From cerebrovascular disease to cerebral small vessel disease subtypes: multiscale tools for decoding vascular-centered heterogeneity

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Abstract

Cerebral small vessel disease (cSVD) is a major contributor to ischemic stroke, intracerebral hemorrhage, vascular cognitive impairment, dementia, and age-related functional decline. Traditional cSVD research and clinical classification have largely relied on neuroimaging markers, including white matter hyperintensities, lacunes, recent small subcortical infarcts, cerebral microbleeds, enlarged perivascular spaces, and brain atrophy. Although these markers provide a robust phenotypic framework, they do not fully explain the molecular mechanisms, cellular heterogeneity, regional vulnerability, or divergent clinical trajectories of cSVD. Increasing evidence suggests that cSVD should be viewed as a vascular-centered neurovascular unit disorder involving endothelial dysfunction, blood-brain barrier disruption, pericyte injury, vascular smooth muscle cell degeneration, extracellular matrix remodeling, impaired perivascular clearance, glial activation, immune involvement, and metabolic stress.

In this review, we summarize cSVD from a multiscale and vascular-centered perspective. First, we discuss the position of cSVD within cerebrovascular disease, its major pathological categories, molecular biological basis, and imaging features. Second, we review the research objects and tools used to study cSVD across vascular, structural, regional, molecular, imaging, clinical, genetic, and computational levels. Third, we examine data-type-specific analytical strategies, including imaging data, clinical and demographic variables, genetic and molecular evidence, and multimodal integration. Finally, we discuss how cSVD research may move from traditional

disease classification toward molecular subtype classification. We propose that future cSVD classification should integrate lesion localization, vascular pathology, brain-region vulnerability, cell-type-specific molecular programs, clinical trajectories, and therapeutic target accessibility. Within this framework, vascular-centered molecular barcode discovery and subtype-aware drug mapping may provide new routes toward mechanism-guided stratification and precision intervention. Overall, this review emphasizes the need to reinterpret cSVD not as a single radiological endpoint, but as a set of molecularly distinguishable and therapeutically targetable cerebrovascular injury programs.

Keywords: cerebral small vessel disease; neurovascular unit; blood-brain barrier; regional vulnerability; multimodal integration; molecular subtyping; precision intervention

1. Introduction

Cerebral small vessel disease (cSVD) is an important contributor to the global burden of cerebrovascular disease and age-related cognitive decline. It primarily affects older adults and is closely associated with chronic vascular and metabolic disorders, including hypertension, diabetes, cardiovascular disease, and systemic inflammation[1, 2]. As a subtype of cerebrovascular disease, cSVD accounts for a substantial proportion of ischemic stroke and intracerebral hemorrhage, and is also one of the major causes of vascular cognitive impairment and dementia[3]. With population aging and the increasing prevalence of vascular risk factors, the clinical and socioeconomic burden of cSVD is expected to continue rising. Therefore, elucidating the mechanisms of cSVD and developing strategies for early detection, stratification, and intervention have become urgent tasks in cerebrovascular research.

Neuroimaging provides the main clinical framework for detecting and classifying cSVD. Imaging features include white matter hyperintensities, small subcortical infarcts, lacunes, cerebral microbleeds, enlarged perivascular spaces, and brain atrophy[3]. Among these markers, white matter hyperintensities are particularly common and are closely associated with stroke risk, cognitive impairment, and functional decline[3]. Beyond conventional MRI, advanced imaging techniques such as diffusion tensor imaging, neurite orientation dispersion and density imaging, cerebrospinal-fluid-based spatial statistics, and connectomic analyses have further expanded the ability to detect white matter microstructural abnormalities, vascular

dysfunction, cerebrospinal fluid dynamics, and lesion-related network disturbances[4-6]. Imaging-genetic studies have also linked white matter microstructural phenotypes to genetic loci involved in vascular integrity and early mechanisms of chronic small vessel disease[4]. However, despite these advances, imaging markers alone cannot fully explain the molecular mechanisms, cellular heterogeneity, or divergent clinical trajectories of cSVD. At the biological level, cSVD is increasingly understood as a disorder of the neurovascular unit rather than a purely radiological entity. Endothelial dysfunction, blood-brain barrier disruption, pericyte injury, vascular smooth muscle cell degeneration, extracellular matrix remodeling, impaired perivascular clearance, astrocytic reactivity, microglial activation, and peripheral immune involvement may collectively drive sustained vascular and white matter injury[2, 3, 7]. Chronic neuroinflammation is an especially important pathological feature, characterized by persistent microglial activation, immune cell infiltration, cytokine signaling, and neurovascular unit dysfunction [7]. These processes may aggravate vascular injury, white matter degeneration, and cognitive decline, yet the specific cellular subsets and molecular programs that drive different cSVD phenotypes remain incompletely defined.

In recent years, advances in genomics, transcriptomics, proteomics, metabolomics, single-cell sequencing, spatial transcriptomics, and multimodal data integration have provided new opportunities to resolve the heterogeneity of cSVD. Integrative multi-omics studies have begun to reveal inflammatory pathways, gut microbiota–

brain interactions, extracellular matrix dysregulation, vascular remodeling programs, and immune-related molecular features in cSVD[8, 9]. For example, the gut microbiota of patients with atherosclerotic cSVD may promote IL-17A production through RORγt-related neutrophil activation[9], whereas IL-2 receptor signaling may participate in post-ischemic white matter injury by regulating CD8+ T lymphocyte differentiation[10]. Meanwhile, monogenic and hereditary forms of cSVD highlight the importance of specific vascular molecular pathways, including extracellular matrix remodeling, TGF- β signaling, COL4A1-related basement membrane dysfunction, and ARHGEF15-mediated vascular pathology[11, 12]. These findings indicate that cSVD is not a single disease process, but rather a heterogeneous spectrum of vascular-centered brain injury states. However, translating multi-omics findings into clinically useful biomarkers and therapeutic strategies remains a central bottleneck. Molecular studies are often limited by batch effects, species differences, tissue accessibility, small sample sizes, model heterogeneity, and inter-individual variability. Imaging studies provide robust phenotypic information, but often lack mechanistic resolution. Genetic studies can identify risk loci, but may not clarify cell-type-specific mechanisms. Single-cell and spatial analyses reveal disease-associated cellular states, but remain difficult to directly connect with clinical imaging manifestations and therapeutic decision-making.

Therefore, the field still lacks a unified framework capable of integrating lesion localization, vascular pathology, cellular subpopulations, molecular features, and

therapeutic targets. In this review, we systematically summarize cSVD from a vascular-centered and multiscale perspective. We first outline the position of cSVD within cerebrovascular disease, its major pathological categories, molecular biological basis, and imaging features. We then discuss the tools used to study cSVD across vascular, structural, regional, molecular, imaging, clinical, genetic, and computational scales. Finally, we explore how cSVD research may move from traditional disease classification toward molecular subtype classification, and how integrative multi-omics frameworks may support biomarker discovery, disease stratification, and the development of precision therapy.

2. From cerebrovascular disease to cerebral small vessel disease: classification, causes, and pathological basis

2.1. Cerebrovascular disease and the position of cSVD

Cerebrovascular disease refers to a group of central nervous system disorders caused by structural or functional abnormalities of cerebral blood vessels, among which stroke is one of the most important clinical manifestations. According to the underlying pathological process, stroke is commonly classified into ischemic stroke and hemorrhagic stroke. Common etiological subtypes of ischemic stroke include cardioembolism, large-artery atherosclerosis, lacunar or small-vessel-disease-related stroke, as well as cryptogenic stroke or stroke of other determined causes[13]. In contrast, hemorrhagic stroke mainly includes intracerebral hemorrhage and

subarachnoid hemorrhage, which may be associated with hypertensive small arteriopathy, cerebral amyloid angiopathy, vascular malformations, large-artery disease, or other forms of vascular wall fragility[14, 15].

Within this spectrum of cerebrovascular disease, cerebral small vessel disease (cSVD) occupies a distinct position. cSVD primarily affects small arteries, arterioles, capillaries, small veins, and perivascular structures in the brain, and represents an important pathological basis for lacunar infarction, intracerebral hemorrhage, white matter hyperintensities, cerebral microbleeds, enlarged perivascular spaces, and vascular cognitive impairment. Epidemiological data indicate that stroke continues to impose a substantial global disease burden. According to NHANES data, the prevalence of stroke among adults aged 20 years or older in the United States is approximately 3.3%, while the 2021 Global Burden of Disease data show that stroke-related deaths and prevalent cases remain at high levels worldwide [16]. In this context, cSVD is not only an important risk factor for stroke occurrence and recurrence, but also a key pathological process linking chronic vascular injury, brain structural alterations, cognitive decline, and age-related neurological deterioration.

However, compared with large-vessel disease, cSVD research has long been constrained by multiple limitations. Cerebral small vessels are small in diameter, deeply distributed, and difficult to sample directly. Conventional imaging captures downstream tissue damage more readily than early mechanisms. At the same time, the clinical manifestations and imaging phenotypes of cSVD are highly heterogeneous,

while genetic definition, molecular stratification, and translational tools remain insufficient. Therefore, the study of cSVD needs to move beyond traditional radiological description and further expand into multiscale levels, including vascular structure, molecular mechanisms, cellular states, regional vulnerability, and therapeutic targets. For these reasons, elucidating the basic mechanisms of cSVD and developing more precise strategies for classification, prediction, and intervention have become important directions in cerebrovascular research.

2.2. Major categories of cSVD

Cerebral small vessel disease is not a single disease, but rather a heterogeneous group of pathological conditions involving small arteries, arterioles, capillaries, small veins, and perivascular structures in the brain. Anatomically, the cerebral small vessel network can be broadly divided into the pial and cortical surface vascular networks located on the brain surface, and the penetrating arteries, arterioles, and capillary networks that supply the deep brain parenchyma. Compared with surface vascular networks, penetrating vessels lack sufficient collateral circulation after entering the brain parenchyma. As a result, they are more susceptible to focal or diffuse injury under the influence of chronic hypertension, hemodynamic stress, vascular wall degeneration, or inherited vascular structural abnormalities. This anatomical feature helps explain why cSVD commonly manifests as lesions in the deep white matter, basal ganglia, thalamus, brainstem, and subcortical regions[17].

