximately 90% sensitivity and approximately 90% specificity for CADASIL.[14]

## Histopathologic Findings in CADASIL

Pathologic characteristics of CADASIL include GOMs, as mentioned earlier, that surround the vascular smooth muscle cells of arterioles[13] and the degeneration of vascular smooth muscle cells in arterial walls.[20] In addition, CADASIL brains show significantly thickened arteriolar walls and reduced diameters of small penetrating arteries.[22] It is thought that these thickened arteries and GOMs result from disease-related abnormal protein accumulation that includes molecules such as NOTCH3 ectodomain.[23]

## Protein Processing Involved in CADASIL

Notch signaling plays a critical role in development and involves processing of NOTCH proteins. NOTCH3 is a transmembrane protein, composed of both an extracellular and a membrane tethered intracellular domain.[24] On activation by Notch signaling ligands, a series of proteolytic cleavages occur by ADAM-TACE and γ-secretase, releasing the NOTCH3 intracellular domain to the nucleus to affect gene transcription.[24]

Although CADASIL is characterized by mutations in *NOTCH3*, it seems unlikely that a loss of NOTCH3 signaling is the sole driver of disease. Several studies report connections between CADASIL-causing *NOTCH3* mutations and NOTCH3 receptor function, which alter canonical Notch signaling.[25–28] However, other mouse model studies do not support this idea.[29] For example, despite having increased susceptibility to stroke, *Notch3* knockout mice do not demonstrate classic features of CADASIL.[6] On the other hand, mice that overexpress mutant *Notch3* better model the human disorder, although it is also unlikely that CADASIL leads to a gain of Notch signaling function because CADASIL mutations do not enhance downstream signaling.[29] The early accumulation of GOMs and NOTCH3 aggregates, found in these mouse models and patients with CADASIL, therefore suggests a neomorphic role of mutant NOTCH3, although it remains unknown whether these aggregates cause disease or are consequences of disease.

In addition to physiological NOTCH3 processing involved in Notch signaling, post-translational modification of NOTCH3 protein has also been identified as a disease-specific feature of CADASIL. Using conformation-specific antibodies, Zhang et al[30] identified a reduced form of NOTCH3 that accumulates specifically in disease-affected CADASIL vessels compared with normal-appearing vessels from age-matched control subjects. It is conceivable that a cysteine-involving mutation results in a change in NOTCH3 tertiary structure or aggregation state. Support for disease-related post-translational alterations of NOTCH3 also comes from Arboleda-Velasquez et al,[31] who demonstrated impairment of glycosylation and cleavage in mutant CADASIL protein. Both reduced glycosylation and aberrant multimerization of mutant NOTCH3 may play a role in abnormal protein accumulation in CADASIL. In addition, studies identify accumulation of NTF in pathologic vessels compared with normal-appearing vessels, suggesting enhanced cleavage of NOTCH3 protein in disease that is unrelated to Notch signaling.[19] *In vitro* studies demonstrate that reduction of NOTCH3, which breaks NOTCH3 disulfide bonding and destabilizes protein structure, enhances nonenzymatic NOTCH3 fragmentation in CADASIL vessels.[19] Given the disease-specific nature of these NOTCH3 forms, there are likely multiple abnormalities of post-translational processing of NOTCH3 in CADASIL.

## NOTCH3 Assemblies

Both wild-type NOTCH3 and mutant NOTCH3 are capable of forming dimers, oligomers, and higher-order multimers *in vitro*.[32] However, mutant NOTCH3 demonstrates increased propensity to form higher-order oligomers compared with wild-type protein.[31–33] NOTCH3 oligomerization does not require presence of cofactors and is mediated in part by disulfide bonding.[32,33] Furthermore, NOTCH3 binding partners, such as thrombospondin 2 and Fringe, have also been identified to co-aggregate with mutant NOTCH3, suggesting a role of protein aggregation in disease.[31,33]

In addition, NTF, which localizes to pathologically affected vessels in CADASIL, has also been shown to undergo spontaneous thiol-mediated oligomerization *in vitro*.[34] NTF can form covalent conjugates with vascular catecholamines that enhance multimerization of the NOTCH3 fragmentation product.[34] More recent studies have highlighted the presence of NTF multimers in CADASIL tissue, implicating a role for NTF multimers in disease (unpublished data).

## Protein Accumulation and Signaling Dysregulation in CADASIL

CADASIL brains demonstrate abnormal protein accumulation that is often thought to be linked to disease. For example, CADASIL vessels feature accumulation of the NOTCH3 ectodomain without simultaneous accumulation of the intracellular domain, NOTCH3 aggregates, abnormal NOTCH3 conformations enriched in disease, and NOTCH3 fragments, including an NTF.[19,23,34] In addition, other proteins, such as proteoglycans, collagens (COLs), and other extracellular matrix (ECM) proteins, have been shown to accumulate within the vessels as well, suggesting formation of extracellular protein complexes in disease.[15,35–37] The extracellular matrix is a network of highly cross-linked proteins that plays important roles in cell structure and support.[38] ECM proteins are also thought to influence fundamental cellular processes, such as differentiation,

survival, and proliferation.[38] Traditionally, the ECM is thought to consist of collagens, proteoglycans, and glycoproteins.[38] However, there are also ECM-associated proteins, such as ECM regulators, enzymes, or proteins, that modify the ECM and ECM-affiliated proteins.[38]

The observed enrichment of ECM proteins in CADASIL can be explained by increased secretion of proteins or decreased protein turnover in disease. In mouse models of CADASIL, NOTCH3 ectodomain accumulation is one of the earliest observed events in disease pathogenesis, suggesting a role in disease initiation.[39]

One model of enhanced protein recruitment suggests that *NOTCH3* mutations lead to abnormal accumulation of mutant NOTCH3 protein, which then plays a role in recruiting and sequestering other proteins. Recent studies have identified the enrichment of ECM proteins in CADASIL, such as TIMP3, VTN, collagens, LTBP1, clusterin, decorin, biglycan, and laminins, among others.[15,35–37,40] *In vitro* studies suggest potential physical interaction between NOTCH3 and some of the proteins, including TIMP3 and LTBP1.[15,17] Transforming growth factor (TGF)-β signaling activity is linked to regulation of fibrotic events in the vasculature, and is shown to be activated by fibronectin, fibrillin-1, and other members of the LTBP family.[17] This supports a model where dysregulation of TGF-β signaling results from aggregation and/or accumulation of NOTCH3 in the cerebrovasculature of patients with CADASIL and promotes abnormal recruitment of ECM proteins.[15] The resulting aggregation of proteins can result in a change in biological function and contribute to disease. Interestingly, TGF-β activity has been implicated in other vasculopathies, including cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) and CAA.[17]

