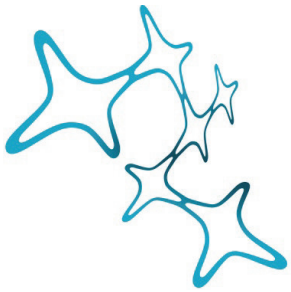
DIFFUSION IMAGING MARKERS OF CEREBRAL SMALL VESSEL DISEASE

– VALIDATION AND APPLICATION –



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SUMMARY

Diffusion magnetic resonance imaging (MRI) is widely used as a research tool to assess (subtle) alterations of the cerebral white matter. Measures derived from diffusion MRI appear to be valuable markers for cerebral small vessel disease (SVD). However, SVD is frequently co-occurring with Alzheimer's disease (AD), and disturbed white matter integrity and altered diffusion measures are considered key findings in both conditions. Yet, the contribution of SVD and AD to diffusion alterations is unclear, which hampers the interpretation of research studies in patients with mixed disease, e.g. memory clinic patients.

Study 1 of this thesis aimed to clarify the effect of SVD and AD on diffusion measures by including multiple (memory clinic) samples covering the entire spectrum of SVD, mixed disease, and AD. We calculated diffusion measures from diffusion tensor imaging (DTI) and free water imaging. Within each sample of the disease spectrum, we applied simple regression analyses and multivariable random forest analyses between AD biomarkers (amyloid-beta, tau), conventional MRI markers of SVD, and global diffusion measures. Furthermore, we investigated regional associations between tau on positron emission tomography (PET) and diffusion measures in voxel-wise analyses. Our main findings are that conventional MRI markers of SVD were strongly associated with diffusion measures and showed a higher contribution than AD biomarkers in multivariable analyses across all memory clinic samples. Regional analyses between tau PET and diffusion measures were not significant. We conclude that SVD rather than AD determines diffusion alterations in memory clinic patients. Our findings validate diffusion measures as markers for SVD.

Study 2 applied diffusion MRI markers to study gait impairment in SVD. Gait impairment is a commonly reported clinical deficit in SVD patients, but the underlying mechanisms are still debated. The proposed mechanisms include SVD-related white matter alterations resulting in impaired supraspinal locomotor control, cognitive deficits (e.g. planning and execution of movements), and factors independent of SVD, such as age-related instability (e.g. joint wear, sarcopenia) and comorbidities (e.g. neurodegenerative pathology). A reason for the lack of knowledge on gait impairment in SVD is that studies in elderly, sporadic SVD patients are typically confounded by effects of normal-aging and age-related comorbidities. Therefore, Study 2 of this thesis aimed to study the effect of pure SVD on gait performance in a relatively young sample of genetically defined SVD patients without age-related confounding. We performed comprehensive gait assessment using an electronic walkway to obtain multiple spatio-temporal gait parameters standardized based on data from healthy controls. Importantly,

we tested the association between diffusion MRI markers of SVD-related white matter alterations and gait performance, since (strategic) white matter alterations are discussed as a major cause of gait decline in the elderly. Furthermore, we assessed the relation between cognitive deficits and gait performance. Our main finding is that, despite severe white matter alterations in pure SVD patients, gait performance was relatively preserved. Cognitive deficits in our study participants were not related to gait impairment. Thus, our results query isolated white matter alterations, in the absence of comorbidities, as a main factor of gait impairment in SVD and suggest that their combination with age-related comorbidities and/or normal-aging may play a crucial role in gait decline.

In conclusion, diffusion measures are valid MRI markers of SVD-related white matter alterations. They have significant value both in future research on altered white matter and potentially also in the diagnostic work-up of memory clinic patients, to differentiate between vascular and neurodegenerative disease. Researchers may select target populations for clinical trials based on diffusion measures, e.g. to identify patients with a low SVD burden as targets for prevention and early intervention in SVD. Clinicians and researchers should always consider SVD as the origin of diffusion alterations in patients with mixed pathology. The field of application of diffusion measures is wide and may provide new insights into effects of subtle white matter alterations on clinical deficits, as shown in Study 2 on gait impairment in pure SVD. Future studies should investigate measures from advanced diffusion models and diffusion-based brain network analysis, to further elucidate the mechanisms of clinical deficits in SVD patients.

ABBREVIATIONS

AD	Alzheimer's disease
Aβ	amyloid-beta
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CMB	cerebral microbleeds
CSF	cerebrospinal fluid
DKI	diffusion kurtosis imaging
DTI	diffusion tensor imaging
FA	fractional anisotropy
FAt	tissue compartment of FA
FAu	uncorrected FA
FLAIR	fluid-attenuated inversion recovery
FW	free water compartment
MD	mean diffusivity
MDt	tissue compartment of MD
MDu	uncorrected MD
MRI	magnetic resonance imaging
NODDI	neurite orientation dispersion and density imaging
PET	positron emission tomography
P-tau	tau phosphorylated at threonine 181
PVS	enlarged perivascular spaces
STRIVE	Standards for Reporting Vascular Changes on Neuroimaging
SVD	cerebral small vessel disease
T-tau	total-tau
WMH	white matter hyperintensity

1. INTRODUCTION

Cerebral small vessel disease (SVD), a disorder of the cerebral microvessels, contributes to about 50% of all dementias, up to 25% of ischemic strokes, and most hemorrhagic strokes (Wardlaw *et al.*, 2019). SVD is present to some extend in more than 90% of individuals aged 60 or older (de Leeuw *et al.*, 2001). Beside cognitive decline, SVD is associated with various clinical deficits, such as gait impairment, depression, and urinary disturbances (Pantoni, 2010). Eventually, the disease leads to a complete loss of autonomy in daily living and thus is a challenge for our aging society and health-care systems. Comprehensive understanding of the disease mechanisms and a reliable diagnosis are inevitable to develop prevention and intervention strategies.

Functionally relevant *in vivo* disease markers foster the understanding of disease mechanisms and improve the diagnosis. Measures derived from diffusion magnetic resonance imaging (MRI) appear to be promising markers for SVD-related white matter damage: They capture subtle white matter alterations preceding and accompanying the occurrence of visible, conventional SVD markers on MRI (e.g. white matter hyperintensities, lacunes), and typically outperform conventional SVD markers in explaining clinical deficits and in detecting disease progression (Tuladhar *et al.*, 2015; Baykara *et al.*, 2016). However, SVD is frequently co-occurring with Alzheimer's disease (AD) in elderly populations (Kapasi *et al.*, 2017), and disturbed white matter integrity and altered diffusion measures are considered key findings in both conditions (Wardlaw *et al.*, 2013b; Nasrabady *et al.*, 2018). The contribution of SVD and AD to diffusion alterations is largely unknown, therefore, diffusion measures as markers for white matter alterations appear unspecific for SVD and AD. This research gap was addressed in Study 1 of this thesis. Study 2 applied diffusion MRI markers to investigate the role of SVD-related white matter alterations in gait impairment, a frequently observed clinical deficit in SVD patients.

In the following sections, SVD, its pathology, and conventional SVD markers on MRI are briefly introduced. AD as co-existing pathology is described and the challenge to disentangle the contribution of SVD and AD to white matter alterations and clinical deficits is pointed out. Diffusion measures calculated from two different diffusion MRI models are presented and their use in research on white alterations in SVD and AD are reviewed. The lack of knowledge on the origin of diffusion alterations is highlighted, which motivated Study 1 (validation of diffusion measures as SVD markers). Finally, gait impairment is explored, a common clinical deficit in SVD patients and cognitively impaired elderly individuals. Different methods to study

gait impairment and its possible mechanisms in SVD patients are summarized. The main causes of gait impairment in SVD patients and especially the role of white matter alterations are unclear, which motivated Study 2 (application of diffusion MRI markers).

1.1 Pathology and types of cerebral small vessel disease

The term ‘cerebral small vessel disease’ refers to clinical and imaging abnormalities caused by a disorder of the small vessels of the brain, i.e. penetrating arterioles, capillaries, and venules (Wardlaw *et al.*, 2019). The exact pathogenesis is still largely unclear, but endothelial dysfunction is a potential major initiating event of a pathological cascade. Vessel walls become damaged, thickened, and stiff resulting in reduced vasodilation, impaired cerebral blood flow (hypoperfusion), and compromised interstitial fluid drainage (fluid stagnation). Eventually, these vascular changes will lead to downstream tissue alterations, such as white matter lesions visible on conventional MRI, as described in section 1.2.1 (Wardlaw *et al.*, 2013a, 2019).

SVD is an umbrella term comprising several subtypes. The most common type is typically referred to as ‘sporadic SVD’ and mostly related to age and classic vascular risk factors, in particular hypertension (ter Telgte *et al.*, 2018). Study 1 includes memory clinic samples of sporadic SVD patients with concomitant AD pathology to study the effect of each of these conditions on diffusion measures.

Less common types are inherited, genetically defined forms of SVD, of which the most frequent one is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) caused by *NOTCH3* gene mutations. Lesions visible on conventional MRI, as described in section 1.2.1, and clinical characteristics in CADASIL mostly resemble those of sporadic SVD patients (Chabriat *et al.*, 2009). CADASIL is considered a model disease of pure SVD due to its early disease onset at the age of 35 to 50 years and the absence of age-related co-pathologies (Chabriat *et al.*, 2009). Study 1 and 2 include CADASIL patients to study the effect of pure vascular pathology on diffusion measures and gait performance.

Another type of SVD is cerebral amyloid angiopathy, characterized by progressive accumulation of amyloid-beta (Aβ) mostly in cortical and leptomeningeal vessels (Bourassa *et al.*, 2019) resulting in lobar, i.e. cortical and juxtacortical, macro- and microhemorrhages (Knudsen *et al.*, 2001). In contrast, a different lesion pattern is characteristic for sporadic SVD,

where mostly deep perforating vessels are affected, resulting in macro- and microhemorrhages in deep brain structures, i.e. basal ganglia, thalamus, brain stem, and cerebellum (Wardlaw *et al.*, 2013a). A typical imaging feature of cerebral amyloid angiopathy is cortical superficial siderosis, a distinct pattern of blood-breakdown product deposition in cortical areas, which is rarely found in the elderly population (< 2%) and absent in CADASIL (Linn *et al.*, 2010; Charidimou *et al.*, 2015; Wollenweber *et al.*, 2017). As a post-hoc analysis in Study 1, we considered the presence of cerebral amyloid angiopathy in a sample of genetically defined AD patients.