According to etiology and pathological basis, cSVD can be broadly divided into sporadic and hereditary categories. Sporadic cSVD is the most common form and is usually associated with aging, hypertension, diabetes, dyslipidemia, chronic inflammation, and other vascular risk factors. Its core pathological features include arteriolosclerosis, lipohyalinosis, vascular wall thickening, luminal narrowing, blood-brain barrier injury, and impaired perivascular clearance. These pathological changes are more commonly associated with imaging manifestations such as white matter hyperintensities, lacunar infarcts, cerebral microbleeds, enlarged perivascular spaces, and brain atrophy. In contrast, cerebral amyloid angiopathy (CAA) is a form of cSVD characterized by amyloid deposition in cortical and leptomeningeal small vessel walls, and is commonly associated with lobar microbleeds, lobar intracerebral hemorrhage, cortical superficial siderosis, and cognitive decline, thereby showing a stronger hemorrhagic tendency[3].

Hereditary cSVD is driven by mutations in specific vascular-related genes. Representative disorders include CADASIL caused by NOTCH3 mutations, HTRA1-related cSVD, COL4A1/2-related vascular basement membrane disease, and other less common monogenic small vessel diseases. These disorders highlight the key roles of vascular smooth muscle cells, extracellular matrix, basement membrane structure, TGF- β -related pathways, and vascular wall homeostasis in the development of cSVD. CADASIL and HTRA1-related cSVD typically present with extensive white matter lesions, lacunar infarction, and progressive cognitive

impairment, but they may also be accompanied by cerebral microbleeds or other hemorrhagic markers. This suggests that hereditary cSVD is not purely an ischemic disease, but rather a multi-phenotypic spectrum of cerebrovascular injury caused by structural and functional abnormalities of the vascular wall[3, 18].

From the perspective of clinical and imaging consequences, different types of cSVD can jointly lead to chronic cerebral hypoperfusion, white matter injury, lacunar infarction, cerebral microbleeds, brain atrophy, gait disturbance, mood changes, and cognitive decline. Binswanger disease and multiple lacunar infarction represent classic patterns of subcortical vascular cognitive impairment, respectively emphasizing the disruption of brain network integrity caused by diffuse white matter lesions and multiple small infarcts[19]. Therefore, although different categories of cSVD differ in etiology, vascular involvement, and imaging manifestations, they may ultimately promote long-term neurological decline through shared mechanisms such as vascular wall injury, blood-brain barrier disruption, chronic hypoperfusion, inflammatory responses, and neural network disconnection.

2.3. Molecular biological basis of cSVD

At the molecular level, cSVD can be understood as a heterogeneous set of pathological programs centered on vascular wall homeostasis, blood-brain barrier integrity, extracellular matrix remodeling, inflammatory and immune responses, and metabolic stress. Although different types of cSVD vary in etiology and imaging

presentation, they share a common feature: long-term structural and functional injury of cerebral small vessels, which further affects white matter integrity, regional brain metabolism, neural network connectivity, and cognitive function. Therefore, the molecular biological basis of cSVD should not be viewed simply as a collection of isolated targets, but rather as a combination of multiple vascular-centered pathways operating under different genetic backgrounds, regional brain environments, and systemic risk factors.

Hereditary cSVD provides an important window into the molecular homeostasis of the vascular wall. CADASIL is one of the most typical monogenic forms of hereditary cSVD and is mainly driven by dominant mutations in the NOTCH3 gene. Related studies have shown that some NOTCH3 mutations can lead to abnormal aggregation of the extracellular domain, thereby inducing pathological changes in vascular smooth muscle cells and small arterial walls. Studies based on CADASIL animal models also suggest that interventions targeting NOTCH3 aggregation, receptor function restoration, and abnormal transcriptional regulation may provide potential therapeutic directions for hereditary small vessel disease[20, 21]. Meanwhile, different NOTCH3 mutation locations and mutation types may correspond to different clinical and imaging phenotypes. Mutations located in high-risk EGF-like repeat regions are associated with more severe white matter lesions, lacunes, cerebral microbleeds, and intracranial hemorrhage risk, suggesting that hereditary cSVD may contain both ischemic and hemorrhagic injury components[22].

In addition to NOTCH3-related pathways, abnormalities of the extracellular matrix and vascular basement membrane are also important molecular foundations of cSVD. COL4A1 and COL4A2 encode the α1/α2 chains of type IV collagen, and their genetic variants can be observed in both hereditary and sporadic cSVD, with associations with stroke, dementia, and intracerebral hemorrhage risk. Related models suggest that COL4A1/2-associated abnormalities may affect cerebral small vessel stability and perfusion regulation by disrupting basement membrane structure, endothelial function, vascular wall mechanical properties, and arteriolar vasomotor responses[23]. These findings indicate that the basement membrane and extracellular matrix are not merely structural scaffolds of the vascular wall, but also key molecular platforms for maintaining small vessel function, blood-brain barrier homeostasis, and cerebral perfusion stability.

Endothelial cells and blood-brain barrier-related pathways constitute another core layer of cSVD. Endothelial dysfunction can affect vascular permeability, vasomotor regulation, inflammatory cell migration, and the extent of tissue injury after ischemia. For example, studies related to the Foxf2-Tie2 axis suggest that abnormal endothelial regulation may aggravate ischemic injury, whereas reactivation of vascular stability-related signaling may alleviate part of the pathological process[24]. This line of evidence indicates that endothelial cells are not merely passive barrier structures that become damaged, but important nodes connecting genetic regulation, vascular stability, ischemic vulnerability, and therapeutic intervention.

Inflammatory and immune responses also run through the occurrence and progression of cSVD. Multiple peripheral and central molecular indicators have been reported to be associated with cSVD imaging burden or prognosis, including inflammatory proteins, immune-related molecules, and cerebrospinal fluid/plasma protein signals related to white matter hyperintensities, cerebral microbleeds, PVS, and MRI-defined cSVD phenotypes[25-27]. Together, these studies suggest that the molecular phenotype of cSVD may not be driven by a single inflammatory factor, but is instead jointly shaped by vascular injury, immune recognition, altered protein homeostasis, and neurovascular unit responses.

At the cellular level, both peripheral immune cells and central immune cells may participate in cSVD-related injury. Peripheral inflammatory indicators, such as the neutrophil-to-lymphocyte ratio, have been used to evaluate cSVD and stroke prognosis risk. Animal models and human studies also suggest that changes in microglial states, inflammatory monocyte migration, and adaptive immune responses may participate in neurovascular pathology under hypertensive, infectious, or post-ischemic white matter injury contexts[10, 28-31]. These lines of evidence indicate that the immune basis of cSVD needs to be considered in terms of peripheral immune status, blood-brain barrier permeability, and brain-resident glial responses, rather than being explained by a single cell type or a single inflammatory factor.

Metabolic and systemic risk factors further shape the molecular background of cSVD. Clinical metabolic indicators and regional brain metabolic readouts are both

associated with cSVD-related cognitive impairment, suggesting that hypertension, dyslipidemia, homocysteine levels, and regional neurometabolic alterations may jointly influence cognitive vulnerability[32]. In diabetes-related models, metabolic abnormalities can be accompanied by inflammatory activation and changes in programmed cell death pathways, thereby accelerating cerebral small vessel injury and brain structural degeneration[33]. In addition, Mendelian randomization studies suggest that body composition and cardiovascular metabolic factors may influence cSVD-related brain phenotypes such as WMH and lacunar stroke through different pathways[34]. Therefore, systemic metabolic status should be regarded as an important component of the molecular background of cSVD, rather than merely a clinical comorbidity.

Overall, the molecular biological basis of cSVD encompasses multiple layers, including hereditary vascular wall abnormalities, extracellular matrix and basement membrane imbalance, endothelial and blood-brain barrier dysfunction, inflammatory and immune activation, altered glial cell states, metabolic stress, and systemic vascular risk factors. These molecular clues provide an important foundation for understanding the heterogeneity of cSVD, but it remains difficult to establish a single, stable, and universally applicable molecular diagnostic gold standard. The core reason is that different patients vary in genetic background, vascular risk factors, affected brain regions, disease stage, and imaging phenotype. The same molecular signal may also have different meanings across different cell types, brain regions, and disease

stages. Therefore, future molecular research on cSVD needs to move from single candidate targets toward multiscale integrative frameworks, incorporating genetic evidence, vascular cell states, inflammatory and immune programs, regional brain metabolic changes, and imaging phenotypes to support more interpretable molecular subtyping and therapeutic target discovery.

2.4. Imaging basis of cSVD

Imaging is currently the core clinical tool for identifying and evaluating cSVD. Conventional MRI markers include white matter hyperintensities, recent small subcortical infarcts, lacunes, cerebral microbleeds, enlarged perivascular spaces, and brain atrophy. These imaging phenotypes are not only used to determine the presence and severity of cSVD, but are also closely associated with stroke recurrence, cognitive decline, gait disturbance, and long-term functional outcomes. However, conventional imaging markers largely reflect downstream tissue damage and cannot directly distinguish the underlying mechanisms across patients, such as vascular wall abnormalities, blood-brain barrier disruption, inflammatory responses, amyloid vasculopathy, or metabolic vascular injury. Therefore, recent imaging research has begun to move from simple lesion detection toward comprehensive assessment of blood-brain barrier function, vascular wall status, perfusion changes, cerebrospinal fluid dynamics, and brain network injury.

Blood-brain barrier injury is an important direction in cSVD imaging research. BBB-related MRI parameters can be used to assess vascular permeability, water exchange status, and early vascular functional abnormalities around white matter lesions. Related studies have shown that sporadic cSVD, CADASIL, and regions transitioning from normal-appearing white matter to white matter lesions may present different BBB permeability or water exchange patterns[35, 36]. These findings suggest that BBB imaging markers may help capture vascular functional abnormalities that cannot be fully explained by conventional MRI, and may provide auxiliary information for distinguishing different pathological mechanisms of cSVD.