Another model of abnormal protein accumulation involves decreased protein turnover and proposes a loss of serine protease HTRA1 activity in CADASIL. HTRA1 is a negative regulator of TGF-β signaling and an extracellular serine protease that is known to be mutated in CARASIL, an autosomal recessive disorder that shares clinical overlap with CADASIL.[41] In a recent liquid chromatography–tandem mass spectrometry study of CADASIL brain tissue, significantly increased levels of HTRA1 protein and substrates were identified (clusterin, vitronectin, elastin, and LTBP1), suggesting an impairment of HTRA1 activity in CADASIL.[42]

## Cerebral Amyloid Angiopathy

### Clinical Overview of CAA

CAA is a common age-related disease that can occur both with and without Alzheimer disease (AD).[43] Although CAA can be caused by several amyloidogenic proteins, such as cystatin C, transthyretin, and others, this review will focus on amyloid-β (Aβ) CAA. Population-based studies demonstrate that CAA affects roughly 20% to 40% of elderly populations without dementia and 50% to 60% of elderly populations with dementia.[44] In AD, CAA is predicted to be present in roughly 80% to 90% of patients.[45] Classic clinical presentation of CAA involves spontaneous lobar intracerebral hemorrhage, cognitive impairment and/or dementia, and transient focal neurologic episodes.[45]

### Genetics of CAA

Most cases of CAA are sporadic. However, some CAA has been linked to specific genetic loci that include apolipoprotein E (*APOE*) e4 and e2 alleles, mutations in presenilin 1 (*PS1*) and *PS2*, and mutations in amyloid precursor protein (*APP*).[45] Mutations in *APP* that cause familial forms of CAA (eg, hereditary cerebral hemorrhage with amyloidosis, Dutch type) tend to cluster around residues 21 to 23 and have been proposed to decrease proteolytic degradation of Aβ or attenuate Aβ protein clearance from the brain into the circulation.[46] On the other hand, two isoforms of APOE are thought to promote CAA via different mechanisms. For example, *APOE* e4 has been linked to increased amyloid deposition, whereas *APOE* e2 is thought to accelerate the formation of vasculopathies that promote vessel rupture.[47] Both *APOE* e2 and e4 alleles have been associated with early recurrence of lobar intracerebral hemorrhage following a prior lobar intracerebral hemorrhage.[45] In addition, alterations in *TGF-β1* have been linked to CAA, along with polymorphisms in a1-antichymotrypsin, neprilysin, low-density lipoprotein receptor protein 1, *CR1*, and angiotensin-converting enzyme genes.[45]

### CAA Diagnostic Testing

Definitive diagnosis of CAA relies on identification of CAA-related vascular damage, multiple lobar hemorrhages, and absence of alternative pathologies on postmortem examination of brain, as assessed by the Boston criteria.[48] Diagnosis of probable CAA includes use of clinical data and MRI imaging to identify the presence of multiple hemorrhages or microbleeds in regions typical of CAA, and more recently in the modified Boston criteria, the presence of superficial siderosis.[48] Although many imaging features are shared between CADASIL and CAA (cerebral microbleeds and white matter hyperintensities), cortical superficial siderosis is absent in a large cohort of patients with CADASIL from a clinical study examining imaging features of CADASIL, CAA, and control subjects.[49] Thus, this study posits that in individuals with imaging features suggestive of small-vessel disease, presence of cortical superficial siderosis is highly suggestive of CAA.[49]

### Histopathologic Findings in CAA

Histopathologically, CAA is characterized by extracellular Aβ protein deposits in the cerebrovasculature.[50] The most common forms of vascular amyloid deposits include $A\beta_{1\text{-}40}$

and $A\beta_{1-42}$.[50,51] Many sources of Aβ have been proposed to explain its deposition within the vessel wall, including transport across the blood-brain barrier (BBB) from the circulation, the vascular smooth muscle cells, and neurons as a result of impaired perivascular drainage.[52–54] In most cases, there is no direct evidence of overproduction of Aβ within the vessel wall. Instead, the perivascular drainage model suggests that the Aβ accumulation within the vasculature is likely the result of impaired Aβ drainage through perivascular pathways that typically serve as lymphatic-like drainage pathways from the brain.[55] The impaired elimination of proteins results in the accumulation of both soluble and insoluble protein aggregates in the extracellular spaces within arterial and capillary walls. Alterations of these extracellular spaces in disease or aging might further contribute to decreases in drainage capacity and increased Aβ deposition.[55]

CAA vessels also feature marked degeneration of smooth muscle cells within the medial layer of arterioles and hyalinization.[56] Severely affected vessels demonstrate disruption of the vascular architecture, leading to microaneurysm formation, fibrinoid necrosis, and Aβ deposition in the surrounding neuropil (alias dysphoric changes).[43] The two major types of CAA include type 1, which involves cortical capillaries and/or larger vessels, and type 2, which is limited to larger leptomeningeal and cortical arteries.[43,56] CAA type 1 leads to capillary occlusion and alterations in vascular flow, contributing to the development of dementia.[45]

## Protein Processing Involved in CAA

Aβ is generated from cleavage of APP.[45] APP is a membrane glycoprotein intimately involved in neuronal development, maintenance of neuronal homeostasis, cellular signaling, and intracellular transport.[57] APP can generate many different cleavage products, some of which are thought to be major contributors to Aβ deposits throughout the brain.[57] Under physiological conditions, APP is cleaved by α-secretase to shed the ectodomain, which does not produce amyloidogenic fragments.[58] Alternatively, ectodomain shedding can occur by β secretase activity.[58] Aβ is generated by β-secretase and γ-secretase cleavage to generate two predominant forms: $A\beta_{1-40}$ and $A\beta_{1-42}$.[45] $A\beta_{1-40}$ is more often found within the vessel wall, whereas $A\beta_{1-42}$ is more often found in senile plaques and capillaries involved in CAA.[51] The major β-secretase is BACE1, which has a main cleavage site at position 1D of Aβ, but also has an alternative site at 11E, generating $A\beta_{11-40/42}$.[58] A second cleavage process occurs via the γ-secretase complex, consisting of presenilin, nicastrin, anterior pharynx-defective-1, and presenilin enhancer-2.[58] The γ-secretase complex results in the intramembranous proteolysis of the β-secretase cleaved product to generate $A\beta_{1-40/42}$.[58] The γ-secretase complex is also essential for physiological processing of NOTCH3.

## Aβ Assemblies

Aβ monomers can form various types of assemblies, such as oligomers, protofibrils, and amyloid fibrils.[57] In AD, insoluble amyloid fibrils can further assemble into amyloid plaques most commonly found in the neocortex of AD brains, whereas soluble amyloid oligomers can deposit throughout the brain.[57] The dominant form in CAA is fibrillar Aβ, although a recent study found that most mutations in the Aβ sequence that promote CAA and AD do not have obvious stabilizing or destabilizing effects on Aβ fibrils derived from AD brains.[59] Both soluble and insoluble Aβ assemblies are thought to contribute to cerebrovascular dysfunction.