1.2 Conventional MRI markers of cerebral small vessel disease

Whereas alterations of the small vessels themselves cannot be detected *in vivo* in humans with current available imaging methods, their pathological consequences, i.e. brain parenchymal lesions, are easily recognized on images obtained by conventional MRI. The following sections summarize conventional MRI markers of SVD according to the Standards for Reporting Vascular Changes on Neuroimaging (STRIVE) criteria (Wardlaw *et al.*, 2013b). In the following sections, we point out the advantage of a summary SVD score of several conventional MRI markers compared to the use of individual markers, which was used in Study 1 to describe SVD burden.

1.2.1 Individual markers

White matter hyperintensities (WMHs) are hyperintense signal abnormalities of variable size on T2-weighted MRI sequences, e.g. on fluid-attenuated inversion recovery (FLAIR) images (**Fig. 1A**). They are predominantly located in the periventricular and deep white matter and mostly reflect demyelination, axonal loss, and gliosis (Wardlaw et al., 2019). WMHs may be caused by ischemia or a failure in the clearance of interstitial fluid from the white matter, which is associated with blood-brain barrier damage (Weller *et al.*, 2015; Wardlaw *et al.*, 2019). Histopathological studies indicate that parietal WMHs may also result from Wallerian degeneration due to cortical AD pathology (McAleese et al., 2017). Visual rating scales allow to quantify WMH burden, such as the Fazekas scale (Fazekas et al., 1987). WMH volumetry describes WMH burden on a continuous scale and is more sensitive for lesion progression than visual rating scales (Gouw et al., 2008).

Lacunes are fluid-filled round or ovoid subcortical cavities between 3 to about 15 mm in axial diameter (**Fig. 1B**). They appear similar to cerebrospinal fluid (CSF) on MRI and can be recognized on FLAIR images. Lacunes most likely result from acute small subcortical infarcts or hemorrhages, either symptomatic or silent (ter Telgte et al., 2018).

Cerebral microbleeds (CMBs) are small depositions of hemosiderin consistent with vascular leakage of blood cells into the brain tissue (**Fig. 1C**). They appear as round, hypointense lesions of 2 to 10 mm in diameter on T2*-weighted sequences or susceptibility-weighted imaging (ter Telgte et al., 2018). Strictly lobar microbleeds are a feature of cerebral amyloid angiopathy (Knudsen et al., 2001).

Enlarged perivascular spaces (PVS) are enlargements of the spaces around the penetrating vessel (**Fig. 1D**). They are typically located in the basal ganglia and the centrum semiovale and follow the course of the vessel with a linear or dot-like shape depending on the view, i.e. parallel or perpendicular to the vessel orientation. Usually, PVS measure less than 3 mm in diameter in perpendicular view, but can also be larger, especially in the infraputaminal region. PVS are visible on T1-weighted (hypointense) or T2-weighted (hyperintense) images and the differentiation from lacunes can be challenging (Wardlaw et al., 2013b).

Recent small subcortical infarcts are hyperintense lesions equal or smaller than 20 mm on diffusion MRI images. Subcortical microinfarcts of a size less than 5 mm are detectable only with high-resolution imaging, i.e. minimum of 3 Tesla (ter Telgte et al., 2018).

Cortical microinfarcts are ischemic lesions with a size of only a few millimeters and appear as hyperintense lesions on FLAIR and diffusion MRI images. They are best seen in the cortex on high-resolution imaging (ter Telgte et al., 2018).

Brain atrophy describes cortical or subcortical brain volume loss. This marker is not specific for SVD as it may occur in many other disorders or conditions, including AD and traumatic brain injury (Wardlaw et al., 2013b).

1.2.2 Summary score

Most studies rely only on single conventional MRI markers to capture SVD burden. However, a score summarizing individual, conventional SVD markers may provide a more comprehensive overall view of SVD burden than single markers (Staals *et al.*, 2014). SVD is a whole brain disease, as focal lesions occur in white and grey matter and can affect remote brain

structures and networks (Duering *et al.*, 2012; Duering *et al.*, 2015; ter Telgte *et al.*, 2018). Therefore, in Study 1, complementary to WMH volume (continuous scale), we used an established total SVD score (ordinal scale) to quantify SVD burden and to investigate the effect of SVD on diffusion measures (Staals *et al.*, 2014). The summary score ranges between 0 and 4 and captures the severity or presence of WMHs, lacunes, CMBs, and PVS (**Fig. 1**). These four markers show high intercorrelations and are associated with vascular risk factors and general cognitive ability (Staals *et al.*, 2014; Staals *et al.*, 2015).

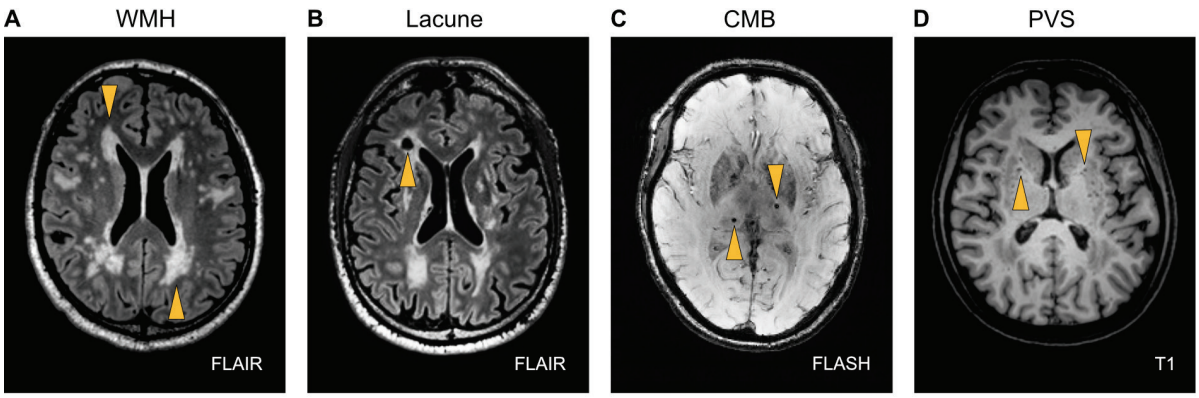

Figure 1. Total cerebral small vessel disease score. The total SVD score summarizes the severity or presence of the following conventional MRI markers of SVD on an ordinal scale from 0 to 4 (Staals *et al.*, 2014): (**A**) WMH burden on Fazekas scale 2-3 (1 point), (**B**) at least one lacune (1 point), (**C**) at least one CMB (1 point), and (**D**) more than 10 PVS in the basal ganglia on a single T1-weighted axial image slice with the highest number of enlarged perivascular spaces (1 point). Abbreviations: CMB = cerebral microbleed; FLASH = fast low angle shot; FLAIR = fluid-attenuated inversion recovery; PVS = enlarged perivascular spaces; WMH = white matter hyperintensity.

1.3 Alzheimer's disease as co-existing pathology with cerebral small vessel disease

AD is a progressive neurodegenerative disorder characterized by extracellular Aβ plaques and intracellular tau neurofibrillary tangles (Zetterberg and Mattsson, 2014). Aβ and tau accumulate in grey matter and are related to synaptic dysfunction and neuronal loss, which typically becomes apparent on conventional MRI as regional and global atrophy including enlarged ventricles and decreased hippocampal volume (Selkoe and Hardy, 2016).

Longitudinal cohort studies increasingly recognized the co-occurrence of SVD and AD (mixed disease) in patients with dementia. Data from the Religious Orders Study (Bennett *et al.*, 2013) and the Memory and Aging Project (Bennett *et al.*, 2012) indicate that almost 75% of subjects (N = 447) with a neuropathologic (*ex vivo*) diagnosis of AD show concomitant vascular

pathology such as macro- or microinfarcts, atherosclerosis, arteriolosclerosis, or cerebral amyloid angiopathy (Kapasi *et al.*, 2017).

Ex vivo neuropathologic examination is the gold standard for an AD diagnosis (DeTure and Dickson, 2019). However, various valid *in vivo* AD biomarkers are available including CSF and PET based assessments of Aβ and pathologic tau: Low concentrations of $A\beta_{1-42}$ (Aβ 42) in CSF indicate cerebral Aβ depositions in the brain tissue (Blennow *et al.*, 2015) and high concentrations of tau phosphorylated at threonine 181 (p-tau) in CSF reflect aggregated tau, i.e. neurofibrillary tangles (Blennow *et al.*, 2015). CSF total tau (t-tau) is not specific for AD but rather a general indicator of neurodegeneration (Wirth *et al.*, 2013). Studies comparing neuropathologic examinations and PET imaging validated cortical amyloid PET ligand binding as surrogate marker for Aβ deposits in the brain parenchyma and vessel walls (Ikonomovic *et al.*, 2008; Clark *et al.*, 2012; Murray *et al.*, 2015) and tau PET ligand binding for pathologic tau (Villemagne *et al.*, 2015), although some off-target binding is possible. In Study 2, we used CSF and PET based assessments of Aβ and pathologic tau as biomarkers of AD in order to investigate the association between AD and diffusion measures.

1.4 Diffusion MRI to assess subtle cerebral white matter alterations

Diffusion MRI is a technique to study white matter fiber organization *in vivo*. It captures subtle white matter alterations, such as microstructural damage invisible on conventional MRI. In the following sections, different measures based on diffusion MRI and their use in research on SVD and AD are described.

1.4.1 Measures from diffusion tensor imaging and free water imaging

Diffusion MRI characterizes the movement of water molecules in brain tissue. Water diffusion in the intracellular space is more restricted than in the extracellular space, because intracellular space contains more natural barriers, such as cell membranes and organelles. Water molecules within white matter fibers, preferentially diffuse along the fiber direction, i.e. anisotropic diffusion, resulting in a higher diffusion coefficient along the fiber compared to diffusion in perpendicular directions (Price *et al.*, 2011).

The most common and most straightforward model for quantifying diffusion and inferring tissue architecture from water movement is diffusion tensor imaging (DTI). The three-

dimensional diffusion in a particular volume element (voxel) can be described by a so-called 'tensor'. Frequently used voxel-averaged measures calculated from the tensor are fractional anisotropy (FA), reflecting the directionality of water diffusion, and mean diffusivity (MD), reflecting the amount of water diffusion. The typical finding in studies on brain pathologies affecting the white matter, including SVD and AD, is a decrease in FA and an increase in MD. These diffusion alterations were thought to result from microstructural tissue damage, such as axonal degeneration (Gouw *et al.*, 2011). However, more recently it has been suggested that they may also stem from alterations in extracellular free water content, e.g. through edema caused by blood-brain barrier damage (Cognat *et al.*, 2014; Duering *et al.*, 2018). In contrast to the simple DTI model, free water imaging, a more complex diffusion model, enables the differentiation between free water-related alterations in the brain parenchyma and microstructural tissue alterations.