In addition to BBB assessment, vascular wall features and upstream arterial morphology may also influence the imaging burden of cSVD. High-resolution vessel wall MRI and magnetic resonance angiography studies suggest that intracranial atherosclerosis, arterial dilation, vascular tortuosity, and hemodynamic status may be associated with white matter hyperintensities, lacunes, cerebral microbleeds, and overall cSVD burden[37, 38]. This indicates that cSVD is not completely restricted to the microvasculature itself, but may be jointly influenced by upstream arterial structure, vascular wall pathology, and the hemodynamic environment.

Imaging markers can also be combined with vascular risk factors and cognitive outcomes to reveal the clinical relevance of cSVD. Associations among central blood pressure, lacunar infarction, cerebral microbleeds, and white matter hyperintensities suggest that vascular risk exposure may affect brain structure and cognitive

performance through imaging-visible small vessel injury[39]. Meanwhile, combined MRI and cognitive scale analyses show that cSVD-related cognitive impairment is not limited to global cognitive decline, but may also involve specific cognitive domains such as executive function, visuospatial ability, naming, and abstract thinking [32]. Therefore, imaging not only reflects brain tissue injury, but also serves as an important intermediate phenotype linking vascular risk factors, regional brain damage, and cognitive decline.

Overall, imaging provides a stable and reproducible phenotypic basis for cSVD research, enabling researchers to quantify lesion burden, localize affected brain regions, evaluate vascular function, and track disease progression. However, imaging markers themselves remain insufficient to fully explain the molecular and cellular heterogeneity of cSVD. Similar white matter hyperintensity or lacunar burdens may arise from different vascular pathological processes, while different imaging manifestations may share common mechanisms such as endothelial dysfunction, blood-brain barrier disruption, vascular wall remodeling, or inflammatory activation. Therefore, the imaging basis of cSVD needs to be further integrated with molecular biology, single-cell analysis, genetics, clinical variables, and computational models, in order to move from imaging phenotypes toward mechanistic stratification and subtype discovery.

3. Multiscale research objects and tool systems derived from cSVD

3.1. Vascular scale: from large vessels to the microvascular unit

From the vascular-scale perspective, the research object of cSVD is not limited to a single type of small vessel, but involves a continuous vascular network extending from large vessels to the microvascular unit. The cerebral vascular system can be broadly divided into large arteries, pial arteries, penetrating arteries, small arteries, capillaries, small veins, veins, and venous sinuses. Among these, the deep microvascular networks, including penetrating arteries, small arteries, capillaries, and small veins, are most directly related to cSVD, because these vessels directly participate in the perfusion of the deep white matter, basal ganglia, thalamus, brainstem, and subcortical regions, and are structurally more vulnerable to chronic hypertension, hemodynamic stress, vascular wall degeneration, and inherited vascular abnormalities[19, 40].

In terms of cellular composition, cerebral small vessels are not merely tubular structures, but neurovascular units composed of multiple cell types and extracellular components. Endothelial cells form the luminal surface of vessels and maintain barrier function; pericytes are mainly distributed along capillaries and microvascular walls, participating in vascular stability, blood-brain barrier regulation, and local blood flow control; vascular smooth muscle cells are primarily located in small arteries and arterioles, where they regulate vascular tone and blood flow responses; astrocytic endfeet, basement membrane, neurons, oligodendrocytes, microglia, and

peripheral immune cells jointly participate in dynamic vascular-neural-immune interactions[41]. Therefore, vascular-scale research on cSVD is not essentially about studying “vascular narrowing” alone, but about understanding how different cell types and structural components within the neurovascular unit become jointly dysregulated.

The vascular network of the central nervous system has different functions across spatial levels. Microvessels within the brain parenchyma are mainly responsible for oxygen and nutrient delivery, metabolic waste clearance, local blood flow regulation, and neurovascular coupling; pial and cortical surface vessels participate in supplying the brain surface and cortical regions; dural vessels, dural venous sinuses, and meningeal lymphatic vessels constitute boundary-associated vascular and clearance systems of the central nervous system, participating in cerebrospinal fluid and interstitial fluid drainage, immune surveillance, and maintenance of central nervous system homeostasis[42-44]. It should be noted that the classical blood-brain barrier is mainly located in brain parenchymal microvessels and is maintained by endothelial tight junctions, basement membrane, pericytes, and astrocytic endfeet. In contrast, meningeal vessels and meningeal lymphatic vessels are better understood as components of the CNS boundary vascular-immune-clearance network rather than as classical BBB structures[45].

When cerebral small vessels undergo chronic pathological changes or acute rupture, their effects are not limited to the local vessels themselves, but further extend to surrounding brain tissue and neural networks. Genetic factors, aging, hypertension, metabolic abnormalities, and inflammatory states can chronically alter vascular wall structure, vascular tone, blood-brain barrier function, and local perfusion stability, thereby increasing the risk of white matter injury, lacunar infarction, cerebral microbleeds, and cognitive decline. Persistent or intermittent hypoperfusion may particularly affect the deep white matter and subcortical regions, because these regions are more sensitive to changes in small vessel perfusion and often lack sufficient compensatory blood supply[40].

Microvascular degeneration can also be understood as a dynamic process rather than a single injury event. Studies have shown that cerebral vessels may undergo sporadic occlusion and degeneration, involving a sequence of changes such as blood flow interruption, endothelial cell collapse, pericyte relocation or loss, and retraction of glial endfeet. Vascular degeneration can be linked to mitochondrial metabolic abnormalities and glutamate-related functional disturbances, ultimately affecting neuronal activity[46]. This process suggests that small vessel injury can gradually amplify from the microcirculatory level into white matter damage, local edema, neuronal dysfunction, and network disconnection.

Abnormal protein homeostasis in the vascular wall further illustrates the connection between the vascular scale and the molecular scale. For example, in hereditary small vessel diseases such as CADASIL and CARASIL, NOTCH3-related deposition and HTRA1 dysfunction can both affect cerebral vascular wall structure. Mutant extracellular protease HTRA1 may co-occur with NOTCH3-related deposition in the cerebral vascular system, suggesting that different hereditary forms of cSVD may share common mechanisms of vascular wall protein deposition and homeostatic disruption[21]. Therefore, studying cSVD from the vascular scale requires attention not only to vessel diameter, blood flow, and perfusion, but also to vascular wall cells, extracellular matrix, protein deposition, the blood-brain barrier, and the overall stability of the neurovascular unit.

Overall, the vascular scale provides the most fundamental spatial framework for understanding cSVD. The core lesions of cSVD occur in small vessels and microvascular units, but their effects can extend stepwise along the vascular tree, neurovascular unit, CNS boundary clearance systems, and brain regional networks. Therefore, it is insufficient to understand cSVD merely as small vessel stenosis or radiological white matter lesions. A more appropriate interpretation is that cSVD is a vascular-centered disease process that originates from the microvascular unit and progressively affects the structure and functional networks of brain tissue.

3.1.1. Molecular biological tools

When studying cSVD at the vascular scale, molecular biological tools are mainly used to analyze vascular wall cell states, blood-brain barrier integrity, inflammatory and immune responses, extracellular matrix remodeling, and molecular programs related to white matter injury. Traditional molecular biological methods include Western blot, qPCR, ELISA, immunofluorescence staining, immunohistochemistry, flow cytometry, basic animal behavioral testing, and histopathological assessment. These methods are suitable for different analytical levels: qPCR and transcriptomics can reflect changes in gene expression; Western blot, ELISA, and proteomics can detect protein expression and changes in secreted factors; immunofluorescence and immunohistochemistry can preserve spatial localization information; flow cytometry can be used to analyze the proportions and states of specific immune or vascular-related cell populations; and metabolomics can reflect the links among vascular injury, energy metabolism, and neuronal dysfunction[47, 48].

Animal models and genetic manipulation models remain important foundations for studying the molecular mechanisms of cSVD. Through hypertension models, diabetes models, chronic hypoperfusion models, ischemia models, and specific gene knockout or mutation models, researchers can observe vascular injury, white matter degeneration, inflammatory activation, and cognitive functional changes under controlled conditions. For example, after cerebral ischemia, IL-2R α knockout mice

show better performance in behavioral tests and exhibit greater white matter protection in diffusion tensor imaging, electrophysiology, and MBP assessment, suggesting that IL-2R α -related immune regulation may participate in post-ischemic white matter injury[10]. The advantage of such studies is that they can establish causal links from genetic or pathway alterations to tissue injury and behavioral phenotypes.

With the development of single-cell and omics technologies, molecular research on cSVD has begun to shift from single candidate targets toward the analysis of cell types and cellular states. scRNA-seq, snRNA-seq, spatial transcriptomics, proteomics, metabolomics, and multi-omics integration can identify disease-related vascular cell states, glial responses, immune cell infiltration, ligand-receptor communication, and region-specific molecular alterations at higher dimensionality. Compared with traditional bulk methods, single-cell and spatial technologies can distinguish the different contributions of endothelial cells, pericytes, vascular smooth muscle cells, astrocytes, microglia, oligodendrocytes, and peripheral immune cells in cSVD, making them more suitable for explaining cellular heterogeneity and brain-region vulnerability in cSVD.

However, in the cSVD field, the application of scRNA-seq and snRNA-seq remains far less extensive than in cancer, immune diseases, and neurodegenerative diseases. The main limitations include the difficulty of obtaining human cerebral small vessel

samples, the deep and scattered distribution of lesions, slow disease progression, marked inter-patient variability, temporal gaps between postmortem tissue and living pathology, and species differences between animal models and human sporadic cSVD. Therefore, current molecular research on cSVD still relies heavily on GWAS, colocalization analysis, Mendelian randomization, imaging genetics, blood or cerebrospinal fluid proteomics, metabolomics, and imaging-based risk stratification studies. Blood-based biomarker studies have good clinical accessibility and can be used for early detection and risk stratification, but their cellular origin, brain-region specificity, and correspondence with imaging phenotypes still need to be further clarified[27, 49-51].