Accumulation of Aβ has been proposed to exert neuronal toxicity through various means, and it is thought that assemblies of oligomeric Aβ result in activation of microglia and astrocytes, oligomerization and aggregation of tau protein, and progressive neuronal loss.[57] Alternatively, accumulation of fibrillar Aβ in the vasculature has been proposed to impact blood vessel integrity and function.[60,61] Deposition of amyloid in capillaries has been linked to degeneration of the lumen, endothelium, and basal lamina, resulting in ischemia and neurodegeneration.[61] Amyloid fibrils found in the perivascular space, indicating dysphoric changes, have also been linked to neurite dystrophy.[61] In larger vessels, Aβ deposition is associated with degeneration of vascular smooth muscle cells.[61] Furthermore, it is thought that accumulation of Aβ in the vessel wall can impair perivascular draining of Aβ, further promoting accumulation of protein.[60]

## Protein Accumulation and Signaling Dysregulation in CAA

Numerous protein-based studies have highlighted CAA-specific differential regulation of proteins in addition to amyloidogenic proteins, with many of the differentially regulated proteins related to extracellular structure and matrix organization. Several proposed mechanisms exist to explain the increase in ECM proteins in CAA. For example, this could be explained by either increased secretion of extracellular matrix components or decreased turnover.

One model suggests that tissue injury or cellular processes result in enhanced synthesis of ECM proteins. Disruption of the BBB has been implicated in CAA both with and without AD pathology, with identification of BBB leakage markers, such as fibrinogen, and a decrease in tight junction proteins (occludin, claudin-5, and tight junction protein 1) important for maintenance of the BBB.[62–64] Thus, it is conceivable that enhanced cross-linking of ECM proteins might result as a response to strengthen weakened vessel walls and prevent further BBB leakage.[65] Covalent protein cross-linking results in increased formation and stability of extracellular protein complexes. Tissue transglutaminase is a known modifier of proteins implicated in AD,[66–68] and enhanced tissue transglutaminase activity

has been identified in hereditary cerebral hemorrhage with amyloidosis, Dutch type, and CAA with AD pathology. Tissue transglutaminase cross-links proteins, such as Aβ and APP, and additional ECM proteins, such as fibronectin and laminin, potentially leading to their enhancement in CAA.[69,70]

In the ECM, tissue transglutaminase and tissue injury are also capable of activating TGF-β, which stimulates synthesis of ECM proteins and protease inhibitors that prevent enzymatic breakdown of the ECM.[71,72] In support of this notion, expression of *TGF-β1* and *TGFβR2* is increased in hereditary cerebral hemorrhage with amyloidosis, Dutch type, a genetic form of CAA that results in both accelerated and more severe CAA pathology and symptoms.[73] In addition, immunohistochemical staining of downstream TGF-β pathway signaling molecules, such as phosphorylated SMAD2/3, and TGF-β regulated proteins, such as fibronectin, collagen, and TIMP3, has been identified.[69,70,73,74] Similarly, TGF-β activity has been implicated in other ECM affecting diseases, such as AD, CADASIL, and CARASIL.[17,73]

Another potential explanation for the increase in ECM proteins is the decreased turnover and removal of ECM components. This could be due to aggregation and sequestration of chaperones, contributing to further aggregation and deposition of proteins. In CAA, enrichment of extracellular chaperones, such as clusterin, APOE, and HTRA1, was identified.[70,74] APOE features chaperone function that targets Aβ assemblies, and clusterin and HTRA1 are also thought to be involved in removal of protein aggregates.[75,76] Sequestration of protein chaperones can thus contribute to aggregation of Aβ and other extracellular proteins. In addition, tissue endogenous inhibitors (TIMPs) and matrix metalloproteinases (MMPs) are involved in regulation of the ECM, and dysregulation of these proteases can result in decreased turnover of ECM components and/or damage the integrity of the BBB, contributing to lobar hemorrhage.[65,77,78] MMP2 and MMP9 are enhanced in CAA with AD pathology, and matrix metalloproteinase inhibitor TIMP3 is enhanced in CAA both with and without underlying AD pathology.[40,70,78,79] MMP2 and MMP9 have also been shown to proteolyze various Aβ peptides in AD.[80,81] Thus, dysregulation of proteases can contribute to accumulation of ECM proteins identified in CAA.

## CADASIL and CAA

### CADASIL and CAA Exhibit Distinct Histopathologies

CADASIL and CAA can be clearly distinguished by histologic evaluation. Key histologic findings of CADASIL include accumulation of NOTCH3 protein ectodomain within the vasculature and the pathognomonic presence of ultrastructural extracellular GOMs.[6,23] In addition, CADASIL vessels demonstrate dramatic intimal hyperplasia and accumulation of intimal proteins (Figure 1A).[83,84] In comparison, CAA is characterized by cerebrovascular accumulation of amyloidogenic protein, such as Aβ (Figure 1A).[50,51] Routine histopathologic distinction is straightforward, because CADASIL vessels have not been shown to contain amyloid.[10]

### Shared Features between CADASIL and CAA

There is significant clinical overlap in CADASIL and CAA disease presentation, with both disorders resulting in increased risk of dementia, stroke, and intracerebral hemorrhage.[14,43] At the histopathologic level, CADASIL and CAA both harbor abnormal accumulation of proteins within the vessel wall, although in CADASIL the major protein is NOTCH3 ectodomain and in CAA the major protein is Aβ (Figure 1A).[23,50] Furthermore, both diseases feature degeneration of the vascular smooth muscle cells with accompanying hyalinization of vessels (Figure 1A).[14,43] Finally, many characteristics of CADASIL at the molecular level are highly reminiscent of known amyloid pathology. For example, as discussed above, abnormal protein cleavage, processing, and oligomerization have been identified in both CADASIL and CAA. Both disorders are also thought to recruit abnormal accumulation of additional proteins other than NOTCH3 and Aβ. Given the numerous common qualities, shared molecular mechanisms may be involved in CADASIL and CAA. Thus, published work was reviewed to compare and contrast the protein profiles of the two cerebrovascular diseases.