Free water imaging decomposes the diffusion signal into two compartments, a free water compartment (FW), i.e. unrestricted extracellular water diffusion, and a free water corrected tissue compartment (**Fig. 2**) (Pasternak *et al.*, 2009). FW and tissue FA and MD (FAt, MDt) are voxel-averaged measures frequently derived from free water imaging. Several studies on different brain pathologies, including SVD and AD, indicate that free water imaging sensitively captures clinically relevant (subtle) white matter alterations (Pasternak *et al.*, 2009; Pasternak *et al.*, 2012; Maier-Hein *et al.*, 2015; Planetta *et al.*, 2015; Duering *et al.*, 2018). Measures from DTI and free water imaging were used in Study 1 to investigate their association with SVD and AD.

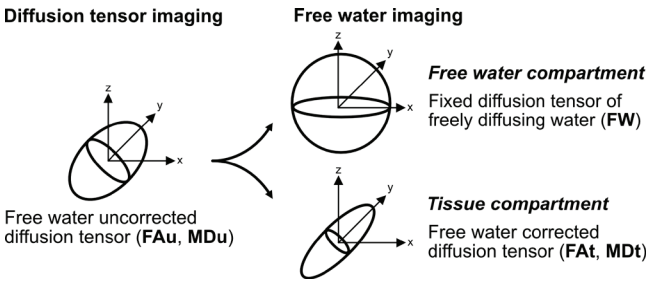

Figure 2. Free water imaging principle. The free water compartment represents water molecules that are not restricted or directed. It is modelled by an isotropic tensor with a fixed diffusion coefficient of freely diffusing water at 37°C body temperature. The tissue compartment represents all remaining water molecules within or in close proximity to cellular structures and is modelled by a unconstrained tensor fit (adapted from Duering *et al.* (2018)). Abbreviations: FAt = tissue compartment of fractional anisotropy; FAu = uncorrected fractional anisotropy; FW = free water compartment; MDt = tissue compartment mean diffusivity; MDu = uncorrected mean diffusivity.

1.4.2 Diffusion alterations in cerebral small vessel disease and Alzheimer's disease

In SVD patients, DTI measures outperform conventional MRI markers in explaining cognitive deficits and in detecting disease progression (van Norden *et al.*, 2012; Baykara *et al.*, 2016; Konieczny *et al.*, 2020). DTI can reveal white matter alterations in SVD, i.e. a decrease in uncorrected FA (FAu) and an increase in uncorrected MD (MDu), not only in visibly lesioned areas but also in areas with unaltered signal on conventional MRI, i.e. in normal appearing white matter. In fact, conventional SVD markers are now known to be only the "tip of the iceberg" of the total SVD-related brain damage (ter Telgte *et al.*, 2018). Consequently, diffusion measures appear to be highly sensitive markers for SVD burden. However, SVD and AD are often concomitant (as described in section 1.3) and subtle white matter alterations have also been described in AD. Several studies in sporadic AD patients show that DTI measures are associated with AD biomarkers of CSF and PET (Racine *et al.*, 2014; Melah *et al.*, 2016; Hoy *et al.*, 2017; Jacobs *et al.*, 2018; Strain *et al.*, 2018; Racine *et al.*, 2019; Vipin *et al.*, 2019; Araque Caballero *et al.*, 2020). To date, the contribution of SVD and AD to diffusion alterations is unclear, which was addressed in Study 1 of this thesis.

As mentioned above, the simple DTI model appears unspecific for SVD and AD. Recent studies, including one from our group, indicate that free water imaging might be able to disentangle SVD- and AD-related subtle white matter alterations (Maier-Hein *et al.*, 2015; Duering *et al.*, 2018). We previously showed that diffusion alterations in SVD are predominantly driven by an increase in FW, possibly caused by vasogenic edema or vacuolization within myelin sheaths, and less by altered fiber geometry, i.e. microstructural damage (Duering *et al.*, 2018). Conversely, a study in AD patients suggests that AD pathology is mainly represented in free water corrected tissue measures indicating microstructural damage. Alterations in the tissue measures were detected in early stages of AD and predicted the conversion from mild cognitive impairment to AD dementia (Maier-Hein *et al.*, 2015). Of note, free water uncorrected measures, i.e. simple DTI measures, revealed no difference between converters from mild cognitive impairment to AD dementia and non-converters. Taken together, SVD and AD might have distinct signatures when analyzed with free water imaging. We therefore studied associations between SVD, AD, and diffusion measures, not only from DTI but also from free water imaging.

1.5 Aim Study 1: Validation of diffusion MRI markers

SVD and AD together cause the majority of dementia cases and are often co-occurring. Both diseases are thought to affect the brain's microstructural white matter integrity. The contribution of each of these diseases to subtle white matter alterations is unknown and hampers an accurate diagnosis and disease management. Therefore, Study 1 aimed to clarify the pathologic contribution of SVD and AD to subtle white matter alterations as assessed by DTI and free water imaging, and thereby validate diffusion measures as *in vivo* markers for SVD and/or AD. Additionally, we tested whether SVD and AD have differential effects on measures from free water imaging (Maier-Hein *et al.*, 2015; Duering *et al.*, 2018).

1.6 Application of diffusion MRI markers in research on gait impairment in cerebral small vessel disease

White matter alterations in SVD are associated with a variety of clinical deficits including gait (walking) impairment. Diffusion measures as sensitive markers for subtle white matter alterations are valuable tools to study the effect of SVD burden on gait performance.

Gait impairment is highly prevalent in the elderly affecting around 35% of individuals aged 70 or older and more than 46% aged 85 or older (Verghese *et al.*, 2006). These individuals are at high risk for falls and fractures, which increase institutionalization and mortality (Bridenbaugh and Kressig, 2015; van der Holst *et al.*, 2016). SVD in the elderly appears to play an important role in the development of gait impairment. After cognitive disturbances, gait impairment is the second most common clinical deficit in SVD (Okroglic *et al.*, 2013). Yet, relative to the number of studies on cognitive disturbances, studies on gait impairment in SVD are scarce and the etiology of gait impairment is still unclear. Therefore, there is an urgent need to identify the underlying mechanisms of gait impairment in SVD in order to develop prevention, intervention, and rehabilitation strategies.

In the following sections, methods of gait assessment used in Study 2 are introduced and possible contributing factors of gait impairment in SVD are discussed.

1.6.1 Gait assessment

The assessment of gait and balance can be useful in the diagnostic work-up, because specific features of gait are characteristic for different diseases. For instance, gait characteristics of SVD

patients with diffuse white matter alterations are small steps, wide-based gait, and variable timing and amplitude of steps, whereas Parkinson's disease patients with affected substantia nigra often show narrow-based gait and freezing of gait (Snijders *et al.*, 2007). Clinical assessment most commonly includes visual observation during ordinary gait, typically while walking in the corridor. Standard rating scales allow to score gait and balance, e.g. the Tinetti mobility index (Tinetti, 1986). However, observational gait assessment requires training and clinical experience. While quantitative screening test, e.g. the Timed-Up-and-Go (Podsiadlo and Richardson, 1991) and the Short Physical Performance Battery (Guralnik *et al.*, 1994), capture gait velocity and balance with high reliability, these tests do not allow to evaluate gait quality. In contrast, advanced tools, such as pressure-sensitive insoles or pressure-sensitive carpets record multiple spatio-temporal gait parameters and automatically evaluate gait performance, independent of a time-consuming subjective rating. Despite being cost-intensive, these advanced tools enable comprehensive gait analysis with high concurrent validity and test-retest reliability and foster standardized methodology of research on gait impairment (Bilney *et al.*, 2003; Menz *et al.*, 2004). Therefore, in Study 2, gait parameters were assessed using a computerized walkway (GAITRite, MAP/CIR Inc. Havertown, PA, USA).

1.6.2 Factors of gait impairment in cerebral small vessel disease

Various factors of gait impairment in SVD patients have been described in previous research (**Fig. 3**). First, altered white matter is considered an important contributor to gait decline in SVD as indicated by associations between conventional MRI markers of SVD and gait performance (de Laat *et al.*, 2010b; de Laat *et al.*, 2011; Smith *et al.*, 2015; Pinter *et al.*, 2017). Compared to conventional MRI markers, stronger associations can be found with diffusion measures as markers of subtle white matter alterations in SVD (Van Der Holst *et al.*, 2018). Some studies described strategic white matter regions to be associated with gait impairment, such as the internal capsule, bilateral frontal periventricular white matter, and the corpus callosum, especially the genu (de Laat *et al.*, 2010a; Srikanth *et al.*, 2010; Van Der Holst *et al.*, 2018).

Second, more recently, cognitive deficits have been related to gait impairment. Normal walking relies on the interaction of various cognitive functions, including executive control, i.e. planning and execution of movements, and postural control (Snijders *et al.*, 2007). Studies using the so-called 'dual task paradigm' indicate that the ability to maintain normal walking deteriorates while performing a secondary cognitive task. The demands of walking and the secondary task are thought to exceed limited cognitive resources resulting in gait decline,

specifically in cognitively impaired individuals, who are not able to cognitively compensate gait difficulties (Muir *et al.*, 2012). In Study 2, we implemented two different cognitively challenging dual task conditions, to study the effect of cognition on gait.

Third, age-related instability, such as degenerative, musculoskeletal disorders or comorbidities such as neurodegenerative disease or vestibular dysfunction, independent of SVD, may directly affect gait (Aboutorabi *et al.*, 2016). Other consequences of normal-aging, such as visual or oculomotor changes, are considered as indirect causes of gait decline, when forcing individuals to walk more cautiously, e.g. by reducing stride length and velocity (Bridenbaugh and Kressig, 2015).

A reason for the lack of knowledge on mechanisms of gait impairment in SVD is that gait studies typically include elderly SVD patients (aged 60 years or older). Results may therefore be confounded by effects of normal-aging and age-related comorbidities. In Study 2, to shed more light onto pure SVD-related effects on gait performance, we studied CADASIL patients with genetically defined SVD, with a high SVD burden already at young age (for details on CADASIL see section 1.1).