In addition to detection tools, targeted delivery and labeling technologies also provide new possibilities for cerebral small vessel research. Because cSVD lesions are deeply distributed, have uncertain temporal windows, and show substantial individual variability, traditional tissue sampling and imaging detection make it difficult to capture small vessel cellular states in real time and with high precision. AAV capsid engineering offers a potential solution to this problem. Studies have shown that specific AAV capsids can efficiently transduce brain vascular pericytes and smooth muscle cells, thereby providing a tool basis for the localization, labeling, intervention, and functional study of cerebral small vessel-related cells[52]. In the future, such technologies may be combined with single-cell sequencing, spatial omics, in vivo

imaging, and gene regulation tools to track the dynamic changes of specific vascular cell populations during the occurrence and progression of cSVD.

Overall, molecular biological tools provide multilayer evidence for cSVD research, ranging from genes, proteins, metabolites, and cellular states to spatial localization. However, existing tools still cannot independently solve the core challenges of cSVD: heterogeneous lesion distribution, inconsistent disease stages, marked differences in patient background, incomplete correspondence between animal models and human disease, and the lack of direct mapping between imaging phenotypes and molecular mechanisms. Therefore, future molecular tool systems for cSVD need to move from single-point validation toward multiscale integration. Genetic evidence, blood and cerebrospinal fluid biomarkers, single-cell and spatial references, animal models, targeted delivery tools, and imaging phenotypes should be combined to more effectively support disease stratification, mechanistic interpretation, and therapeutic target discovery.

3.1.2. Imaging tools

Imaging tools are central means for linking vascular pathology, brain tissue injury, and clinical phenotypes in cSVD research. Conventional MRI can identify the major imaging markers of cSVD, including white matter hyperintensities, lacunes, recent

small subcortical infarcts, cerebral microbleeds, enlarged perivascular spaces, and brain atrophy. These markers provide the basis for disease identification, burden scoring, and longitudinal follow-up. However, as the research question shifts from “whether cSVD is present” to “which vascular mechanisms drive cSVD,” reliance on conventional MRI alone is no longer sufficient to resolve disease heterogeneity. Therefore, techniques such as arterial spin labeling, diffusion tensor imaging, functional MRI, intravoxel incoherent motion imaging, high-field MRI, 4D flow MRI, APT imaging, and retinal microvascular imaging are jointly expanding the research boundaries of cSVD[53].

From the perspective of perfusion and hemodynamics, arterial spin labeling can noninvasively assess cerebral blood flow, intravoxel incoherent motion imaging can reflect microcirculatory perfusion and water molecular diffusion components, and 4D flow MRI can quantitatively analyze blood flow velocity, flow volume, wall shear stress, pulsatility index, and resistance index in the intracranial arterial and venous systems. In patients with cSVD accompanied by cognitive impairment, one study assessed white matter hyperintensity burden using 2D T2-FLAIR sequences, measured hemodynamic parameters in the internal carotid artery, middle cerebral artery, basilar artery, transverse sinus, straight sinus, and superior sagittal sinus using 4D flow MRI, and further calculated cerebrospinal fluid hydrodynamics in the cerebral aqueduct using 2D phase-contrast MRI. Such approaches provide a tool basis

for noninvasively assessing the relationships among arterial supply, venous drainage, and cerebrospinal fluid circulation in cSVD[54].

From the perspective of microstructure and perivascular clearance, diffusion tensor imaging and its derived metrics can be used to evaluate white matter integrity, perivascular-space-related diffusion, and brain network injury. 7T MRI combined with the DTI-ALPS index and high-resolution susceptibility-weighted imaging can simultaneously assess diffusion along perivascular spaces, deep medullary vein visibility and continuity, white matter hyperintensities, and perivascular space burden. Related studies suggest that cerebral venous integrity may participate in maintaining perivascular or glymphatic clearance activity and may be linked to cSVD-related white matter injury and perivascular space alterations[55]. This indicates that cSVD imaging tools should not focus only on arterial-side lesions, but should also incorporate the venous system, perivascular spaces, and cerebrospinal fluid–interstitial fluid exchange processes.

Metabolism-related imaging provides another class of tools for understanding the relationship between cSVD and cognitive impairment. Amide proton transfer imaging can indirectly evaluate brain tissue metabolic status by reflecting mobile amide proton concentration and tissue pH-related changes. In studies of type 2 diabetes-related cSVD and cognitive impairment, researchers measured APT values in the hippocampus, temporal white matter, temporal gray matter, occipital white matter,

occipital gray matter, and cerebral infarct regions, and found that patients with type 2 diabetes and mild cognitive impairment showed reduced APT values in the left temporal white matter and right temporal gray matter, with these changes associated with poorer cognitive performance and more severe cSVD[56]. This suggests that APT imaging may provide a noninvasive indicator for capturing metabolic vascular injury and brain-region-specific cognitive vulnerability.

In addition to brain imaging, peripheral microvascular imaging can also serve as a complementary window for cSVD research. Retinal microvessels share certain similarities with cerebral small vessels in embryological origin, vascular structure, and microcirculatory regulation; therefore, retinal vascular geometric features may reflect systemic microvascular status. One study combined retinal microvascular features with MRI-defined cSVD markers, assessing white matter hyperintensity volume, lacunes, and composite extreme cSVD phenotypes, and found that abnormal retinal microvascular geometry was associated with higher MRI-defined cSVD burden and executive function decline[57]. Such approaches suggest that vascular research in cSVD can extend from intracranial imaging to accessible peripheral microvascular systems, providing additional information for early screening and risk stratification.

Overall, imaging tools provide a multidimensional, noninvasive, and longitudinally trackable research platform for cSVD. Conventional MRI defines lesion burden,

advanced MRI evaluates perfusion, microstructure, perivascular clearance, cerebrospinal fluid dynamics, and metabolic changes, while peripheral microvascular imaging provides complementary information on systemic microcirculatory status. However, imaging tools themselves still mainly capture structural and functional phenotypes, making it difficult for them to independently resolve the underlying cell types, molecular programs, and therapeutic targets. Therefore, imaging tools need to be integrated with genetics, blood and cerebrospinal fluid biomarkers, single-cell or spatial omics, and computational models to further advance cSVD from imaging burden assessment toward mechanistic stratification and subtype discovery.

3.2. Brain structural scale: from local lesions to brain network alterations

At the brain structural scale, the impact of cSVD is not limited to single visible lesions, but can gradually extend from local vascular injury to white matter integrity, gray matter structure, regional brain atrophy, and large-scale brain network connectivity. Traditional imaging markers, such as white matter hyperintensities, lacunes, cerebral microbleeds, and enlarged perivascular spaces, are often regarded as local or regional manifestations of cSVD-related injury. However, increasing evidence suggests that these lesions are not isolated events, but may reflect broader processes involving neurovascular unit imbalance, blood-brain barrier disruption, myelin loss, regional metabolic abnormalities, and network disconnection. Therefore,

understanding cSVD at the structural scale requires linking local lesions, tissue microstructure, and brain network alterations.

First, local vascular dysfunction can lead to region-specific blood-brain barrier disruption and brain tissue injury. In animal models of cSVD, studies using Bruker 11.7 T MRI T1 mapping to assess blood-brain barrier integrity have shown different degrees of BBB leakage across brain regions, with the most prominent T1 changes observed in the hippocampus. In spontaneously hypertensive rats, myelin basic protein expression is reduced, accompanied by hippocampal atrophy, reduced hippocampal volume, loss of myelin integrity, increased vascular permeability, and microvascular pathology[58]. These findings suggest that structural injury in cSVD shows clear regional differences, and that altered vascular permeability may play an important role in white matter damage and hippocampal vulnerability.

White matter injury is one of the core structural phenotypes of cSVD, but white matter hyperintensities do not fully represent its microscopic pathology. In patients with familial cSVD, studies combining X-ray scattering techniques and susceptibility-weighted imaging have assessed myelin and iron content in white matter hyperintensity regions, normal-appearing white matter, and key white matter tracts in CADASIL, and compared these measures with diffusion indicators such as mean diffusivity and free water. The results showed that abnormalities in CADASIL are not confined to white matter hyperintensity regions; signal changes suggesting

myelin loss can also be observed in normal-appearing white matter, indicating that cSVD-related white matter injury may precede or extend beyond lesions visible on conventional imaging[59]. In addition, associations have also been reported among physical activity, lipid status, and myelin integrity. Studies suggest that myelin loss is common in patients with cSVD, while physical activity and serum HDL levels are associated with higher in vivo myelin content, and HDL may modulate the protective effect of physical activity on myelin[60]. These findings suggest that white matter and myelin alterations are not only structural consequences of cSVD, but may also be modulated by systemic metabolic status and lifestyle factors.

Beyond white matter, gray matter structural abnormalities and brain atrophy are also important components of cSVD-related cognitive impairment. In the Hamburg City Health Study, multidomain neuropsychological testing and multimodal neuroimaging were used to integrate white matter hyperintensity burden, Fazekas score, perivascular space counts, and peak width of skeletonized mean diffusivity into a composite cSVD burden score. T1-weighted, FLAIR, and diffusion-weighted MRI were then used to assess the structural integrity of cortical and subcortical gray matter, including the hippocampal region. The results suggested that abnormalities in gray matter integrity may serve as an important link between small vessel disease and cognitive impairment[61]. Another study based on cortical DTI and quantitative MRI found increased cortical diffusivity and quantitative T values in patients with cSVD. Quantitative T values were positively associated with white matter hyperintensity

volume, while cortical diffusivity and T values were related to axonal injury in white matter tracts, further indicating that cSVD can affect both white matter and cortical structures[62].

Metabolic vascular injury can also manifest at the structural level as brain atrophy, ventricular enlargement, and cellular ultrastructural abnormalities. In a diabetes-related cSVD model, cranial MRI, HE staining, and transmission electron microscopy were used to compare wild-type C57BL/6 mice with leptin receptor-deficient db/db mice. The results showed that 16-week-old db/db mice developed temporal lobe atrophy and fourth ventricle enlargement, with capillary proliferation and cortical injury in the frontal cortex, as well as mitochondrial swelling, degeneration, and nuclear membrane rupture in hippocampal cells [33]. This evidence suggests that diabetes-related cSVD affects not only the vasculature itself, but may also promote cognitive decline through metabolic stress, mitochondrial injury, and cortico-hippocampal structural disruption.