### Overlap of CADASIL and CAA Protein Profiles

A recent review of CADASIL and CAA proteomics-based studies by Haffner[85] identified six proteins that demonstrated increased abundance in both diseases compared with controls: SAP, TIMP3, VTN, APOE, clusterin, and HTRA1.[85] To expand on this, both proteomics-based studies and immuno-based studies of human CADASIL and CAA tissue were examined. In addition, CAA studies that did not include patients with underlying AD pathology were specifically targeted to compare CADASIL and CAA as primarily vascular disorders. The criteria for inclusion of studies in this review can be found in Table 1.[15,17–19,23,26,30,36,37,40,42,54,62–64,69,70,73,74,77–79,84,86–103] Limitations of including immuno-based studies include the lack of specificity for some protein subtypes. For example, numerous immunohistochemical studies have observed enrichment of various collagens in both CADASIL and CAA. However, unlike with proteomics-based approaches, immunohistochemical studies often cannot discern the specific collagen subtype (eg, COL1A1 versus COL1A2). In these cases, only the more specific collagen subtypes from liquid chromatography–tandem mass spectrometry studies were included, when

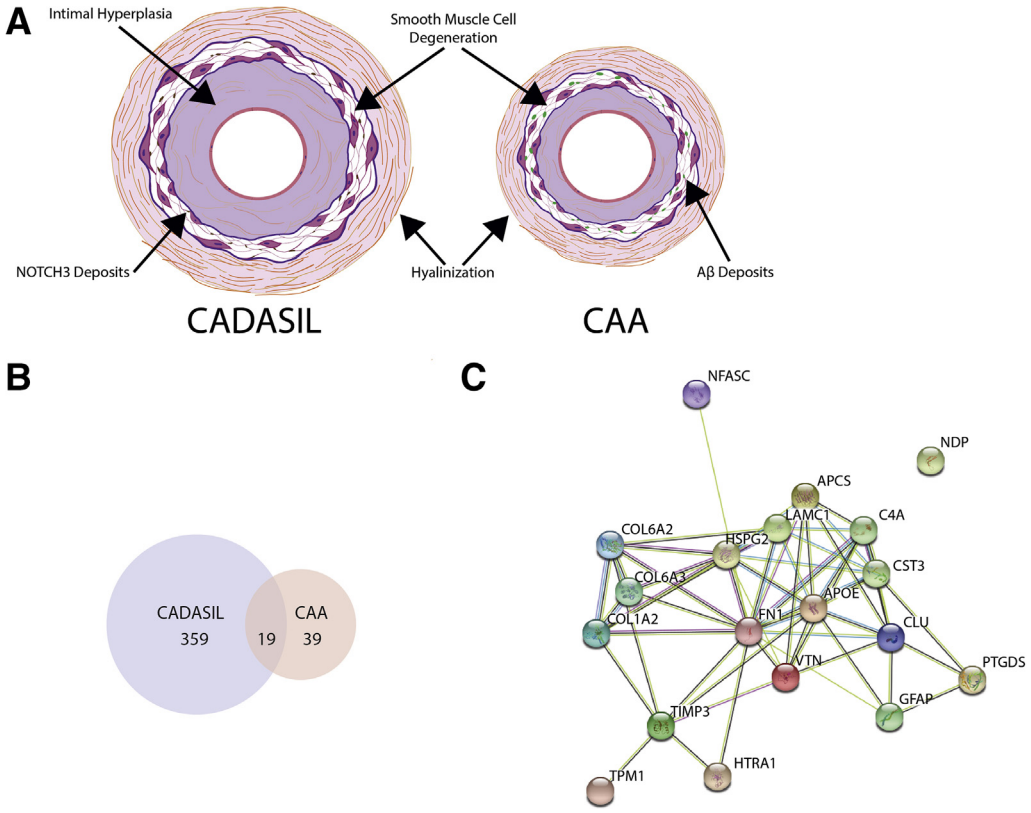


**Figure 1** Differences and similarities between cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and cerebral amyloid angiopathy (CAA) histopathology and protein profiles. **A:** The histopathologies of CADASIL and CAA vessels are compared. Both CADASIL and CAA feature abnormal protein accumulation. In CADASIL, the major protein involved is NOTCH3 ectodomain (brown deposits), whereas in CAA, the major protein involved is amyloid-β (Aβ; green deposits). CADASIL vessels also demonstrate dramatic intimal hyperplasia with accumulation of intimal proteins. Both CADASIL and CAA vessels involve significant smooth muscle cell degeneration in the medial layer and hyalinization of the vessel walls. **B:** Examination of published literature indicated 378 proteins differentially regulated in CADASIL and 58 proteins differentially regulated in vascular CAA. Of these, approximately 33% of the proteins differentially regulated in vascular CAA overlap with those differentially regulated in CADASIL. **C:** STRING version 11 analysis shows a high degree of interconnectedness among the shared proteins.[82] Only norrin (NDP) does not have any known or predicted interactions with the other proteins. APCS, serum amyloid protein; APOE, apolipoprotein E; CLU, clusterin; COL1A2, collagen α-2(I) chain; COL6A2, collagen α-2(VI) chain; COL6A3, collagen α-3(VI) chain; C4A, complement C4-A; CST3, cystatin C; FN1, fibronectin; GFAP, glial fibrillary acidic protein; HSPG2, basement membrane–specific heparan sulfate proteoglycan core protein; HTRA1, serine protease HTRA1; LAMC1, laminin subunit γ 1; NFASC, neurofascin; PTGDS, prostaglandin H2 D isomerase; TIMP3, metalloproteinase inhibitor 3; TPM1, tropomyosin α 1 chain; VTN, vitronectin.

available. When unavailable, the general protein (eg, COL4) was included. In later gene expression analyses, all subtypes of the protein were included. Occasionally, studies indicated opposite directions of change for some proteins (eg, glial fibrillary acidic protein and basement membrane–specific heparan sulfate proteoglycan core protein). These cases were noted, and the proteins were graded in the direction supported by most studies (≥50%).

Analysis of existing literature identified significant overlap of proteins differentially regulated in CADASIL and CAA compared with controls. The vast majority of these proteins come from liquid chromatography–tandem mass spectrometry studies. The advantages of proteomics-based studies include the ability to assess global protein content from disease tissue.[76] In CAA without underlying AD pathology, 58 proteins from eight studies were found to be enhanced or decreased. A comparison between proteins differentially regulated in CADASIL and CAA studies highlighted 19 shared proteins changed in the same direction, which made up roughly 33% of the enhanced or decreased CAA proteins (Figure 1B and Table 2[15,17,18,26,42,69,70,74,86,89,104]). Functional STRING version 11 (https://string-db.org/) analysis demonstrated that 18 of the 19 shared proteins are known or predicted to interact with each other, with an average per protein 5.6 predicted and known interactions with other shared proteins listed in Table 2 (Figure 1C).[82] Only norrin did not demonstrate any known or predicted interactions with the other overlapped proteins.[82] Interestingly, the vast majority of the shared enriched proteins are components of the ECM.[82] The high degree of similarity and interconnectedness of shared proteins suggests mechanistic overlap between the two vascular angiopathies at the molecular level.