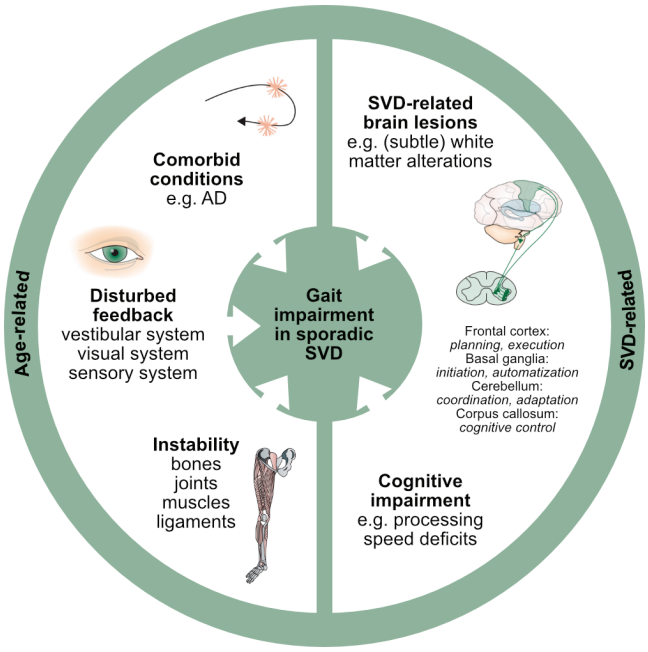

Figure 3. Factors influencing gait impairment in sporadic cerebral small vessel disease. SVD- and age-related factors may contribute to gait impairment in SVD patients (adapted from Snijders *et al.* (2007)). Abbreviations: AD = Alzheimer's disease; SVD = cerebral small vessel disease.

1.7 Aim Study 2: Studying the effect of pure cerebral small vessel disease on gait

The etiology of gait impairment in SVD is largely unclear. SVD-related white matter alterations and cognitive deficits, as well as age-related instabilities and comorbidities might foster gait impairment in SVD (**Fig. 3**). Study 2 aimed to examine the effect of pure SVD without age-related confounding on gait performance by studying CADASIL patients (for details on CADASIL see section 1.1). These patients have a high SVD burden already at young age. Importantly, we applied diffusion measures, which have been validated in Study 1 as markers for subtle SVD-related white matter alterations, to investigate the association between disturbed white matter and gait. Also, we aimed to study the association between cognitive deficits, i.e. processing speed as the main cognitive deficit in SVD, and gait.

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2. STUDIES

This chapter comprises two research articles on the validation (Study 1) and application (Study 2) of diffusion MRI markers. The original numbering of tables, figures, and supplementary material within each article has been retained.

2.1 Study 1: Validation of diffusion MRI markers

The following section includes the research article entitled "Small vessel disease rather than Alzheimer's disease determines diffusion MRI alterations in memory clinic patients". The manuscript has been submitted to a journal and is currently under review.

Small vessel disease rather than Alzheimer's disease determines diffusion MRI alterations in memory clinic patients

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[**] Data used in preparation of this article were obtained from the Dominantly Inherited Alzheimer Network (DIAN) database, the DZNE-Longitudinal Cognitive Impairment and Dementia Study (DELCODE) database, and the

Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within DIAN, DELCODE, and ADNI contributed to the design and implementation of the respective studies and/or provided data but did not participate in analysis or writing of this report. A complete listing of the DIAN consortium, the DELCODE study group, and ADNI investigators can be found in the Supplement (DIAN and DELCODE) and at http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement _List.pdf (ADNI).

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2.1.1 Abstract

Introduction: Microstructural alterations as assessed by diffusion tensor imaging (DTI) are key findings in both Alzheimer's disease (AD) and small vessel disease (SVD). We determined the contribution of each of these conditions to diffusion alterations.

Methods: We studied six samples (N = 365 participants) covering the spectrum of AD and SVD, including genetically defined samples. We calculated diffusion measures from DTI and free water imaging. Simple linear, multivariable random forest, and voxel-based regressions were used to evaluate associations between AD biomarkers (amyloid-beta, tau), SVD imaging markers, and diffusion measures.

Results: SVD markers were strongly associated with diffusion measures and showed a higher contribution than AD biomarkers in multivariable analysis across all memory clinic samples. Voxel-wise analyses between tau and diffusion measures were not significant.

Discussion: In memory clinic patients, the effect of SVD on diffusion alterations largely exceeds the effect of AD, supporting the value of diffusion measures as markers of SVD.

2.1.2 Introduction

Alzheimer's disease (AD) and cerebral small vessel disease (SVD) are the two leading causes of cognitive decline and dementia (O'Brien and Thomas, 2015). Altered white matter microstructure is considered a key finding in both conditions (Wardlaw *et al.*, 2013; Nasrabady *et al.*, 2018) and has consistently been associated with cognitive deficits (Baykara *et al.*, 2016; Araque Caballero *et al.*, 2018; Mito *et al.*, 2018). The most commonly used method to study white matter microstructure *in vivo* is diffusion tensor imaging (DTI), which quantifies diffusion properties of water molecules in brain tissue (Amlien and Fjell, 2014; Pasi *et al.*, 2016). The typical finding described in both AD and SVD is an increase in the extent of water diffusion (mean diffusivity) and a decrease in diffusion directionality (fractional anisotropy). Despite the wide use of diffusion alterations as efficient disease markers and their strong associations with clinical deficits, little is known about their underlying pathology (Tuladhar *et al.*, 2015; Baykara *et al.*, 2016; Araque Caballero *et al.*, 2018).

In memory clinic patients, AD and SVD often co-exist (Kapasi *et al.*, 2017). The extent to which each of these conditions contribute to diffusion alterations is largely unknown and has so far not been examined in a systematic study covering the entire spectrum of AD, mixed disease, and SVD. Free water imaging, an advanced diffusion model, improves the specificity of the DTI model and could therefore provide additional insight into the origin of diffusion magnetic resonance imaging (MRI) alterations (Pasternak *et al.*, 2009). Recent studies suggest that by modelling two distinct diffusion compartments, free water imaging might be suited to disentangle the effects of AD and SVD (Maier-Hein *et al.*, 2015; Hoy *et al.*, 2017; Duering *et al.*, 2018; Vipin *et al.*, 2019).

The aim of this study was to determine the contribution of AD and SVD to microstructural alterations as assessed by diffusion MRI, using conventional DTI and free water imaging. We examined associations between biomarkers of AD, MRI markers of SVD, and diffusion measures. Six study samples (N = 365 participants) were included to cover the entire spectrum of AD, mixed disease, and SVD, and to account for both cerebrospinal fluid (CSF) and positron emission tomography (PET) markers. Analyses were performed separately within each sample in order to validate results and address generalizability using the independently recruited samples. Our analysis also included patient samples with pure, genetically defined AD or SVD, which enabled us to examine effects of both diseases on diffusion measures without confounding pathology.

2.1.3 Methods

Participants

We studied six independent samples (N = 365 participants) covering the spectrum of AD, mixed disease, and SVD: four memory clinic samples with mixed disease with a recruitment focus on either AD or SVD, one sample each of genetically defined AD and SVD. Memory clinic samples were drawn from single or multi-center studies, which were selected based on availability of (diffusion) MRI sequences and CSF or PET data. The compilation of samples, subject selection criteria, and exclusions are shown in **Fig. 1,** and further elaborated below. MRI, CSF, and PET data from subjects of the included samples were obtained within one year. Diagnostic criteria used in the AD and SVD focused memory clinic samples are summarized in **Supplementary Table 1**. All studies were approved by the ethics committees of the respective institutions and all subjects provided written informed consent.

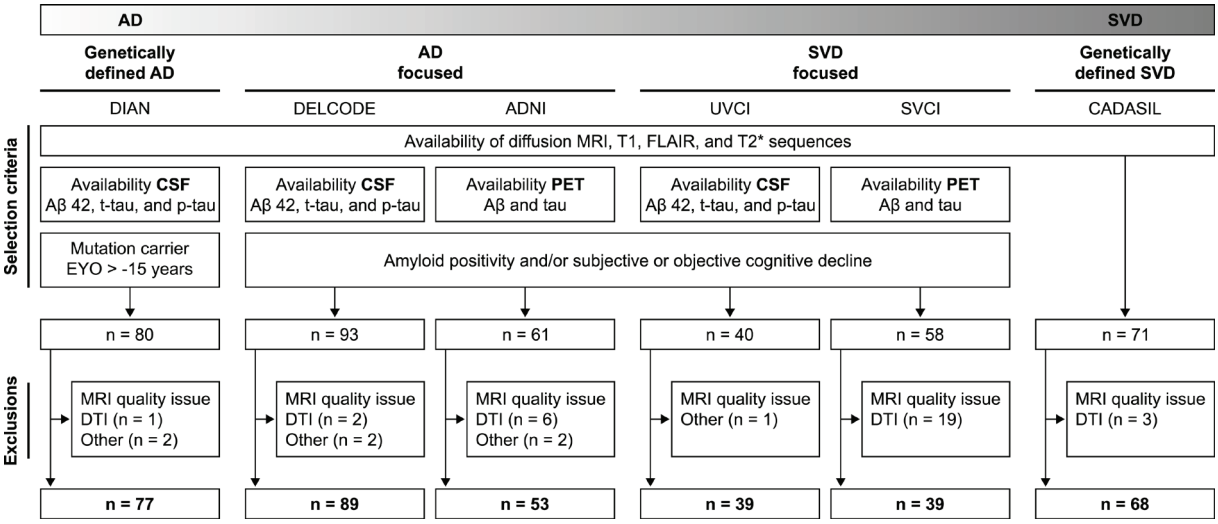

Figure 1. Study concept and participant selection flowchart. Samples cover the entire spectrum of AD, mixed disease, and SVD. Abbreviations: AD = Alzheimer's disease; DTI = diffusion tensor imaging; EYO = estimated years from symptom onset; FLAIR = fluid-attenuated inversion recovery; p-tau = phosphorylated-tau$_{181}$; SVD = small vessel disease; t-tau = total tau.

Alzheimer's disease focused samples

We included 89 participants from the German multicentric DZNE-Longitudinal Cognitive Impairment and Dementia Study (DELCODE; downloaded in December 2018) with available CSF amyloid-beta$_{1\text{-}40}$ (Aβ 40), amyloid-beta$_{1\text{-}42}$ (Aβ 42), total-tau (t-tau), and phosphorylated-

tau_{181} (p-tau) data. The sample consisted of Aβ 42-positive healthy controls (Aβ 42 cut-off see **Supplementary Text 1**) and patients with subjective cognitive decline, amnestic mild cognitive impairment, and mild dementia (Jessen *et al.*, 2018).