At a higher level, cSVD can also disrupt large-scale brain network connectivity. Cognitive decline is not always directly explained by a single lesion, but may arise from network disconnection caused jointly by white matter injury, gray matter alterations, and vascular pathology. In delayed post-stroke cognitive decline, a prospective study showed that functional segregation formed during post-stroke compensation may be disrupted by underlying amyloid pathology or cSVD.

Disruption of modular organization, reduced system segregation, and abnormal between-network communication may represent important pathophysiological bases for delayed post-stroke cognitive decline, while white matter hyperintensities and subthreshold amyloid deposition may jointly contribute to these network alterations [63]. In asymptomatic patients with cSVD, resting-state fMRI and independent component analysis also showed that white matter lesion volume was associated with within-network and between-network connectivity across multiple large-scale functional networks, including the sensorimotor network, frontoparietal network, auditory network, default mode network, executive control network, salience network, and dorsal attention network. These connectivity alterations may further affect executive function and information processing speed[64].

Overall, the structural impact of cSVD forms a continuum from local lesions to whole-brain networks. Microvascular pathology and blood-brain barrier disruption may first cause regional white matter injury, myelin loss, and local brain-region vulnerability. Subsequently, reduced gray matter integrity, brain atrophy, metabolic cellular injury, and ventricular enlargement may further reflect the extension of disease into brain structural levels. Ultimately, white matter tract injury and disruption of cortico-subcortical circuits may lead to large-scale functional network reorganization, manifesting as cognitive, gait, and executive dysfunction. Therefore, cSVD should not be regarded merely as focal small vessel lesions on imaging, but

rather as a multiscale cerebrovascular injury process that spans local tissue, regional structures, and brain network levels.

3.2.1. Research tools

To understand how cSVD extends from local vascular lesions to white matter injury, gray matter atrophy, and brain network alterations, research tools need to cover multiple levels, including microscopic dynamics, tissue structure, perfusion status, and network function. Unlike approaches that simply describe imaging markers, structural-scale research tools place greater emphasis on tracking disease processes: how local vessels undergo occlusion, degeneration, or permeability changes; how brain tissue develops iron deposition, myelin loss, and hypoperfusion; and how these changes further affect large-scale brain network connectivity.

At the level of microscopic dynamics, in vivo two-photon microscopy provides an important tool for observing the cerebral vasculature and cellular responses around lesions. This technique can track central nervous system vascular structure, blood flow changes, dynamic responses after ischemic brain injury, and interactions between the vascular system and immune cells at lesion sites under living conditions[65]. Therefore, two-photon imaging is particularly suitable for studying early vascular responses, blood-brain barrier changes, immune cell recruitment, and dynamic

remodeling of the neurovascular unit, providing direct evidence that links microvascular pathology to tissue injury.

At the level of tissue structure and microenvironment, quantitative susceptibility mapping and related modeling methods can improve the detection of cSVD-related iron deposition and microstructural abnormalities. Studies based on MR paramagnetic imaging and Monte Carlo simulation have developed an imaging marker termed the expected iron coefficient (EIC). By constructing synthetic pathological models to simulate pathological iron deposition represented by impermeable magnetic spheres with different numbers and distributions, and by incorporating parameter-adjustable Monte Carlo diffusion simulations, this approach models sphere size, spatial distribution, and extracellular environment. In clinical datasets, researchers further combined quantitative susceptibility mapping, R2*, iron microenvironment coefficient, and EIC to evaluate iron-related changes in the presence of white matter hyperintensities, thereby improving sensitivity to subtle iron deposition changes in cSVD[66]. Such tools help reveal microenvironmental abnormalities that are difficult to capture using conventional MRI.

At the level of perfusion and longitudinal follow-up, arterial spin labeling MRI is an important noninvasive tool for assessing structural progression in cSVD. ASL-MRI can quantitatively measure cerebral blood flow without exogenous contrast agents, making it suitable for older adults, patients with chronic diseases, and cSVD cohorts

requiring repeated follow-up[67]. Because cSVD is often accompanied by chronic hypoperfusion, local blood flow dysregulation, and white matter injury progression, ASL can be used to evaluate whether perfusion insufficiency precedes structural damage and to track changes in cerebral blood flow after therapeutic or lifestyle interventions.

At the brain network level, functional connectivity analysis and dynamic network modeling can link local structural injury to cognitive functional changes. Traditional resting-state fMRI analyses mostly focus on average functional connectivity, whereas cSVD-related network injury may have time-varying characteristics. The alternating hidden Markov model (aHMM) initializes HMM covariance matrices using sliding-window functional connectivity estimates and reduces the number of parameters in each model estimation through an alternating optimization process. Studies based on simulated data and Human Connectome Project resting-state fMRI data have shown that aHMM is more robust than traditional sliding-window and HMM methods in dynamic functional connectivity estimation under variations in noise, parameter number, and sample size[68]. Such methods provide computational tools for analyzing functional network state transitions, disruption of system segregation, and abnormal between-network communication in cSVD.

Overall, structural-scale research tools for cSVD are moving from static lesion description toward dynamic, multilayered, and computational analysis. In vivo

two-photon microscopy can observe microvascular and immune cell dynamics; QSM/EIC and related methods can improve the detection of iron deposition and microenvironmental abnormalities. ASL-MRI can support noninvasive longitudinal assessment of perfusion status; and dynamic functional connectivity models can capture temporal changes at the brain network level. In the future, integrating these tools with white matter microstructural imaging, gray matter morphometric analysis, cognitive assessment, and molecular data will help explain more systematically how cSVD develops from local vascular pathology into whole-brain structural and functional network disorders.

3.3. Brain-region pathological scale: cSVD phenotypes differ across brain regions

At the brain-region pathological scale, one of the core features of cSVD is that injury is not uniformly distributed across the whole brain, but instead shows clear regional vulnerability. Regions such as the deep white matter, basal ganglia, thalamus, hippocampus, temporal lobe, cortico-subcortical circuits, and perivascular spaces may exhibit different imaging phenotypes and clinical consequences because of differences in blood supply, collateral circulation, blood-brain barrier status, metabolic demand, white matter fiber connectivity, and local cellular composition. Therefore, regionalized research on cSVD does not only ask where lesions are located, but also

why different brain regions show different structural, functional, and cognitive vulnerabilities to small vessel injury.

First, regional differences are closely related to vascular supply and cerebral blood flow regulation. Different cerebral perfusion territories have distinct blood flow regulation patterns and injury consequences. Studies based on transfer function analysis suggest that abnormalities in dynamic cerebral autoregulation in middle cerebral artery and posterior cerebral artery territories may correspond to different stroke risks and prognostic features[69]. Although such studies are not specifically focused on cSVD, their findings provide a hemodynamic basis for understanding regional perfusion vulnerability in cSVD.

Hereditary cSVD also suggests that different brain regions may share certain vascular functional abnormalities. In patients with HTRA1-related cSVD and CADASIL, MRI studies have shown whole-brain alterations in blood-brain barrier water exchange-related metrics, suggesting that small vessel diseases with different genetic backgrounds may converge at the level of BBB function, white matter injury, and vascular wall pathology[18]. This indicates that although hereditary cSVD is driven by different genetic abnormalities, its regional phenotypes may not be entirely independent, but may instead arise through shared pathways of vascular dysfunction.

As a core imaging marker of cSVD, white matter hyperintensities also have region-specific structural-functional effects. Structural decoupling analysis shows that

high WMH burden may be accompanied by abnormal structure-function coupling across multiple cortico-subcortical regions, including the frontal cortex, orbitofrontal regions, cingulate cortex, thalamus, and striatum, and may be associated with cognitive domains such as memory and processing speed. Related gene enrichment results further suggest that pathways involving neural function, ion transport, synaptic signaling, neuronal projection development, and cellular secretion may participate in this process[70]. This indicates that WMH burden is not merely a matter of white matter lesion volume, but may affect cognitive performance by disrupting structural-functional coordination in specific regions.

The temporal lobe, hippocampus, and thalamus are important vulnerable regions in cSVD-related cognitive impairment. Spatial correlation analyses combining the Allen Human Brain Atlas and structural MRI show that cSVD-related cognitive impairment can be accompanied by reduced gray matter volume in regions such as the temporal lobe, hippocampus, parahippocampal region, fusiform gyrus, and thalamus, and that these changes are associated with memory and executive function deficits[71]. This evidence further suggests that the cognitive consequences of cSVD are not determined solely by white matter injury; gray matter atrophy, regional transcriptomic background, and region-specific neural network vulnerability may also participate in disease progression.

The thalamus deserves particular attention in the regional pathology of cSVD, because it is both a deep small-vessel perfusion territory and an important relay node in cortico-subcortical circuits. High-field MRI and vascular imaging studies suggest that thalamic perfusion patterns, motor network functional connectivity, and gray matter density are associated with each other, and that this relationship may be modulated by overall cSVD burden[72, 73]. In other words, at low lesion burden, certain vascular supply patterns may still be associated with better motor network function; however, as cSVD burden increases, this local perfusion-network relationship may become weakened.

Further studies of thalamic subregions also support the view that the thalamus acts as a hub for cSVD-related cognitive injury. Joint analyses of high-resolution MRI, plasma biomarkers, and cognitive testing suggest that patients with cSVD may develop selective thalamic nucleus atrophy, which is associated with processing speed and attentional control. WMH burden in the thalamus and basal ganglia may contribute to this deep gray matter degeneration[74]. These findings indicate that the thalamus is not only an affected region in cSVD, but may also connect white matter lesions, deep gray matter degeneration, motor control, and cognitive decline.

Hemorrhagic small vessel injury can also show regional abnormalities in brain activity. Resting-state fMRI studies show that patients with intracerebral hemorrhage may exhibit alterations in local brain activity and dynamic functional metrics in

regions such as the thalamus, cerebellum, and cingulate cortex, and that some of these changes are related to attention and executive function[75]. Although intracerebral hemorrhage is not equivalent to all cSVD phenotypes, such studies suggest that small-vessel-related hemorrhagic injury may also affect cognitive function through abnormal activity in specific brain regions.