Expansion of the literature search to include CAA studies with underlying AD pathology (which added an additional 20 studies), led to a list of 177 proteins that are increased or decreased in the disease. A comparison between CADASIL-affected and CAA with AD-affected proteins identified 37

**Table 1** Inclusion Criteria for CADASIL, Vascular CAA, and CAA with Underlying AD Pathology Studies

| CADASIL studies | Method | Tissue | Brain region | Sample size | Inclusion criteria |
|---|---|---|---|---|---|
| Chen et al[86] | Plasma biomarker | Plasma | NA | 63 CADASIL, 17 control | $P < 0.05$ |
| Young et al[19] | IHC | Leptomeningeal arteries, small penetrating arteries of white matter | Frontal lobe | 19 CADASIL, 10 control | Qualitative |
| Zellner et al[42] | LC-MS/MS | Isolated cerebral vessels | Frontal lobe | 6 CADASIL, 6 control | $P < 0.05$ |
| Nagatoshi et al[18] | LC-MS/MS | Isolated leptomeningeal arteries/arterioles or superficial temporal artery | NA | 3 CADASIL (2 autopsy, 1 biopsy), 6 control (5 autopsy, 1 biopsy) | Top 25 changed proteins (up/down) in nondetected/detected and detected/detected conditions |
| Zhang et al[37] | WB, IHC | Leptomeningeal arteries, small penetrating arteries of white matter | Frontal lobe | 8 CADASIL, 6 control | $P < 0.05$ |
| Zhang et al[30] | IHC | Leptomeningeal arteries, small penetrating arteries of white matter | Frontal lobe | 8 CADASIL, 6 control | Qualitative |
| Lee et al[36] | WB, IHC | Leptomeningeal arteries, small penetrating arteries of white matter, and capillaries | Anterior temporal lobe | 6 CADASIL, 6 control | $P < 0.05$ |
| Kast et al[17] | WB, IHC | Arterioles | Frontal subcortex | 5 CADASIL, 4 control | Qualitative |
| Monet-Leprêtre et al[15] | Nano—LC-MS/MS | Isolated microvessels | Frontal or occipital lobe | 7 CADASIL, 9 control | >6 Peptide changes from control |
| Dong et al[35] | IHC | Leptomeningeal arteries, small penetrating arteries of white matter, and capillaries | Anterior temporal lobe | 6 CADASIL, 6 control | $P < 0.05$ |
| Zhang et al[87] | IHC | Leptomeningeal arteries, small penetrating arteries of white matter, and capillaries | Anterior temporal lobe | 6 CADASIL, 25 control | Qualitative |
| Arboleda-Velasquez et al[26] | LC-MS/MS | Arterioles | Subcortical white matter | 2 CADASIL (R1031C mutation), 2 control | Absolute value of the difference between the average peptide counts (ie, PSM) in the experimental sample minus the average PSM in the control sample exceeded three times the variation and a value of 4 |
| Joutel et al[23] | WB, IHC | Cerebral arteries, veins, and capillaries | Frontal lobe | 9 CADASIL, 13 control | Qualitative |
| **CAA studies** | **Method** | **Tissue** | **Brain region** | **Sample size** | **Inclusion criteria** |
| Endo et al[70] | LC-MS/MS | Laser capture microdissected tissue; leptomeningeal and cortical vessels | Cortical tissue | 6 CAA, 5 control | ≥50% Detection in CAA patients |
| Hondius et al[74] | LC-MS/MS | Laser capture microdissected tissue | Cortical layers II to VI | 7 AD, 7 CAA, 6 control | $P < 0.05$ |
| Grand Mourset et al[73] | IHC, qPCR | Parenchymal angiopathic arterioles | Frontal and occipital cortex | 11 HCHWA-D, 11 sCAA, 11 control | $P < 0.05$ |
| de Jager et al[69] | IHC | Leptomeningeal and parenchymal vessels | Neocortex | 5 AD, 2 CAA, 5 HCHWA-D, 7 control | Qualitative |
| Carrano et al[64] | IHC | Normal and Ab-laden capillaries | Occipital cortex | 23 CAA | $P < 0.05$ |
| van Horssen et al[88] | IHC | Leptomeningeal and cortical vessels | Frontal cortex | 7 AD, 4 HCHWA-D, 3 control | Qualitative |

(*table continues*)

**Table 1** (*continued*)

| CADASIL studies | Method | Tissue | Brain region | Sample size | Inclusion criteria |
|---|---|---|---|---|---|
| McCarron et al[89] | IHC | Leptomeningeal and cortical vessels | Frontal, parietal, occipital, temporal or GFM | 26 AD, 37 CAA, 20 control | $P < 0.05$ |
| Tagliavini et al[90] | WB, IHC | Isolated leptomeningeal and cortical microvessels | Cortical tissue | 3 AD, 2 HCHWA-D, 2 control | Qualitative |
| **CAA studies with underlying AD pathology** | **Method** | **Tissue** | **Brain region** | **Sample size** | **Inclusion criteria** |
| Jäkel et al[78] | IHC | Leptomeningeal and cortical vessels | Occipital cortex | 18 CAA (nonhemorrhagic), 11 CAA (hemorrhagic), 11 control | $P < 0.05$ |
| Magaki et al[62] | IHC | Gray matter and white matter | Frontal, temporal, parietal, and occipital cortices, hippocampus, entorhinal cortex and amygdala, basal ganglia, brainstem, and cerebellum | 7 AD, 8 type 1 CAA, 10 type 2 CAA, 10 control | $P < 0.05$ |
| Inoue et al[91] | LC-MS/MS | Cerebral neocortical tissue | Neocortical tissue | 8 Severe CAA, 12 mild CAA, and 10 control | $P < 0.05$ |
| Lepelletier et al[77] | IHC | Cortical gray matter | Superior frontal gyrus and inferior temporal gyrus | 17 AD (10 subclinical, 8 clinical), 1 CAA, 12 control | $P < 0.05$ |
| Merlini et al[92] | IHC | Leptomeningeal arterioles, small arteries, medium arteries | GFM and hippocampus | 17 AD, 28 control | $P < 0.05$ |
| Keable et al[93] | IF | Leptomeningeal and intraparenchymal vessels | Occipital cortex | 20 Severe CAA, 14 young controls, 20 aged controls | $P < 0.05$ |
| Manousopoulou et al[40] | LC-MS | Isolated leptomeningeal arteries | Occipital cortex | 4 CAA, 2 old control (females only) | >Average $\pm$ 2.4 log2 ratio |
| Hernandez-Guillamon et al[79] | WB, IHC, ELISA | Hemorrhagic stroke area | Perihematoma and contralateral area | 4 CAA, 3 control | $P < 0.05$ |
| Carrano et al[63] | IHC | Normal and Ab-laden capillaries | Occipital cortex | 6 CAA, 2 control | $P < 0.05$ |
| Xiong et al[94] | WB, IHC, qPCR | Cerebral vasculature | Occipital cortex | 13 AD, 13 CAA/AD, 12 normal | $P < 0.05$ |
| Bell and Zlokovic[54] | WB | Leptomeningeal vessels | Brodmann areas 9/10 | 3 AD, 3 control | $P < 0.05$ |
| Stopa et al[95] | IHC | Arterioles | Frontal cortex | 76 AD, 19 control | $P < 0.05$ |
| Miners et al[96] | IHC | Cerebral vasculature | Frontal cortex | 5 AD, 5 AD with CAA, 5 control | $P < 0.05$ |
| Tian et al[97] | IHC | Leptomeningeal arteries and intraparenchymal blood vessels | Frontal cortex | 70 Late-stage AD patients | $P < 0.05$ |
| Ervin et al[98] | IHC | Arteries | Frontal cortex | 10 AD (APOE4), 10 AD (APOE3), 10 control | $P < 0.05$ |