We further included 53 participants from the multicentric Alzheimer's disease Neuroimaging Initiative (ADNI, phase 3; downloaded in December 2018 at http://adni.loni.usc.edu) with available Aβ [^{18}F]-florbetapir and tau [^{18}F]AV-1451 flortaucipir (PET). The sample consisted of amyloid-positive (cut-off see **Supplementary Text 1**) healthy controls and patients with amnestic mild cognitive impairment and mild dementia (http://adni.loni.usc.edu).

Small vessel disease focused samples

We included 39 participants from the University Medical Center Utrecht, Netherlands (prospective Utrecht Vascular Cognitive Impairment study, UVCI) with available CSF data for Aβ 42, t-tau, and p-tau. The sample consisted of patients with subjective cognitive decline, mild cognitive impairment, and dementia and with no evidence of a primary etiology other than neurodegenerative disease or sporadic SVD and a high burden of SVD on MRI (Aalten *et al.*, 2014).

We further included 39 participants from the Samsung Medical Center, Seoul, Republic of Korea (Seoul Vascular Cognitive Impairment study, SVCI) with available Aβ [^{18}F]-florbetaben and tau [^{18}F]AV-1451 flortaucipir (PET). The sample consisted of patients with objective cognitive impairment and a high burden of SVD on MRI (Kim *et al.*, 2016; Kim *et al.*, 2018).

Genetically defined samples

As a genetically defined AD sample, we included 77 participants from the multicentric Dominantly Inherited Alzheimer Network (DIAN, data freeze 11; downloaded in August 2018).(Moulder *et al.*, 2013) DIAN is a longitudinal cohort study of individuals at risk of developing autosomal dominant AD. Here we included *PSEN1* (n = 59), *PSEN2* (n = 5), and *APP* (n = 13) mutation carriers with available Aβ 40, Aβ 42, t-tau, and p-tau CSF data. In our study, subjects had to be less than 15 years from estimated symptom onset in order to increase sensitivity to detect AD and SVD marker alterations in proximity to the onset of AD symptoms (Fleisher *et al.*, 2015; Araque Caballero *et al.*, 2018).

As a genetically defined SVD sample, we included 68 patients with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL)

recruited from a single-center study in Munich (Baykara *et al.*, 2016). Although CSF or PET data were not available in this dataset, we included CADASIL to judge the effect sizes of SVD markers in genetically defined SVD.

MRI

All MRI data were obtained on 3 Tesla systems. All samples included diffusion MRI, T1-weighted, fluid-attenuated inversion recovery (T2-weighted), and gradient echo (T2*-weighted) sequences. While each study used a standardized protocol, acquisition parameters differed across studies. The MRI protocols have been published previously for DIAN (Araque Caballero *et al.*, 2018), DELCODE (Franzmeier *et al.*, 2019), ADNI (Jiaerken *et al.*, 2018), UVCI (Heinen *et al.*, 2018), SVCI (Kim *et al.*, 2016), and CADASIL (Duering *et al.*, 2018). Diffusion MRI sequence parameters for all samples are summarized in **Supplementary Table 2**. All diffusion images were processed with the same pipeline as described in **Supplementary Text 2.** Global diffusion measures were calculated as mean of all voxels within a white matter skeleton. Regional analyses were based on voxel-wise diffusion measures.

Alzheimer's disease markers

We used Aβ and tau (CSF or PET) as biomarkers of AD. Details on CSF assays, PET tracers, and calculations of PET standardized uptake value ratio (SUVR) scores have previously been published for DIAN (Araque Caballero *et al.*, 2018), DELCODE (Jessen *et al.*, 2018), ADNI (http://adni.loni.usc.edu), UVCI (de Wilde *et al.*, 2017), and SVCI (Kim *et al.*, 2018).

For the main analyses we used continuous CSF and PET measures. For a subgroup analysis in amyloid-positive individuals, we used study specific Aβ cut-off values. See **Supplementary Text 1** for details.

Small vessel disease markers

We used an established total SVD score (ordinal variable) (Staals *et al.*, 2014) and white matter hyperintensity (WMH) volume (continuous variable) as MRI markers of SVD. The total SVD score summarizes the presence or severity of SVD lesions on an ordinal scale, i.e. WMH, lacunes, microbleeds, and enlarged perivascular spaces (Staals *et al.*, 2014). Two trained raters (SF, NV) assessed these lesions according to the STRIVE consensus criteria (Wardlaw *et al.*, 2013): WMHs were rated using the Fazekas scale (Fazekas *et al.*, 1987), the number of lacunes was determined on fluid-attenuated inversion recovery and T1-weighted images, the number of

cerebral microbleeds on T2*-weighted gradient echo images, and the number of enlarged perivascular spaces in the basal ganglia on a single T1-weighted axial image slice with the highest number of perivascular spaces (Potter *et al.*, 2015). WMH volume was calculated from a previously described semi-automated segmentation pipeline (Baykara *et al.*, 2016).

Statistical analyses

All statistical analyses were performed in R (version 3.5.1) (R Core Team, 2013). The statistical significance level was set at $\alpha < 0.05$.

Associations between AD biomarkers, SVD markers, age, and sex (independent variables), and global diffusion measures (dependent variables) were first assessed by simple linear regression analyses within each sample. Variables were power transformed in case of non-normal distribution (Shapiro-Wilk test) (Yeo and Johnson, 2000).

To perform multivariable analysis in the presence of multicollinearity, i.e. intercorrelations among disease markers (**Supplementary Figure 1**), we used random forest regressions (R package 'party'; version 1.3-2) (Strobl et al., 2007). This method allows to assess the contribution of each AD biomarker, SVD marker, age, and sex to diffusion alterations, while accounting for all other variables. For each sample, we calculated 1501 conditional inference trees with unbiased variable selection and default parameters as previously described (Duering et al., 2018). We calculated conditional variable importance together with a 95% confidence interval from 100 repetitions.

An effect of Aβ on diffusion measures might be mediated by vascular pathology, in particular cerebral amyloid angiopathy, i.e. Aβ accumulation in perforating vessels (Charidimou et al., 2017). To address this possibility, we performed a post-hoc mediation analysis (R package 'lavaan'; version 0.6-4) (Rosseel, 2012) in samples where simple regression analysis showed an effect of Aβ on diffusion measures. Diffusion measures were entered as dependent variables, Aβ as independent variable, WMH volume as mediator, and age as covariate. Standard errors were based on bootstrapping (1000 iterations).

Because amyloid pathology has been shown to strengthen the association between tau accumulation and structural tract alterations as assessed by diffusion measures (Jacobs et al., 2018), we performed two additional analyses within each sample. First, we conducted a sensitivity analysis restricted to amyloid-positive individuals by repeating simple regression analyses. Second, we assessed the interaction effect of tau × Aβ on diffusion measures.

Finally, since tau is a localized pathology starting in the entorhinal cortex (Cho et al., 2016), we also performed regional analyses between voxel-wise diffusion measures and tau in the PET samples, i.e. ADNI and SVCI. We used permutation test theory with a standard general linear model as implemented in 'randomise' (FSL). We assessed associations between both global tau PET SUVR scores as well as regional tau PET SUVR scores in the entorhinal cortex and voxel-wise diffusion measures. The number of permutations was set at 5000. Significant voxels within the skeletonized diffusion measure maps were identified using threshold-free cluster enhancement with $P < 0.05$, corrected for multiple comparisons.

2.1.4 Results

Sample characteristics are summarized in **Table 1**. As expected, patients with genetically defined AD or SVD were considerably younger than memory clinic patients.

Small vessel disease shows stronger associations than Alzheimer's disease with diffusion alterations in simple regression analyses

In simple regressions, both SVD markers, i.e. WMH volume and total SVD score, were consistently and strongly associated with conventional DTI measures (FAu, MDu; range of $R^2_{adj.}$ [0.08 – 0.79]) and FW (range of $R^2_{adj.}$ [0.18 – 0.76]) across all six samples (**Fig. 2**, **Supplementary Tables 3-5**). In contrast, AD biomarkers, i.e. CSF and PET data, were not or only weakly associated with conventional DTI measures and FW (range of $R^2_{adj.}$ [0.04 – 0.18]; **Fig. 2**, **Supplementary Tables 3-5**). Results were largely consistent across study samples, with a notable exception in the sample of genetically defined AD (DIAN). Here, effect sizes for Aβ 42 (CSF) were similar to the effect sizes of WMH volume (**Fig. 2**, **Supplementary Table 5**). Associations between Aβ 42, WMH volume and diffusion measures in DIAN and DELCODE were further addressed in a post-hoc mediation analysis (see below).

Table 1. Sample characteristics.