At the network level, cSVD can disrupt cortico-thalamic and cortico-subcortical pathways. Structural network studies show that patients with cSVD may exhibit reduced nodal efficiency and connectivity in cortico-thalamic pathways, accompanied by reduced global network efficiency and increased path length. Some basal ganglia-related nodal metrics are also associated with overall cognitive performance [76]. Therefore, regional cSVD injury does not remain confined to local brain regions, but may further affect whole-brain information integration by disrupting key network nodes and long-range connections.

Perivascular spaces and glymphatic dysfunction further reflect the regional specificity of cSVD. DTI-ALPS-related studies suggest that reduced glymphatic function and enlarged PVS are more prominent in regions such as the basal ganglia, thalamus, caudate nucleus, putamen, and hippocampus, and are associated with declines in global cognition, executive function, processing speed, and visuospatial function [77]. This indicates that PVS and glymphatic abnormalities do not occur uniformly

throughout the brain, but may form specific patterns of clearance impairment and cognitive vulnerability in basal ganglia-thalamus-hippocampus-related regions.

Overall, the brain-region pathological manifestations of cSVD show clear regional heterogeneity. Different brain regions display different sensitivities to small vessel injury because of differences in vascular supply, blood-brain barrier status, white matter connectivity, gray matter structure, metabolic demand, transcriptomic background, and perivascular clearance capacity. The deep white matter and basal ganglia are more likely to manifest WMH, lacunes, and PVS burden; the thalamus may serve as a hub linking perfusion, white matter lesions, motor control, and cognitive function; the hippocampus and temporal lobe are more directly involved in memory-related injury; and cortico-thalamic and basal ganglia-cortical circuits may amplify local vascular pathology into network-level cognitive and motor dysfunction. Therefore, future cSVD research should not only calculate whole-brain lesion burden, but should further develop regionalized, network-based, and molecular mapping frameworks to explain why cSVD phenotypes differ across brain regions and to provide a spatial basis for subsequent molecular subtype discovery.

4. Data-type-based analytical tools for cSVD

4.1. Imaging data analysis basis

Imaging data analysis is one of the most mature and clinically translatable analytical directions in cSVD research. Conventional MRI and advanced MRI techniques can provide multidimensional phenotypes, including white matter hyperintensities, lacunes, cerebral microbleeds, perivascular spaces, brain atrophy, perfusion abnormalities, white matter microstructural injury, and functional connectivity changes. As imaging data increase in scale and phenotypic dimensions expand, traditional manual rating methods are no longer sufficient for large-scale cohorts, longitudinal follow-up, and refined disease stratification. Therefore, cSVD imaging analysis is gradually moving from visual rating and single-index measurement toward automatic segmentation, quantitative feature extraction, machine learning prediction, region-aware modeling, and multimodal imaging integration.

Early lesion detection is an important application scenario for imaging data analysis. Fazekas grade 1 usually represents an early stage of white matter lesions, and automatic recognition at this stage may help shift cSVD from passive diagnosis toward early screening. Studies have applied 3D-CNN to automatic MRI recognition of mild cSVD and have shown relatively high classification performance in both two-dimensional and three-dimensional imaging inputs, suggesting that deep learning may serve as an auxiliary tool for early cSVD imaging screening [78]. Compared with manual rating, the value of such models lies not only in improving recognition efficiency, but also in reducing inter-rater variability and providing a standardized entry point for detecting mild lesions in large-scale imaging cohorts.

Automatic segmentation and quantitative analysis of white matter hyperintensities are core tasks in cSVD imaging analysis. WMH is both the most common imaging marker of cSVD and an important intermediate phenotype linking vascular injury, white matter degeneration, and cognitive decline. Quantitative analysis of WMH has expanded from total volume measurement to lesion distribution, regional burden, cortical proximity, and white matter fiber microstructural changes. For example, the U-fiber analysis toolbox can combine automatic WMH extraction with local white matter fiber feature analysis to evaluate the impact of juxtacortical white matter lesions on U-fiber microstructure, thereby providing more refined imaging phenotypes for understanding how WMH disrupts cortical connectivity[79]. Such tools suggest that cSVD imaging phenotypes should not be compressed only into total WMH volume, but should be further decomposed into features related to region, fiber tracts, and cortical proximity.

In heterogeneous patient populations, the robustness of automatic segmentation models is also critical. Convolutional neural network-based methods such as segcSVD have been used for WMH quantification and have shown tolerance to certain levels of MRI noise and artifacts[80]. This indicates that future cSVD imaging analysis should not only pursue high accuracy in a single test set, but also pay attention to generalizability across different scanners, magnetic field strengths, imaging protocols, and real-world image quality. For multicenter cohorts and

community screening, model stability and cross-device adaptability may have greater translational value than a single performance metric.

Beyond lesion segmentation, imaging data can also be used to construct region-specific predictive models. Deep transfer learning can use pretrained models to extract imaging features and improve model performance in cSVD cohorts with limited sample sizes. Studies have applied transfer learning to hippocampal-structure-related MRI analysis and achieved favorable predictive performance[81]. Such approaches indicate that cSVD imaging analysis does not need to be limited to whole-brain lesion burden; region-aware models can also be built around vulnerable regions such as the hippocampus, thalamus, basal ganglia, deep white matter, and cortico-subcortical circuits, thereby better explaining the relationships between different brain regions and cognition, gait, and executive function.

Imaging analysis in low-field and community settings also has important translational significance. Traditional cSVD imaging studies often rely on high-quality MRI data acquired in hospitals, whereas real-world screening requires low-cost, accessible, and interpretable analytical pathways. Studies have combined low-field mobile MRI, routine clinical variables, and machine learning models to identify cSVD risk in community populations, showing relatively high predictive performance[82]. The importance of this type of work lies not only in the model itself, but also in its

suggestion that cSVD imaging analysis can be extended from tertiary hospitals and research cohorts to community chronic disease management, primary screening, and early risk stratification.

Imaging analysis can also support high-throughput image processing in basic and translational research. In animal models and tissue section studies, machine learning-driven image pipelines can be used to quantify cerebrovascular morphology, oxidative-stress-related signals, and vascular aging features at scale. For example, studies on cerebrovascular aging and mitochondrial oxidative stress have developed high-throughput image analysis workflows to improve vascular image processing efficiency and quantitative stability[83]. Although such tools may not be directly applied to clinical MRI, they can connect molecular mechanisms, vascular morphology, and imaging phenotypes in basic research, thereby providing technical support for cross-scale validation.

At the brain network level, imaging data analysis further extends from static structural burden to dynamic functional connectivity. cSVD-related cognitive decline may be associated with large-scale network disconnection, reduced system segregation, and abnormal network state transitions. Compared with traditional static functional connectivity, dynamic functional connectivity models are more suitable for capturing time-varying features of brain network states. Alternating hidden Markov model-based methods improve dynamic functional connectivity estimation and show

greater robustness under variations in noise, parameter number, and sample size[68]. Such models provide a computational basis for analyzing the relationships among lesion burden, network state transitions, and cognitive fluctuation in cSVD.

Overall, imaging data analysis provides a key entry point for cSVD, from lesion detection to mechanistic inference. Automatic segmentation and deep learning can improve early recognition and WMH quantification; region-aware models can capture structural changes in vulnerable regions such as the hippocampus, thalamus, and deep white matter; low-field MRI combined with machine learning can expand screening scenarios; high-throughput image pipelines can connect animal models with basic mechanisms; and dynamic functional connectivity models can help explain how local lesions affect whole-brain networks. However, imaging data analysis remains primarily based on structural and functional phenotypes, making it difficult to independently determine the underlying cell types, molecular pathways, and therapeutic targets. Therefore, imaging analysis should be further integrated with clinical variables, genetic data, proteomics, metabolomics, single-cell references, and spatial information to advance cSVD from imaging burden assessment toward mechanistic interpretation and subtype discovery.

4.2. Clinical and demographic data analysis

Clinical and demographic data are among the most accessible analytical resources for cSVD risk assessment and early screening. Compared with MRI, DTI, or advanced vascular imaging, variables such as age, sex, blood pressure, diabetes status, blood lipids, renal function, uric acid, creatinine, lifestyle factors, and cognitive scales are easier to obtain in community, outpatient, and chronic disease management settings. Therefore, the value of clinical and demographic data analysis lies not in replacing imaging, but in providing a low-cost, scalable, and interpretable entry point for cSVD risk stratification, while helping identify individuals who may require further MRI or multimodal assessment.

Blood pressure-related indicators are among the core variables in clinical data analysis of cSVD. In a study on cSVD, cognitive impairment, and central systolic blood pressure, researchers combined applanation tonometry, MRI, and cognitive scoring to evaluate blood pressure status, imaging markers of cerebral small vessel disease, and cognitive performance, respectively. The results showed that central systolic blood pressure was associated with cSVD markers such as white matter hyperintensity volume and lobar and subcortical microbleeds, and that these vascular factors may further participate in the process of cognitive decline[39]. This finding suggests that clinical vascular risk indicators are not merely background variables, but may directly reflect small vessel injury burden and brain structural vulnerability.

Because MRI accessibility remains limited in community screening and early risk assessment, the role of non-imaging clinical variables in the initial screening of cSVD has received increasing attention. Based on data from 1,489 participants diagnosed with cSVD using 0.35 T mobile MRI in two community regions in China, one study used 19 interpretable clinical features, including age, uric acid, and creatinine, for predictor selection, and constructed multiple machine learning models, including logistic regression, support vector machine, random forest, extreme gradient boosting, and LightGBM. Ultimately, the best-performing LightGBM model showed strong predictive ability, suggesting that interpretable machine learning models based on routine clinical data can be used to identify individuals at high risk of cSVD[82].

The advantage of clinical and demographic data analysis lies in interpretability and translational convenience. Compared with complex imaging or omics features, blood pressure, age, renal function, metabolic indicators, and cognitive scales are easier for clinicians and patients to understand, and are more suitable for incorporation into primary screening, chronic disease follow-up, and stroke risk management systems. Especially in settings where MRI resources are limited or unsuitable for large-scale screening, models based on clinical variables can serve as a preliminary screening layer to determine whether subsequent imaging examination, cognitive assessment, or molecular biomarker testing is needed.