(*table continues*)

**Table 1** (*continued*)

| CADASIL studies | Method | Tissue | Brain region | Sample size | Inclusion criteria |
|---|---|---|---|---|---|
| Zhang et al[99] | IHC | Leptomeningeal and cortical vessels | Cerebral cortex | 10 CAA (5 AD, 1 Down syndrome, 4 unknown CAA), 8 control | Qualitative |
| Verbeek et al[100] | IHC | Leptomeningeal and cortical vessels | Frontal, parietal, temporal, and occipital cortex | 11 AD, 1 FTD, 11 control | Qualitative |
| Kalaria[101] | WB, IHC | Cerebral microvessels, large meningeal vessels, and choroidal samples | Frontal, occipital, temporal lobes and cerebellar cortex | 22 AD, 8 control | $P < 0.05$ |
| Powers et al[102] | IHC | Arteries and plaque | Cerebral cortex | 2 Presenile AD, 5 senile AD, 5 control | Qualitative |
| Ishii and Haga[103] | IHC | Vessels and plaques | Frontal cortex | 2 AD, 2 control | Qualitative |

A total of 13 CADASIL studies, 8 vascular CAA studies, and 20 CAA with underlying AD pathology studies were examined to identify differentially regulated proteins in disease. Detection methods included LC-MS/MS, IHC, and WB. Sample types include isolated vessels and/or brain tissue. When applicable, the brain region is specified. Sample sizes are noted for each study. When classifying studies examining vascular CAA, patients with known AD or cognitive changes indicative of AD were excluded. From each of these studies, a protein was denoted as differentially regulated if it was significantly changed in disease compared with control ($P < 0.05$), qualitatively changed, as in the case of IHC, or otherwise specified if no statistical analysis was available.

Ab, antibody; AD, Alzheimer disease; APOE, apolipoprotein E; CAA, cerebral amyloid angiopathy; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; ELISA, enzyme-linked immunosorbent assay; FTD, frontotemporal dementia; GFM, gyrus frontalis medialis; HCHWA-D, hereditary cerebral hemorrhage with amyloidosis, Dutch type; IF, immunofluorescence; IHC, immunohistochemistry; LC-MS, liquid chromatography—mass spectrometry; LC-MS/MS, liquid chromatography—tandem mass spectrometry; NA, not applicable; PSM, peptide spectrum matches; qPCR, quantitative PCR; WB, Western blot analysis.

shared proteins effected in the same direction. With the inclusion of AD studies, the proportion of shared proteins with CADASIL gets diluted from approximately 33% of CAA-only proteins to approximately 21% of CAA with underlying AD proteins, a finding consistent with the possibility that AD-associated CAA and sporadic/familial CAA arise from different mechanisms. In both cases, the shared proteins are still mostly involved with ECM remodeling, suggesting that ECM changes could play a role in both pure vasculopathies and AD-related vascular changes.

Classic cerebrovascular ECM structures include vascular basement membranes, which are extracellular layers that separate endothelial cells, contractile cells, and astrocytic end feet.[38] On electron microscopy, vascular basement membranes are often visualized as homogeneous-appearing layers ranging from 50 to 100 nm in thickness.[38] Cerebrovascular basement membranes are involved in blood vessel development and formation and maintenance of the BBB.[105] Vascular basement membranes are known to contain laminins, type IV collagens, and heparan sulfate proteoglycans, such as perlecan, agrin, and type XVIII collagen.[38] Laminins, type IV collagen, and perlecan can also interact with nidogens to provide a scaffold for other basement membrane proteins to interact with.[38]

Interestingly, both CADASIL and AD are associated with changes in the basement membrane. In CADASIL, pathognomonic GOMs found in the basement membranes are known to harbor multiple NOTCH3 forms, TIMP3, VTN, LTBP1, amyloid P (SAP), clusterin, annexin 2, and periostin.[15–19] In addition, in AD, studies have identified thickening of the basement membrane, which can also contain Aβ deposits.[106] Tracer studies in AD demonstrate that parenchymal interstitial fluid is cleared via drainage through the basement membrane.[107] Recent studies suggest that soluble Aβ can follow this perivascular clearance pathway when injected into the cerebrospinal fluid and that it gets trapped within cerebrovascular walls, resulting in CAA-like accumulation of Aβ in arteries.[108] The trapping of Aβ in the same location affected by CAA histopathology poses the question whether some forms of CAA result from physiological clearance of parenchymal Aβ. It is thus tempting to conceptualize a similar mechanism in CADASIL, because NOTCH3 accumulation is also found to occur within basement membranes. Mutant NOTCH3 expressed in vascular smooth muscle cells could also be cleared through basement membranes, and entrapment of the protein within the basement membrane may mimic the physiological effects of Aβ accumulation.[23,109]

**Table 2** Proteins That Are Differentially Regulated in Both CADASIL and CAA

| Regulated proteins in CADASIL and CAA | Coding gene | Subcellular localization | STRING interactions, *N*[82] |
|---|---|---|---|
| **Serum amyloid protein (SAP)**[15,18,42,74] | *APCS* | Extracellular | 7 |
| **Apolipoprotein E**[15,42,70,74,89] | *APOE* | Extracellular | 10 |
| **Complement C4-A**[42,70] | *C4A* | Extracellular | 5 |
| **Clusterin**[15,26,42,70,74] | *CLU* | Extracellular, cytoplasm, mitochondria, endoplasmic reticulum, nucleus | 7 |
| **Collagen α-2(I) chain**[26,42,70] | *COL1A2* | Extracellular | 5 |
| **Collagen α-2(VI) chain**[15,18,42,74] | *COL6A2* | Extracellular | 5 |
| **Collagen α-3(VI) chain**[15,42,74] | *COL6A3* | Extracellular | 5 |
| **Cystatin C**[18,89] | *CST3* | Extracellular | 8 |
| **Fibronectin**[15,17,69] | *FN1* | Extracellular | 14 |
| **Glial fibrillary acidic protein**[15,42,70,86] | *GFAP* | Cytoplasm | 4 |
| **Basement membrane–specific heparan sulfate proteoglycan core protein**[15,26,88,104] | *HSPG2* | Extracellular | 9 |
| **Serine protease HTRA1**[42,74] | *HTRA1* | Extracellular, cytosol, plasma membrane | 2 |
| **Laminin subunit γ 1**[15,26,70] | *LAMC1* | Extracellular | 7 |
| **Norrin**[15,42,74] | *NDP* | Extracellular | 0 |
| *Neurofascin*[26,74] | *NFASC* | Plasma membrane | 1 |
| **Prostaglandin H2 D isomerase**[15,42,70] | *PTGDS* | Extracellular, endoplasmic reticulum, nucleus, Golgi apparatus | 3 |
| **Metalloproteinase inhibitor 3**[15,42,70] | *TIMP3* | Extracellular | 7 |
| *Tropomyosin α 1 chain*[18,74] | *TPM1* | Cytoskeleton | 1 |
| **Vitronectin**[15,18,42] | *VTN* | Extracellular | 7 |