	Genetically defined AD	AD focused		SVD focused		Genetically defined SVD
	DIAN (n = 77)	DELCODE (n = 89)	ADNI (n = 53)	UVCI (n = 39)	SVCI (n = 39)	CADASIL (n = 68)
Age, years	42 (14)	72 (9)	78 (13)	74 (12)	79 (10)	55 (11)
Female, n (%)	40 (52)	36 (40)	25 (47)	13 (33)	28 (72)	44 (65)
Diagnosis, n (%) HC, SCD, MCI, dementia	na	4 (4), 37 (42), 33 (37), 15 (17)	22 (42), na, 23 (43), 8 (15)	0 (0), 3 (8), 18 (46), 18 (46)	0 (0), na, 22 (56), 17 (44)	na
CDR, n (%) 0, 0.5, 1, 2, 3	38 (49), 29 (38), 9 (12), 1 (1), 0 (0)	29 (33), 52 (59), 7 (8), 0 (0), 0 (0)[a]	22 (42), 23 (43), 6 (11), 2 (4), 0 (0)	1 (3), 30 (77), 8 (20), 0 (0), 0 (0)	0 (0), 26 (67), 7 (18), 6 (15), 0 (0)	57 (84), 9 (13), 1 (1), 1 (1), 0 (0)
Aβ positive, n (%)	46 (60)	44 (49)	37 (70)	22 (56)	19 (49)	na
DTI						
FAu, mm^2/s	0.45 (0.03) [0.38, 0.49]	0.46 (0.03) [0.36, 0.52]	0.45 (0.04) [0.38, 0.50]	0.44 (0.04) [0.36, 0.48]	0.42 (0.04) [0.35, 0.50]	0.40 (0.06) [0.27, 0.49]
MDu, 10^{-4} mm^2/s	7.84 (0.64) [7.27, 9.31]	7.68 (0.59) [6.71, 9.72]	8.21 (0.63) [7.35, 9.77]	8.05 (0.82) [7.23, 9.72]	9.66 (0.76) [8.48, 11.0]	9.40 (1.61) [7.79, 12.89]
FAt, mm^2/s	0.55 (0.02) [0.52, 0.58]	0.56 (0.02) [0.52, 0.60]	0.57 (0.02) [0.54, 0.60]	0.56 (0.02) [0.52, 0.57]	0.59 (0.01) [0.56, 0.63]	0.55 (0.02) [0.50, 0.59]
MDt, 10^{-4} mm^2/s	5.92 (0.07) [5.80, 6.01]	5.97 (0.10) [5.51, 6.14]	6.01 (0.63) [5.94, 6.09]	5.82 (0.15) [5.63, 5.99]	6.00 (0.04) [5.91, 6.12]	5.97 (0.03) [5.89, 6.03]
FW, mm^2/s	0.18 (0.05) [0.14, 0.28]	0.16 (0.04) [0.11, 0.29]	0.20 (0.05) [0.13, 0.31]	0.22 (0.06) [0.16, 0.35]	0.25 (0.04) [0.17, 0.31]	0.29 (0.11) [0.17, 0.51]
AD markers						
CSF						
Aβ 40, ng/L	7634 (4516) [2215, 15622]	7942 (3229) [3721, 13358]	-	na	-	-
Aβ 42, ng/L	436 (332) [174, 1424]	498 (380) [183, 1317]	-	619 (279) [363, 1641]	-	-
T-tau, ng/L	97 (132) [8, 563]	425 (369) [98, 1477]	-	524 (368) [140, 1274]	-	-
P-tau, ng/L	56 (66) [14, 163]	51 (39) [16, 192]	-	67 (47) [19, 166]	-	-
PET						
[^{18}F]-florbetapir SUVR	-	-	1.18 (0.36) [0.90, 1.70]	-	na	-
[^{18}F]-florbetaben SUVR	-	-	na	-	1.38 (0.49) [1.11, 2.17]	-
[^{18}F]AV-1451 SUVR	-	-	1.10 (0.13) [0.86, 1.67]	-	1.11 (0.16) [0.89, 1.60]	-
SVD markers						
WMHvol, ml	2.22 (3.05) [0.00, 30.47]	2.78 (5.36) [0.03, 34.50]	3.35 (8.29) [0.00, 77.24]	15.72 (1.85) [1.34, 67.27]	32.19 (21.03) [10.48, 71.20]	71.27 (73.74) [1.09, 257.74]
SVD score, n (%) 0, 1, 2, 3, 4	67 (87), 9 (12), 1 (1), 0 (0), 0 (0)	23 (26), 33 (37), 28 (31), 3 (3), 2 (2)	8 (15), 17 (32), 18 (34), 8 (15), 2 (4)	4 (10), 15 (39), 11 (28), 6 (15), 3 (8)	0 (0), 0 (0), 0 (0), 0 (0), 39 (100)	0 (0), 16 (24), 19 (28), 17 (25), 16 (24)

For numeric variables median (interquartile range) [min, max] is shown, except for age. [a] DELCODE: CDR of 1 subject missing. Abbreviations: AD = Alzheimer's disease; CDR = clinical dementia rating; FAu = uncorrected fractional anisotropy; DTI = diffusion tensor imaging; FAt = free water corrected tissue compartment of fractional anisotropy; FW = free water content; HC = healthy control; MCI = mild cognitive impairment; MDu = uncorrected mean diffusivity; MDt = free water corrected tissue compartment of mean diffusivity; na = not available; p-tau = phosphorylated- tau_{181}; SCD = subjective cognitive decline; SUVR = standardised uptake value ratio; SVD = small vessel disease; SVD score = total small vessel disease score; t-tau = total tau; WMHvol = white matter hyperintensity volume.

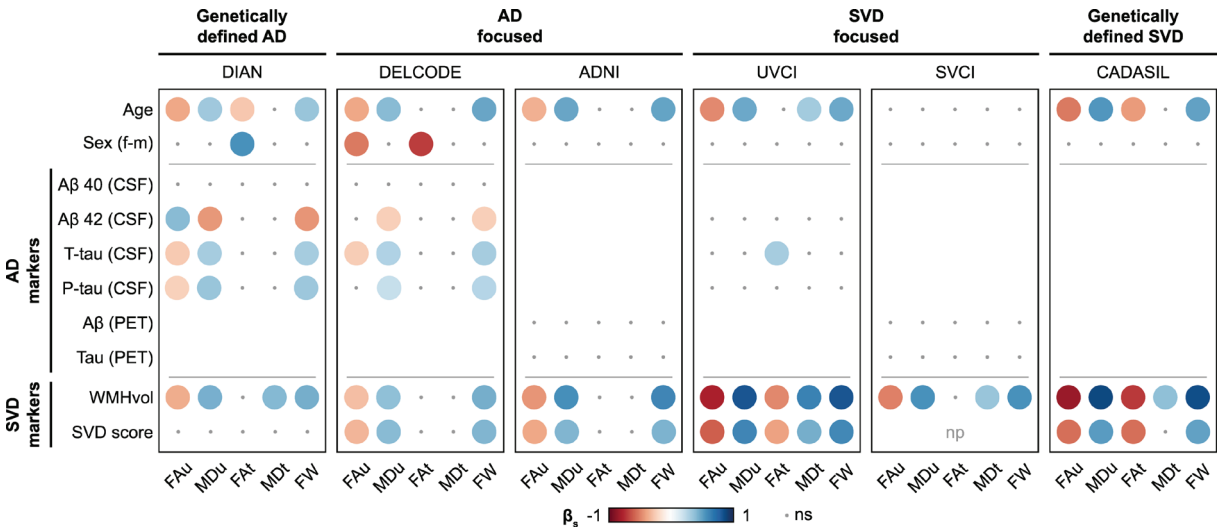

Figure 2. Simple regression analyses. Simple linear regression analyses between diffusion measures and AD biomarkers or SVD markers. Standardized β is represented by color. Abbreviations: AD = Alzheimer's disease; β_s = standardized beta; FAu = uncorrected fractional anisotropy; FAt = free water corrected tissue compartment of fractional anisotropy; FW = free water content; MDu = uncorrected mean diffusivity; MDt = free water corrected tissue compartment of mean diffusivity; np = not possible (all patients had the maximum score); ns = not significant; p-tau = phosphorylated- tau_{181}; SVD = small vessel disease; SVD score = total small vessel disease score; t-tau = total tau; WMHvol = white matter hyperintensity volume.

Small vessel disease and age contribute most to diffusion alterations in multivariable analyses

Using random forest regression as a multivariable method, we assessed the contribution of each AD biomarker, SVD marker, age and sex to diffusion measures, while accounting for multicollinearity. In all memory clinic samples, SVD markers showed higher variable importance than AD biomarkers for alterations of conventional DTI measures (FAu and MDu; **Fig. 3**) and FW (data not shown; nearly identical to MDu). The opposite was found only in DIAN, where AD biomarkers showed higher variable importance. For tissue measures (FAt and MDt), interpretation of random forest regressions was not feasible, because variable importances were zero or almost zero in all samples (data not shown).

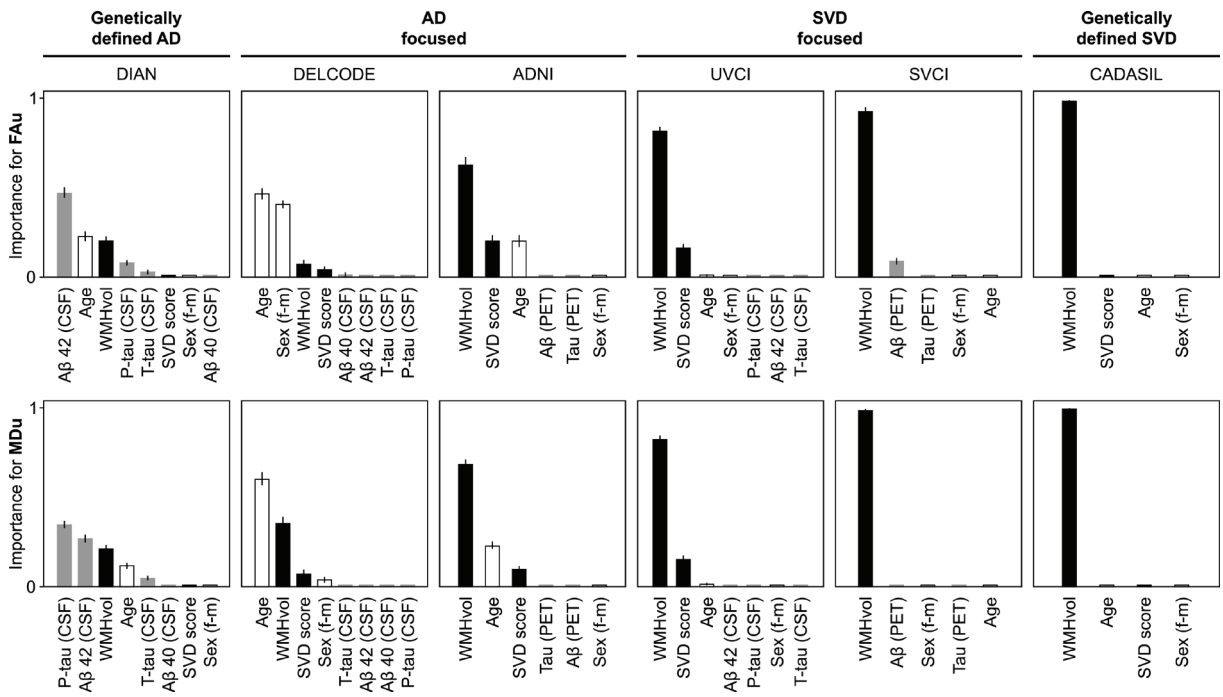

Figure 3. Multivariable analyses. Random forest regression analyses for estimating the relative variable importance of AD biomarkers (grey bars), SVD markers (black bars), age and sex (white bars) with regard to conventional DTI measures (FAu, MDu) while accounting for all other variables (conditional importance). Lines indicate the 95% confidence interval for the conditional variable importance. Abbreviations: AD = Alzheimer's disease; FAu = uncorrected fractional anisotropy; MDu = uncorrected mean diffusivity; p-tau = phosphorylated-tau$_{181}$; SVD = small vessel disease; SVD score = total small vessel disease score; T-tau = total tau; WMHvol = white matter hyperintensity volume.