However, clinical and demographic data also have clear limitations. First, these variables are mostly indirect indicators of risk exposure or disease consequences, and are difficult to use for directly revealing the cell types, molecular pathways, and regional pathological mechanisms of cSVD. Second, the same clinical risk factors may correspond to different imaging phenotypes and pathological subtypes. For example, hypertension can be associated with white matter hyperintensities, lacunes, cerebral microbleeds, and cognitive impairment, but the underlying degrees of endothelial dysfunction, vascular wall remodeling, inflammatory activation, or blood-brain barrier disruption may differ. Third, clinical variables are easily influenced by age, comorbidities, medication use, lifestyle, and population differences; therefore, the generalizability of models across regions, ethnic groups, healthcare systems, and scanning conditions still needs to be validated

Therefore, clinical and demographic data are better suited as a foundational layer in cSVD stratification systems rather than as independent tools for mechanistic interpretation. A more ideal future analytical path is to use clinical variables to capture vascular risk exposure, disease stage, and cognitive trajectories, and then integrate them with imaging burden, genetic risk, proteomics, metabolomics, and single-cell reference information. Such an integrative framework can preserve the accessibility and interpretability of clinical data while compensating for their limited mechanistic resolution, thereby advancing cSVD from risk prediction toward more precise mechanistic subtyping and individualized intervention.

4.3. Genetic and molecular data analysis

Genetic and molecular data analysis provides a mechanistic entry point for cSVD research that differs from imaging and clinical variables. Imaging can define phenotypes such as white matter hyperintensities, lacunes, cerebral microbleeds, perivascular spaces, and brain atrophy, while clinical data can describe vascular risk exposure and cognitive outcomes. However, these types of information usually cannot directly explain the molecular pathways and cellular origins underlying the disease. In contrast, GWAS, Mendelian randomization, colocalization analysis, transcriptomics, proteomics, metabolomics, and single-cell reference mapping can link cSVD-related imaging phenotypes with genetic risk loci, tissue expression patterns, protein alterations, and cell-type-specific mechanisms.

Imaging genetics is an important direction in genetic data analysis of cSVD. Perivascular spaces are one of the core MRI markers of cSVD, but PVS in different brain regions may have different genetic bases and clinical significance. A 2023 study systematically described MRI-based strategies for assessing perivascular spaces and conducted GWAS in approximately 40,000 participants of European ancestry, separately analyzing the genetic associations of basal ganglia PVS and hippocampal PVS, and further identifying the relationship between PVS phenotypes and stroke risk[84]. This study suggests that even for the same imaging marker, different brain

regions may have distinct genetic backgrounds and disease relevance; therefore, genetic analysis of cSVD needs to consider both imaging phenotype and spatial localization.

Mendelian randomization and cross-tissue expression analysis further extend the ability to infer potential causal mechanisms from genetic associations. One study integrated GWAS data from six cSVD MRI markers and used Mendelian randomization to screen candidate genes associated with cSVD. It found that ALDH2 and KLHL24 were associated with cSVD in both blood and brain tissue, whereas ADRB1, BTN3A2, and EFEMP1 showed associations mainly in brain tissue[85]. This analytical strategy can not only help identify risk genes that may participate in cSVD, but also distinguish peripheral blood-detectable signals from brain-tissue-specific signals, thereby providing priorities for subsequent biomarker development and mechanistic validation.

However, genetic association itself is not equivalent to mechanistic explanation. GWAS usually localizes risk loci or statistically associated regions, rather than directly identifying causal cell types, target genes, or pathway directions. Many cSVD-related variants may be located in noncoding regions and require further interpretation through eQTL, pQTL, colocalization analysis, TWAS, chromatin accessibility data, and single-cell expression references[85]. Similarly, MR analysis can strengthen causal inference, but its results are still affected by instrument variable

selection, horizontal pleiotropy, tissue specificity, and differences in phenotype definition[86]. Therefore, genetic data need to be interpreted together with brain tissue expression, blood or cerebrospinal fluid proteins, metabolites, imaging phenotypes, and experimental models in order to move from risk loci toward verifiable molecular mechanisms.

Proteomics and metabolomics provide intermediate layers for molecular stratification of cSVD that are closer to disease phenotypes. Blood and cerebrospinal fluid proteins can be used to capture inflammation, vascular injury, extracellular matrix remodeling, immune activation, and neurodegenerative changes, while metabolomics can reflect energy metabolism, lipid metabolism, oxidative stress, and regional brain functional status[87, 88]. Compared with genomic data, proteins and metabolites are closer to disease activity states and are also more feasible as clinical testing indicators. However, they also face problems such as unclear cellular origin, limited regional localization, and incomplete consistency between peripheral signals and central pathology[27, 50, 51, 89]. Therefore, proteomic and metabolomic results need to be combined with imaging localization and cell-type references to determine whether a given molecular signal reflects vascular endothelial injury, pericyte abnormalities, glial responses, immune infiltration, or systemic metabolic alterations.

Single-cell and spatial reference data are becoming important bridges for interpreting molecular data in cSVD. Because human cSVD lesion tissues are difficult to obtain,

direct large-scale single-cell studies remain limited. Nevertheless, existing single-cell cerebrovascular atlases, disease model transcriptomes, and cross-species reference datasets can be used to map GWAS, proteomic, or transcriptomic signals onto specific cell types. For cSVD, this step is particularly important because endothelial cells, pericytes, vascular smooth muscle cells, astrocytes, microglia, oligodendrocytes, and peripheral immune cells may all participate in disease progression. Only by assigning molecular signals to specific cell types and brain-region structures can we explain why the same imaging phenotype may be driven by different vascular, inflammatory, metabolic, or glial cell programs.

Overall, genetic and molecular data analysis enables cSVD research to move from imaging description toward mechanistic interpretation. GWAS can identify risk loci associated with imaging phenotypes; MR and colocalization analysis can prioritize candidate genes and causal pathways; proteomics and metabolomics can provide molecular readouts of disease activity states; and single-cell and spatial references can help determine the cellular origins and regional context of these signals. However, no single data type can independently define molecular subtypes of cSVD. A more valuable future analytical framework should integrate genetic evidence, tissue expression, proteins and metabolites, cell-type-specific signals, brain-region localization, and imaging phenotypes into a shared space, thereby providing a foundation for mechanistic stratification and molecular subtype discovery in cSVD.

4.4. Multimodal integration and subtype discovery

Although imaging, clinical, genetic, and molecular analyses have each expanded our understanding of cSVD, each data type reflects only one layer of the disease process. Imaging data provide robust phenotypic markers, such as white matter hyperintensities, small subcortical infarcts, cerebral microbleeds, perivascular spaces, brain atrophy, perfusion abnormalities, cerebrospinal fluid pathway dysfunction, and network disturbances. However, these markers usually represent downstream consequences rather than mechanistic causes.

Clinical and demographic models can improve early risk prediction, especially at the community level, but their ability to reveal cellular or molecular mechanisms is limited. Genetic and molecular studies can identify risk loci, proteins, metabolites, inflammatory mediators, and vascular remodeling pathways, but often lack direct spatial correspondence with lesion distribution, brain-region vulnerability, and treatment response. Therefore, the current major challenge is no longer simply the lack of cSVD-related data, but the absence of an integrative framework capable of transforming heterogeneous data layers into biologically meaningful disease subtypes. A multimodal framework for cSVD subtype discovery should integrate at least four complementary dimensions.

First, imaging-derived phenotypes can define the anatomical and structural burden of disease, including lesion volume, lesion location, microstructural injury, vascular dysfunction, cerebrospinal fluid dynamics, and large-scale network disconnection. Second, clinical and demographic variables can provide information about vascular risk exposure, disease stage, cognitive trajectories, and prognosis. Third, genomic, proteomic, metabolomic, and transcriptomic data can identify disease-associated loci, proteins, metabolites, inflammatory pathways, extracellular matrix remodeling programs, and vascular molecular features. Fourth, single-cell, spatial, and cell-type-related analyses can assign molecular signals to specific components of the neurovascular unit, such as endothelial cells, pericytes, vascular smooth muscle cells, astrocytes, microglia, oligodendrocytes, and infiltrating immune cells. By integrating these dimensions, cSVD should not be viewed merely as a collection of radiological markers, but rather as a series of vascular-centered brain injury programs. In practice, subtype discovery can be achieved by transforming heterogeneous cSVD features into a shared feature space.

Imaging features can reflect lesion burden, regional vulnerability, and network disruption; clinical features may capture vascular risk and cognitive decline; genetic and molecular features can indicate pathway activation; and cell-type-specific markers can help reveal the cellular origins of disease-related programs. These features can then be analyzed through unsupervised clustering, matrix factorization, latent variable modeling, network-based integration, imaging-genetic association, or

atlas-based methods to identify recurrent disease patterns. Importantly, the resulting subtypes should not be defined solely by statistical separation. A useful cSVD subtype should have biological interpretability, regional consistency, clinical relevance, and therapeutic feasibility. In this sense, subtype discovery is not simply a classification task, but a translational process that connects phenotype, mechanism, and intervention.

From this perspective, vascular-centered subtype frameworks may provide a practical route for organizing cSVD heterogeneity. Rather than treating cSVD as a single diagnostic entity, such frameworks can encode disease states according to vascular cell involvement, regional vulnerability, molecular pathway activation, cell-cell communication, and therapeutic target feasibility. For example, a subtype-oriented framework may first identify vascular-centered molecular barcodes through genome-wide association analysis (GWAS) and cell-type information, and then connect these barcodes to downstream drug-mapping layers through cell-state scoring, communication indices, ligand-receptor relationships, and target prioritization. This logic provides a conceptual bridge from cSVD phenotype description to mechanism-driven stratification and precision intervention. Therefore, multimodal subtype discovery should become a central direction for future cSVD research, especially when combined with longitudinal imaging cohorts, blood or cerebrospinal fluid biomarkers, single-cell and spatial reference data, and experimentally validated therapeutic targets.