A review of 21 proteomics-based and immuno-based studies on CADASIL and vascular CAA identified 19 shared proteins regulated in the same direction. Boldfaced proteins were enriched in CADASIL and CAA, whereas italicized proteins were decreased in both CADASIL and CAA. Most of these proteins are extracellular proteins, and all proteins on this list, except norrin, have known or predicted interactions with others on this list.[82]

CAA, cerebral amyloid angiopathy; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

As discussed earlier, the observed enrichment of ECM proteins in CADASIL and CAA could result from enhanced protein synthesis or recruitment and/or decreased protein turnover due to formation of stable protein complexes or sequestration of chaperones and proteases. Given that both CADASIL and CAA are linked to degeneration of the medial layer of vessels, one model suggests that enhanced cross-linking of ECM proteins could be a pathophysiological response to strengthen weakened vessel walls.[65] Enrichment of multiple overlapping proteins involved in ECM organization in CADASIL and CAA (Table 2) was as follows: laminin subunit γ 1 (increased in two CADASIL and one CAA study),[15,26,70] various collagens (COL1A2 was increased in two CADASIL and two CAA studies; COL6A2 was increased in four CADASIL and one CAA study; and COL6A3 was increased in two CADASIL and one CAA study),[15,18,26,42,70,74] fibronectin (increased in two CADASIL and one CAA study),[15,17,69] VTN (increased in three CADASIL and one CAA study),[15,18,42,70] HTRA1 (increased in one CADASIL and one CAA study),[42,74] and basement membrane–specific heparan sulfate proteoglycan core protein (increased in two CADASIL and one CAA study; decreased in one CAA study but included in Table 2 because it was found to be increased in two CAA/AD studies as well).[15,31,70,88]

Support for increased ECM protein synthesis and recruitment comes from identification of TGF-β signaling substrates and downstream signaling molecules in both CADASIL and CAA.[15,17,69,70,73,74] TGF-β activity is involved in regulation of fibrotic events in the vasculature.[17] TGF-β signaling targets, fibronectin,[15,17,69] collagens,[15,18,26,42,70,74] and TIMP3 (increased in two CADASIL and one CAA study)[15,42,70] were found to be enriched in both CADASIL and CAA (Table 2). Dysregulation of TGF-β signaling could result from accumulation and aggregation of NOTCH3 and/or Aβ in the cerebrovasculature that then promotes abnormal recruitment of additional ECM proteins.

Decreased ECM protein turnover as a result of sequestered chaperones or dysregulation of chaperones is supported by the enrichment of known proteases and their substrates. Enhancement of HTRA1[42,74] and its substrates, clusterin (increased in three CADASIL and three CAA studies)[15,31,42,70,74] and VTN,[15,18,42,70] was identified in both CADASIL and CAA (Table 2).[41,42,75,76] HTRA1 is a serine protease, and *HTRA1* mutations have also been linked to CARASIL, an autosomal recessive cerebrovascular disorder that shares clinical features with CADASIL.[41] Sequestration, inactivation, and/or dysregulation of such chaperones and proteases can therefore contribute to abnormal ECM protein accumulation in multiple vascular angiopathies, including CADASIL and CAA.

Given that both CADASIL and CAA ultimately result in vascular smooth muscle cell degeneration, it cannot be discounted that the observed accumulation of ECM proteins

could be an indirect consequence of disease and/or the loss of vascular smooth muscle cells due to disease. One explanation could be that in both CADASIL and CAA, the disease process results in widespread vascular smooth muscle cell death, leaving only the ECM and ECM proteins remaining. On the other hand, mouse models suggest that NOTCH3 accumulation is one of the earliest features in mouse models of disease, supporting a direct role of NOTCH3 accumulation in disease.[29,39] Furthermore, reduction of ECM protein TIMP3 in a mouse model of CADASIL does not affect NOTCH3 deposition in the cerebrovasculature or GOMs but rescues measures of cerebrovascular reactivity in a CADASIL mouse model, suggesting a role of ECM protein accumulation in disease pathogenesis.[110] Therefore, a simplified model might suggest that abnormal protein accumulation in CADASIL (NOTCH3) or CAA (Aβ) could lead to abnormal recruitment of ECM proteins, which could then contribute to CADASIL or CAA pathogenesis. Nevertheless, it is not clear whether mouse models of CADASIL fully recapitulate human CADASIL pathomechanisms, and additional studies will be required to discern mechanistic details.

## Many Proteins Altered in CADASIL and CAA Are Long-Lived

Long-lived proteins, proteins that demonstrate limited turnover over months to years, have been linked to age-associated defects.[111] Toyama et al[111] utilized a proteomics-based approach to identify long-lived proteins in rat brains. Of the proteins identified to be differentially regulated in CADASIL and CAA and considered to be long-lived, there was an overlap among collagens, laminins, nuclear proteins, and myelin-associated proteins. A total of 63% of the long-lived extracellular structural proteins were enhanced in CADASIL, and 25% were enhanced in CAA, whereas 60% of the long-lived myelin-associated proteins were decreased in CADASIL. The loss of long-lived myelin-associated proteins in CADASIL is not surprising because CADASIL is a known white matter disease, with myelin degradation found in both human and mouse CADASIL brains.[112,113] Using pulse-chase experiments, Toyama et al[111] propose that protein longevity is likely not a result of protein expression changes but rather a result of deposition into large stable structures. Thus, accumulation