White matter hyperintensities partially mediate the effect of Aβ on diffusion alterations in genetically defined Alzheimer's disease

For diffusion measures significantly associated with Aβ 42 (CSF) in the simple regression analysis, i.e. in DIAN and DELCODE, we performed a post-hoc mediation analysis to explore whether these associations might be mediated by vascular pathology, such as cerebral amyloid angiopathy. In DIAN, the effect of Aβ 42 on MDu and FW was indeed partially mediated by WMH volume (MDu: β_s = -0.06, SE = 0.03, P = 0.030; FW: β_s = -0.06, SE = 0.03, P = 0.026). However, we also found a direct effect of Aβ 42 on MDu and FW (MDu: β_s = -0.30, SE = 0.12, P = 0.005; FW: β_s = -0.30, SE = 0.11, P = 0.005). For FAu, mediation analysis was not significant. In DELCODE, where simple regression analysis showed only weak effects of Aβ 42, none of the mediation analyses were significant (all P > 0.136).

Tau is not associated with diffusion alterations in amyloid-positive individuals

It was recently reported that Aβ might strengthen the association between tau accumulation and diffusion alterations (Jacobs et al., 2018). We addressed this aspect in a sensitivity analysis restricted to amyloid-positive individuals (see **Table 1** for subsample sizes). Simple linear regressions between tau and diffusion measures in amyloid-positive individuals were not significant, except for DIAN (n = 46; p-tau and MDu, $\beta_s = 0.32$, $R^2_{adj.} = 0.08$, P = 0.031; p-tau and FW, $\beta_s = 0.31$, $R^2_{adj.} = 0.07$, P = 0.038). In correspondence with the full DIAN sample, tau showed effect sizes comparable to those found for WMH volume (WMH volume and MDu, $\beta_s = 0.35$, $R^2_{adj.} = 0.10$, P = 0.017; WMH volume and FW, $\beta_s = 0.37$, $R^2_{adj.} = 0.12$, P = 0.011). None of the tau × Aβ interaction models with diffusion measures as dependent variables were significant in any of the samples (all P > 0.051).

Regional tau is not associated with diffusion alterations

Tau is a localized pathology starting in the entorhinal cortex (Cho et al., 2016) and previous literature suggests localized effects of tau on white matter microstructure (Kantarci et al., 2017; Jacobs et al., 2018; Strain et al., 2018). We therefore performed regional analyses in the PET samples, i.e. ADNI and SVCI, which allow to assess local tau load. Associations between regional tau PET SUVR scores in the entorhinal cortex or global tau PET SUVR scores and voxel-wise diffusion measures were not significant.

2.1.5 Discussion

We investigated the effect of AD and SVD on brain microstructure assessed by diffusion measures. As a unique feature, our study included six independently recruited samples covering the entire spectrum of AD, mixed disease, and SVD. The main finding is that in memory clinic patients SVD rather than AD determines diffusion alterations. Results were consistent across all memory clinic samples, illustrating the robustness and generalizability of our findings.

The strong effect of SVD on diffusion measures was evident in all of the six study samples. In contrast, an association between AD and diffusion measures was only detectable in DELCODE and DIAN. While in DELCODE effect sizes of AD biomarkers were considerably smaller than those of SVD markers, effect sizes of Aβ 42 and WMH volume were similar in DIAN. Multivariable analyses using random forest regression showed a higher importance of SVD markers for diffusion alterations in all memory clinic samples. The only sample in which AD biomarkers had a higher variable importance was DIAN. As expected for a genetically defined

sample, these patients are considerably younger than typical memory clinic patients and less likely to show age-related comorbidities, such as SVD. Still, mediation analysis in DIAN suggested a vascular contribution to diffusion alterations also in this population, as the effect of Aβ on diffusion alterations was partly mediated by WMH volume. This might indicate a contribution of cerebral amyloid angiopathy, a specific subtype of SVD caused by deposition of Aβ in perforating vessels (Charidimou *et al.*, 2017). Overall, we conclude that while the effect of AD on diffusion measures is apparent in DIAN patients with pure AD, the presence of SVD in the other samples masks the effect of AD on diffusion measures.

Seemingly in contrast with our results, associations between AD biomarkers and alterations of white matter microstructure as assessed by DTI have been previously reported in memory clinic patients (Racine *et al.*, 2014; Melah *et al.*, 2016; Hoy *et al.*, 2017; Jacobs *et al.*, 2018; Strain *et al.*, 2018; Racine *et al.*, 2019; Vipin *et al.*, 2019), although some studies found no association (Kantarci *et al.*, 2014; Pietroboni *et al.*, 2018). Importantly, however, only one of these studies accounted for SVD. Hence, the effect of AD on diffusion alterations might have been overestimated. Only Strain *et al.* (2018) considered biomarkers of both diseases and found an association between tau PET (but not Aβ PET) in temporal regions and diffusion measures in temporal white matter projections, independently of WMHs. In line with our results, the effect size for WMH volume was larger than effect sizes of AD biomarkers. By considering both diseases, we conclude that SVD determines diffusion alterations to a much larger extent than AD, even in samples where AD was the clinically predominant disease. The strong effect of SVD has implications for future studies, which will need to take SVD into account as an important confounder.

In the current study, neither the regional analysis nor the analysis in amyloid-positive individuals, where the effect of tau was expected to be stronger (Jacobs *et al.*, 2018), indicated a significant association between tau and diffusion measures. In post-mortem studies, white matter alterations in AD patients have been attributed to axonal degeneration secondary to cortical deposition of hyperphosphorylated tau (McAleese *et al.*, 2015; McAleese *et al.*, 2017). Yet, post-mortem studies by design examine patients in very late stages of AD, while our memory clinic patients were mostly in earlier disease stages. Thus, it is conceivable that our patients have not yet reached the disease stage where associations between tau and axonal degeneration can be detected.

Our finding that diffusion alterations are predominantly driven by SVD is also supported by a genome-wide association study in the population-based UK Biobank. Polygenic risk scores for

altered DTI measures were associated with SVD-related stroke and major depressive disorder, but not with AD (Rutten-Jacobs *et al.*, 2018). The study thus provided genetic evidence that mechanisms underlying diffusion alterations are shared with cerebrovascular disease.

Another aim of this study was to investigate whether free water imaging allows to disentangle the contribution of SVD and AD. The finding that SVD markers showed strongest associations with FW corroborates previous results indicating that diffusion alterations in SVD patients are predominantly driven by an increase in the free water content (Duering *et al.*, 2018). However, our current analysis did not provide evidence that AD biomarkers are reflected in the tissue compartment. The latter result is in contrast to studies suggesting that AD-related neurodegeneration of the white matter might be specifically represented in free water corrected tissue measures: Tissue measures were associated with conversion from mild cognitive impairment to dementia in AD patients (Maier-Hein *et al.*, 2015) and showed Aβ-related longitudinal changes (Vipin *et al.*, 2019). It should be noted that the current study was cross-sectional and thus we cannot exclude that the tissue compartment holds valuable information for longitudinal studies (Maier-Hein *et al.*, 2015; Vipin *et al.*, 2019).

A limitation of our study is that elevated tau (especially in CSF) is not specific for AD as it could also indicate other tauopathies, such as Pick's disease, corticobasal degeneration, or progressive supranuclear palsy. However, the tau PET tracer ([^{18}F]AV-1451) employed mostly binds to tau deposits specific for AD (Lowe *et al.*, 2016). Also, the focus on recruitment of clinical AD, e.g. by including amnestic mild cognitive impairment in DELCODE and ADNI, clearly enriched for AD rather than other tauopathies. Another limitation is the lack of AD biomarkers in the CADASIL sample. Yet, the purpose of the CADASIL sample was to judge the effect sizes of SVD markers in genetically defined disease, i.e. in young patients with pure SVD. Interestingly, we found similar effect sizes as in SVD focused samples with mixed pathology, in particular the UVCI sample. As a further limitation, multi-shell diffusion data, which would be necessary for more complex parametrization of the fluid compartments (Hoy *et al.*, 2014; Rydhög *et al.*, 2017; Sepehrband *et al.*, 2019) was not available in the study samples.

The main strength of our analysis is the inclusion of multiple samples from different countries and ethnicities, covering the entire spectrum of AD, mixed disease, and SVD. This has enabled us to independently validate results and to assess both CSF and PET biomarkers of AD in a robust manner. The differences in study protocols among the six samples, such as MRI acquisition, biomarker assessment techniques, and recruitment strategies suggest that our

results are generalizable to other populations along the spectrum of AD and SVD. We also included younger individuals with genetically defined disease to minimize confounding by other age-related pathologies. Finally, the state-of-the art diffusion imaging analysis pipeline included modern pre-processing techniques and rigorous control for confounding by CSF partial volume effects, which is crucial in patients with atrophy and therefore enlarged CSF spaces.

In conclusion, we demonstrate that SVD rather than AD determines diffusion alterations in memory clinic patients. Our results validate diffusion measures as markers for SVD and as valuable tools to assess the vascular contribution to AD and dementia, which still needs to be adequately explored (Sweeney *et al.*, 2019). Building upon our findings, future studies could assess if more advanced parameterization of diffusion processes, such as biophysical diffusion models, further increases the sensitivity in earlier or even asymptomatic stages.

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2.1.7 Conflict of interest

Nothing to report.