5. Future perspective: from cSVD classification to cSVD subtypes

5.1. From disease classification to subtype classification

Current cSVD classification still relies mainly on phenotypes and etiologies. In clinical and imaging practice, cSVD is usually described according to visible imaging markers, including white matter hyperintensities, lacunes, cerebral microbleeds, enlarged perivascular spaces, recent small subcortical infarcts, and brain atrophy. At the same time, pathological and genetic categories such as sporadic hypertensive arteriopathy, cerebral amyloid angiopathy, CADASIL, HTRA1-related cSVD, and COL4A1/2-related disease provide important etiological frameworks. These classifications have greatly improved disease recognition, risk assessment, and clinical communication. However, they primarily describe the causes, anatomical consequences, or imaging endpoints of disease, rather than the underlying molecular mechanisms that drive disease heterogeneity.

A core limitation of traditional cSVD classification is that similar imaging phenotypes may arise from different biological mechanisms, whereas different imaging manifestations may share common vascular or inflammatory pathways. For example, white matter hyperintensities may reflect chronic hypoperfusion, blood-brain barrier disruption, demyelination, gliosis, venous dysfunction, impaired perivascular clearance, or immune-mediated neurovascular injury. Similarly, lacunes, microbleeds,

and enlarged perivascular spaces may represent partially overlapping but mechanistically distinct processes involving endothelial dysfunction, pericyte injury, vascular smooth muscle cell degeneration, extracellular matrix remodeling, amyloid deposition, or inflammatory activation. Therefore, imaging burden alone cannot fully explain why patients with similar cSVD imaging severity may differ in cognitive trajectory, stroke risk, treatment response, or rate of progression.

This limitation suggests that future cSVD classification should move from disease-label-based classification toward mechanism-oriented subtype classification. Future research frameworks should not only ask whether a patient has cSVD, but should also examine which vascular-centered injury mechanism predominates in a given patient or disease model. Such programs may include endothelial barrier dysfunction, vascular wall cell degeneration, extracellular matrix remodeling, inflammatory activation, impaired cerebrospinal fluid drainage or perivascular clearance, metabolic vascular injury, amyloid-related vasculopathy, or region-specific neurovascular vulnerability. From this perspective, cSVD should be understood as a heterogeneous spectrum of vascular-centered brain injury states rather than as a single radiological entity.

Molecular subtype classification requires the integration of multilayered evidence. Imaging data can define lesion burden, lesion localization, microstructural injury, perfusion changes, and network-level effects. Clinical and demographic data reflect

vascular risk exposure, disease stage, cognitive decline, and prognosis. Genetic and molecular data reveal disease-associated loci, proteins, metabolites, inflammatory mediators, and pathway-level alterations. Single-cell and spatial information further assign these signals to specific components of the neurovascular unit, including endothelial cells, pericytes, vascular smooth muscle cells, astrocytes, oligodendrocytes, microglia, and peripheral immune cells. By integrating these layers of information, cSVD subtypes can be defined not only by the morphological features of lesions, but also by where they occur, which vascular or glial cell states are involved, which pathways are activated, and which therapeutic targets may be relevant.

5.2. Vascular-centered molecular barcode and subtype-aware intervention

A practical molecular subtype should meet several criteria. First, the subtype should have biological interpretability, meaning that it should correspond to identifiable mechanisms related to vascular, inflammatory, metabolic, or neurovascular unit processes. Second, it should have regional informational value, because cSVD does not affect all brain regions uniformly; the hippocampus, thalamus, basal ganglia, deep white matter, cortico-subcortical circuits, and perivascular spaces may exhibit different vulnerability patterns. Third, it should have clinical relevance, meaning that it can be associated with cognitive decline, stroke recurrence, gait disturbance,

imaging progression, or treatment response. Fourth, it should have therapeutic feasibility, allowing molecular features to be connected with targetable drugs, ligand-receptor interactions, pathway regulation, or intervention strategies. Without these criteria, subtype discovery may remain at the level of statistical classification, with limited translational value.

A proof-of-concept example of this transition is provided by the cSVI-subtype framework. The core idea of this framework is not simply to generate a longer list of candidate genes, but to organize human genetic evidence, vascular-related single-cell states, and cell-cell communication information into compact molecular subtype barcodes. Under this logic, GWAS-related signals can first be used to constrain the candidate molecular space, and then, with the aid of cross-species single-cell disease models and vascular-centered evidence layers, candidate molecules can be mapped onto vascular cell states carrying subtype information. In this way, cSVI-subtype compresses heterogeneous cSVD evidence into interpretable A1/N3KO-like and B1/BCAS-like molecular barcodes, demonstrating how traditional imaging and genetic phenotypes can be further transformed into subtype-level molecular definitions[90].

The downstream cSVI-DM framework further demonstrates how subtype evidence can be used for therapeutic hypothesis generation. Rather than using the final barcode directly as a single drug-screening marker, cSVI-DM preserves the different

biological sources of upstream subtype evidence and maps them onto layered intervention hypotheses. Evidence related to vascular cell states can indicate directions for state remodeling; evidence related to cell-cell communication can indicate directions for network or signaling rebalancing; and target-level evidence can be further connected to small-molecule candidates, pharmacological resources, and structural validation workflows. In this way, cSVI-DM advances subtype classification from “describing disease differences” to “generating layered intervention hypotheses,” and provides A1/NCL and B1/FLT1-KDR candidate patterns as preliminary examples[91].

Taken together, cSVI-subtype and cSVI-DM constitute a vascular-centered path from subtype discovery to subtype-guided intervention mapping. The former emphasizes how genetic, cellular-state, and cell-cell communication evidence can be integrated into interpretable molecular barcodes, whereas the latter emphasizes how the biological origins of subtype evidence can be preserved during drug mapping, rather than compressing all signals into a generic drug ranking. This two-step logic is particularly important for cSVD, because different disease layers may correspond to different intervention strategies: abnormalities in vascular cell states may require state remodeling, abnormal cell-cell communication may require network rebalancing, and terminal receptor- or protein-level signals may support target-level pharmacological testing. Although these two frameworks remain at the proof-of-concept stage and require further validation in larger population cohorts, longitudinal imaging datasets,

single-nucleus or spatial transcriptomic references, and experimental therapeutic models, they provide a concrete example of how cSVD research can move from radiological disease classification toward molecular subtype classification and precision intervention.

5.3. Validation roadmap and translational challenges

The transition from disease classification to subtype classification also changes how cSVD research should be validated. Traditional studies usually validate associations between risk factors and imaging burden, whereas subtype-oriented research should evaluate whether molecularly defined subtypes can predict disease progression, regional vulnerability, cognitive outcomes, or treatment response in independent cohorts. Therefore, longitudinal imaging cohorts, blood and cerebrospinal fluid biomarkers, single-cell and spatial transcriptomic reference data, animal models, and pharmacological intervention experiments are essential for assessing whether the proposed subtypes are stable, reproducible, and clinically useful.

Within this framework, cSVD classification is no longer limited to distinguishing hereditary from sporadic disease, or ischemic from hemorrhagic manifestations. Instead, the field can move toward a multilayered classification system that jointly considers clinical diagnosis, imaging burden, anatomical distribution, molecular

mechanisms, cellular origins, and therapeutic sensitivity. This shift may provide routes toward earlier diagnosis, more precise risk stratification, and mechanism-based intervention. More importantly, it may allow us to move beyond viewing cSVD as a final common radiological endpoint, and instead understand it as a series of molecularly distinguishable and therapeutically targetable cerebrovascular injury programs.

Conclusion

cSVD has traditionally been described through clinical manifestations, imaging markers, and etiological classification. This approach has achieved substantial progress in disease recognition and risk assessment, but remains insufficient to explain the biological heterogeneity of cSVD. Similar imaging phenotypes may arise from different mechanisms, whereas different imaging manifestations may share vascular, inflammatory, metabolic, or neurovascular-unit-related pathways. Therefore, cSVD should not be regarded merely as a collection of downstream imaging markers, but rather as a heterogeneous spectrum of vascular-centered brain injury states.

A multiscale perspective provides a more comprehensive approach to understanding this heterogeneity. At the vascular level, cSVD involves pathological alterations in endothelial cells, pericytes, vascular smooth muscle cells, the extracellular matrix, blood-brain barrier function, and perivascular clearance. At the structural and regional levels, cSVD affects white matter integrity, gray matter

structure, myelin content, brain atrophy, cerebrospinal fluid drainage, and large-scale functional networks in a region-specific manner. At the molecular and cellular levels, genetic risk, inflammatory activation, immune cell involvement, metabolic stress, glial responses, and cell-type-specific disease programs further contribute to differences in disease progression. These layers cannot be fully captured by any single type of imaging, clinical, genetic, or molecular data.

Therefore, the next stage of cSVD research should move from disease-label-based classification toward mechanism-oriented molecular subtype classification. A useful cSVD subtype should have biological interpretability, regional informational value, clinical relevance, and therapeutic feasibility. Multimodal integration provides the methodological basis for this transition by incorporating imaging phenotypes, clinical variables, genetic and molecular features, single-cell or spatial references, and therapeutic target information into a shared subtype-definition framework. In this context, vascular-centered molecular barcodes and subtype-aware intervention maps may help transform heterogeneous cSVD evidence into compact, testable, and translationally relevant disease programs.

The cSVI-subtype and cSVI-DM frameworks illustrate a proof-of-concept route through which this transition may be achieved. cSVI-subtype aims to compress evidence from human genetics, vascular single-cell states, and cell-cell communication into subtype-resolved molecular barcodes, whereas cSVI-DM extends subtype evidence into layer-specific therapeutic hypotheses through macro, balanced,

and micro intervention channels. Although these frameworks still require further validation in larger populations, including longitudinal imaging datasets, blood and cerebrospinal fluid biomarker studies, single-nucleus or spatial transcriptomic references, animal models, and experimental therapeutic systems, they indicate that cSVD classification can progressively evolve from radiological description toward molecular stratification and precision intervention.

Future cSVD research should prioritize reproducible subtype discovery, longitudinal validation, cross-cohort transferability, and experimental validation of subtype-specific mechanisms and therapeutic sensitivity. These efforts may support earlier diagnosis, more precise risk stratification, and mechanism-based intervention. Ultimately, understanding cSVD as a group of molecularly distinguishable cerebrovascular injury programs may provide a stronger foundation for biomarker development, therapeutic discovery, and precision cerebrovascular medicine.

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Declarations

Consent to participate

Not applicable.

Consent for publication

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Conflict of Interest

The author declares no competing interests.

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