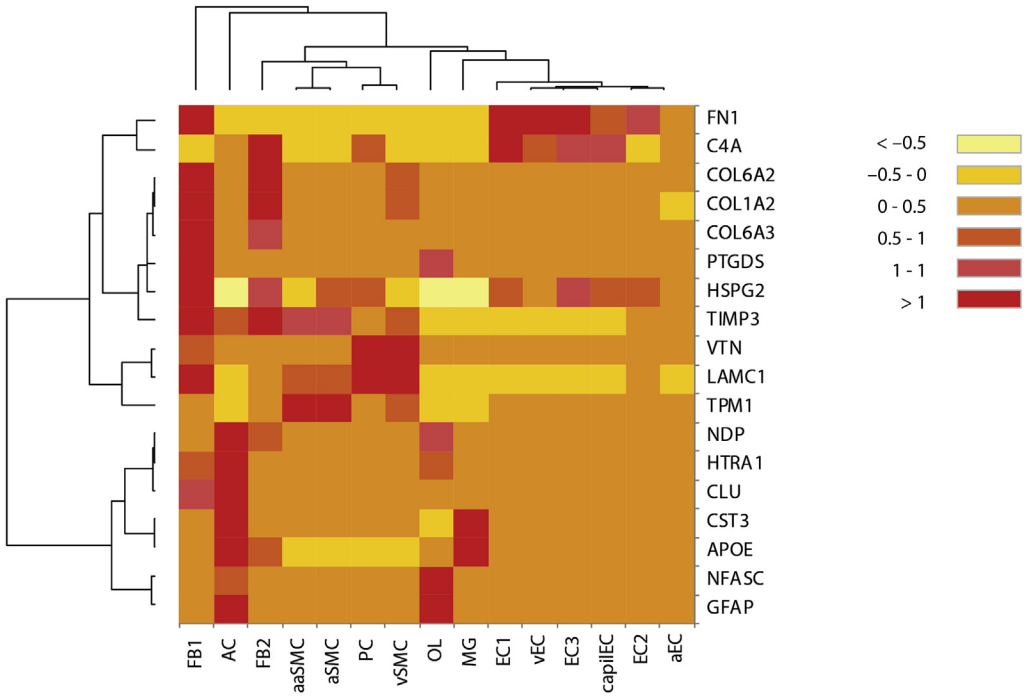


**Figure 2** Identifying vascular cell types expressing genes encoding for overlapped proteins in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and cerebral amyloid angiopathy (CAA). To determine which vascular cells produce the overlapping proteins, vascular gene expression data were compared using an online database of single-cell RNA-sequencing data from adult murine brains (*http://betsholtzlab.org/VascularSingleCells/database.html*, last accessed August 23, 2020).[114,115] Average counts from each cell type were used to generate the heat map using XLSTAT software version 2020.5 (Addinsoft Corp., Paris, France). Heat map colors were defined by SD. In normal murine brains, the primary cell types responsible for production of most of these proteins were fibroblasts. Only vitronectin (*VTN*), laminin subunit γ (*LAMC1*), and tropomyosin α 1 chain (*TPM1*) were primarily expressed by pericytes or venous, arterial, and arteriolar smooth muscle cells (vSMCs, aSMCs, and aaSMCs, respectively). AC, astrocyte; aEC, arterial EC; APOE, apolipoprotein E; capilEC, capillary EC; CLU, clusterin; COL1A2, collagen α-2(I) chain; COL6A2, collagen α-2(VI) chain; COL6A3, collagen α-3(VI) chain; C4A, complement C4-A; CST3, cystatin C; EC, endothelial cell; FB, vascular fibroblast-like cell; FN1, fibronectin; GFAP, glial fibrillary acidic protein; HSPG2, basement membrane–specific heparan sulfate proteoglycan core protein; HTRA1, serine protease HTRA1; MG, microglia; NDP, norrin; NFASC, neurofascin; OL, oligodendrocyte; PC, pericyte; PTGDS, prostaglandin H2 D isomerase; TIMP3, metalloproteinase inhibitor 3; vEC, venous EC; EC1,2,3 endothelial cell subtypes.[114,115]

of long-lived extracellular proteins is consistent with the idea that ECM protein complexes participate in both CADASIL and CAA.

### Identifying Vascular Cell Types That Express Genes Overlapped in CADASIL and CAA

To determine if there is a primary cell type involved in these two vasculopathies, vascular gene expression of the overlapping proteins in Table 2 was investigated at the cellular level using an online database of single-cell RNA-sequencing data from normal adult murine brains (*http://betsholtzlab.org/VascularSingleCells/database.html*, last accessed August 23, 2020).[114,115] CADASIL is thought to predominantly be a vascular smooth muscle cell disease, because *NOTCH3* is primarily expressed in vascular smooth muscle cells.[23,109] In addition, pathologic features of both CADASIL and CAA include vascular smooth muscle cell degeneration. However, evaluation of the cells expressing the overlapping genes in mice suggests that the vast majority of these genes are primarily expressed by fibroblasts (Figure 2).[114,115] This agrees with the observation that most overlapping proteins are ECM proteins and fibroblasts are the principal source of ECM proteins.[116] Although somewhat surprising, given two seemingly smooth muscle cell disorders, one explanation could be that in both CADASIL and CAA, diseased smooth muscle cells stimulate fibroblasts to secrete additional ECM proteins, supporting the idea that accumulation of ECM proteins could be a consequence of primary vascular smooth muscle cell pathology. For example, the secretion of additional ECM proteins could be a response to bolster the integrity of the degenerating vessel wall. Nevertheless, because the current analysis involves single-cell RNA sequencing data from normal mice, the possibility that diseased vascular smooth muscle cells transform from a contractile to productive mode, similar to that in atherosclerosis and in development, can not be excluded.[117] Interestingly, TGF-β, which we propose is involved in both CADASIL and CAA pathomechanisms, is a key mediator of vascular smooth muscle cell phenotype switching.[117] Furthermore, it is important to keep in mind that the expression patterns observed in mice may not necessarily reflect those in people, especially in patients with CADASIL or CAA. Additional characterization of mouse and human vasculature at the molecular level would be required in the future to determine the full extent of translatability of preclinical models.

## Conclusion

In summary, CADASIL and CAA are two pathologically distinct cerebrovascular diseases that feature small-vessel involvement. However, a high degree of clinical and molecular overlap suggests a potential for shared disease pathways that have been previously recognized. Through an expanded review of existing literature, 19 shared proteins were found to be regulated in both CADASIL and CAA. The vast majority of these proteins are known components of the extracellular matrix and have been demonstrated or predicted to interact with each other. Proposed models to explain the observed enrichment of ECM proteins include both increased protein secretion and decreased protein turnover due to formation of stable protein complexes or, alternatively, sequestration or dysregulation of chaperones and/or proteases. Finally, single-cell RNA sequencing of vascular cells suggests that the vast majority of the genes accounting for the overlapped proteins between CADASIL and CAA are expressed by fibroblasts. In the short-term, this information suggests that basic knowledge about each disease may inform the other. In the long-term, interrelations between the proteins shared in the two disorders raise the possibility of shared interventional approaches. Future studies remain necessary to clarify the role of ECM proteins in cerebrovascular diseases and cerebral small-vessel disease.

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