2.1.8 References

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2.1.9 Supplementary material

Supplementary Table 1. Diagnostic criteria in memory clinic samples

	AD focused		SVD focused	
	DELCODE[a]	ADNI, phase 3[b]	UVC[c]	SVCI[d,e]
HC	No subjective/ objective cognitive decline	MMSE ≥ 24; CDR = 0	na	na
SCD	Subjectively reported cognitive worsening; age-, sex-, and education-adjusted CERAD neuropsychological test battery > -1.5 SD	na	Subjective cognitive decline; no objective cognitive impairment on a standardized neuropsychological test battery	na
MCI	Age-, sex-, and education-adjusted performance CERAD episodic memory tests < -1.5 SD	Subjective memory complaints without significant functional impairment; MMSE ≥ 24; objective memory impairment on the revised Wechsler Memory Scale; CDR = 0.5; memory CDR ≥ 0.5.	Subjective and objective cognitive decline in at least one cognitive domain without significant functional impairment	Objective memory decline below the 16th percentile (- 1.0 SD) of age- and education-matched norms in at least one cognitive domain tested by the Seoul Neuropsychological Screening Battery; Petersen's criteria
Dementia	NIA-AA for probable AD; MMSE ≥ 18	NINCDS-ADRDA criteria	NINCDS-ADRDA criteria	NIA-AA for probable AD

[a]Jessen F, Spottke A, Boecker H, et al. Design and first baseline data of the DZNE multicenter observational study on predementia Alzheimer's disease (DELCODE). Alzheimers Res Ther. 2018;10(1):15; [b]http://adni.loni.usc.edu; [c]Aalten P, Ramakers IHGB, Biessels GJ, et al. The Dutch Parelsnoer Institute-Neurodegenerative diseases; methods, design and baseline results. BMC neurol. 2014;14(1):254; [d]Kim HJ, Yang JJ, Kwon H, et al. Relative impact of amyloid-β, lacunes, and downstream imaging markers on cognitive trajectories. Brain. 2016;139(9):2516-27; [e]Kim HJ, Park S, Cho H, et al. Assessment of extent and role of tau in subcortical vascular cognitive impairment using 18F-AV1451 positron emission tomography imaging. JAMA neurol. 2018;75(8):999-1007. Abbreviations: AD = Alzheimer's disease; CERAD = Consortium to Establish a Registry for Alzheimer's disease; CDR = clinical dementia rating; CSF = cerebrospinal fluid; ELISA = enzyme-linked immunosorbent assays; HC = cognitively healthy control; MCI = mild cognitive impairment; MMSE = Mini-Mental-State Examination; na = not available; NIA-AA = National Institute on Aging research criteria for probable AD; NINCDS-ADRDA = National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association; PET = positron emission tomography; SCD = subjective cognitive decline; SUVR = standardized uptake value ratio; SD = standard deviation; SVD = small vessel disease.

Supplementary Table 2. Diffusion parameters

	DIAN	DELCODE	ADNI	UVCI	SVCI	CADASIL
Scanner	Siemens systems	Siemens systems	GE Healthcare systems	Philips Achieva	Philips Achieva	Siemens Verio
TR [ms]	11000	12100	7200	6600	7696	12700
TE [ms]	87	88	56	73	60	81
Slice [mm]	2.50	2.00	2.00	2.50	2.00	2.00
In-plane [mm]	2.50 x 2.50	2.00 x 2.00	2.00 x 2.00	1.72 x 1.72	1.72 x 1.72	2.00 x 2.00
b-value [s/mm^2]	1000	700, 1000	1000	1200	600	1000
Directions	64	30, 30	48	45	45	30

Abbreviations: TE = echo time; TR = repetition time.

Supplementary Table 3. Simple regression models in Alzheimer's disease focused samples

	FAu			MDu			FAt			MDt			FW		
	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*
DELCODE (n = 89)															
Age	-0.38	0.13	**0.000**	0.42	0.17	**0.000**	-0.21	0.03	0.051	0.15	0.01	0.171	0.49	0.23	**0.000**
Sex (f-m)	-0.52	0.05	**0.016**	0.28	0.01	0.198	-0.69	0.11	**0.001**	0.11	-0.01	0.599	0.23	0.00	0.279
Aβ 40 (CSF)	0.04	-0.01	0.745	-0.03	-0.01	0.770	0.07	-0.01	0.492	0.00	-0.01	0.963	0.00	-0.01	0.969
Aβ 42 (CSF)	0.17	0.02	0.102	-0.23	0.04	**0.029**	0.09	0.00	0.386	-0.11	0.00	0.314	-0.24	0.05	**0.025**
T-tau (CSF)	-0.25	0.05	**0.019**	0.29	0.07	**0.005**	-0.14	0.01	0.201	0.16	0.02	0.123	0.33	0.10	**0.002**
P-tau (CSF)	-0.20	0.03	0.063	0.23	0.04	**0.033**	-0.09	0.00	0.405	0.13	0.00	0.238	0.27	0.06	**0.009**
WMHvol	-0.30	0.08	**0.004**	0.40	0.15	**0.000**	-0.05	-0.01	0.631	0.14	0.01	0.206	0.47	0.21	**0.000**
SVD score	-0.32	0.09	**0.002**	0.41	0.16	**0.000**	-0.14	0.01	0.206	0.18	0.02	0.088	0.44	0.18	**0.000**
ADNI (n = 53)															
Age	-0.35	0.10	**0.011**	0.49	0.23	**0.000**	0.10	-0.01	0.464	0.10	-0.01	0.476	0.51	0.24	**0.000**
Sex (f-m)	-0.21	-0.01	0.460	0.42	0.03	0.125	0.28	0.00	0.322	0.29	0.00	0.301	0.39	0.02	0.158
Aβ (PET)	0.14	0.00	0.312	-0.07	-0.02	0.635	0.23	0.04	0.091	-0.19	0.02	0.164	-0.05	-0.02	0.744
Tau (PET)	0.05	-0.02	0.745	-0.04	-0.02	0.777	0.02	-0.02	0.875	0.14	0.00	0.323	-0.05	-0.02	0.702
WMHvol	-0.43	0.17	**0.001**	0.58	0.32	**0.000**	0.12	0.00	0.376	0.10	-0.01	0.490	0.62	0.38	**0.000**
SVD score	-0.38	0.12	**0.006**	0.43	0.17	**0.001**	-0.02	-0.02	0.863	0.26	0.05	0.061	0.45	0.19	**0.001**

P < 0.05 in bold. Abbreviations: β_s = standardised beta; FAu = uncorrected fractional anisotropy; FAt = free water corrected tissue compartment of fractional anisotropy; FW = free water content; MDu = uncorrected mean diffusivity; MDt = free water corrected tissue compartment of mean diffusivity; P-tau = phosphorylated-tau$_{181}$; $R^2_{adj.}$ = adjusted explained variance; SVD score = total small vessel disease score; T-tau = total tau; WMHvol = white matter hyperintensity volume.

Supplementary Table 4. Simple regression models in small vessel disease focused samples

	FAu			MDu			FAt			MDt			FW		
	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*
UVCI (n = 39)															
Age	-0.46	0.19	**0.003**	0.49	0.22	**0.002**	-0.32	0.08	0.050	0.33	0.09	**0.039**	0.49	0.22	**0.002**
Sex (f-m)	0.15	0.00	0.363	-0.08	-0.02	0.607	0.22	0.02	0.177	-0.21	0.02	0.199	-0.11	-0.02	0.518
Aβ 42 (CSF)	0.02	-0.03	0.923	-0.18	0.01	0.262	-0.24	0.03	0.135	-0.03	-0.03	0.850	-0.18	0.01	0.262
T-tau (CSF)	0.21	0.02	0.207	-0.07	-0.02	0.678	0.32	0.08	**0.044**	-0.08	-0.02	0.632	-0.05	-0.02	0.743
P-tau (CSF)	0.16	0.00	0.334	-0.07	-0.02	0.651	0.23	0.03	0.159	-0.08	-0.02	0.604	-0.05	-0.02	0.760
WMHvol	-0.80	0.62	**0.000**	0.85	0.72	**0.000**	-0.50	0.23	**0.001**	0.62	0.37	**0.000**	0.85	0.71	**0.000**
SVD score	-0.59	0.33	**0.000**	0.62	0.37	**0.000**	-0.39	0.13	**0.013**	0.46	0.19	**0.003**	0.62	0.36	**0.000**
SVCI (n = 39)															
Age	-0.16	0.00	0.333	0.11	-0.02	0.521	-0.18	0.01	0.279	0.08	-0.02	0.616	0.11	-0.01	0.490
Sex (f-m)	0.05	-0.03	0.894	-0.03	-0.03	0.943	0.04	-0.03	0.902	0.36	0.00	0.323	-0.05	-0.03	0.888
Aβ (PET)	-0.27	0.05	0.093	0.30	0.06	0.068	-0.11	-0.01	0.505	0.19	0.01	0.244	0.30	0.06	0.064
Tau (PET)	-0.11	-0.01	0.499	0.09	-0.02	0.572	-0.06	-0.02	0.729	0.10	-0.02	0.529	0.09	-0.02	0.579
WMHvol	-0.49	0.22	**0.001**	0.58	0.32	**0.000**	-0.17	0.00	0.288	0.37	0.11	**0.022**	0.57	0.31	**0.000**
SVD score	np	np	np	np	np	np	np	np	np	np	np	np	np	np	np

P < 0.05 in bold. Abbreviations: β_s = standardised beta; FAu = uncorrected fractional anisotropy; FAt = free water corrected tissue compartment of fractional anisotropy; FW = free water content; MDu = uncorrected mean diffusivity; MDt = free water corrected tissue compartment of mean diffusivity; np = not possible (all patients had the maximum score); P-tau = phospho-tau$_{181}$; $R^2_{adj.}$ = adjusted explained variance; SVD score = total small vessel disease score; T-tau = total tau; WMHvol = white matter hyperintensity volume.

Supplementary Table 5. Simple regression models in genetically defined samples

	FAu			MDu			FAt			MDt			FW		
	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*	β_s	$R^2_{adj.}$	*P*
DIAN (n = 77)															
Age	-0.38	0.13	**0.001**	0.35	0.11	**0.002**	-0.27	0.06	**0.018**	0.05	-0.01	0.669	0.37	0.12	**0.001**
Sex (f-m)	0.25	0.00	0.267	0.06	-0.01	0.805	0.58	0.07	**0.010**	0.44	0.04	0.055	0.05	-0.01	0.821
Aβ 40 (CSF)	0.08	-0.01	0.468	-0.08	-0.01	0.468	0.07	-0.01	0.564	-0.07	-0.01	0.555	-0.07	-0.01	0.522
Aβ 42 (CSF)	0.41	0.16	**0.000**	-0.43	0.17	**0.000**	0.22	0.03	0.057	-0.18	-0.01	0.053	-0.43	0.18	**0.000**
T-tau (CSF)	-0.26	0.05	**0.024**	0.33	0.10	**0.003**	-0.09	0.00	0.427	0.14	0.01	0.228	0.32	0.09	**0.004**
P-tau (CSF)	-0.23	0.04	**0.047**	0.37	0.12	**0.001**	0.01	-0.01	0.918	0.21	0.04	0.056	0.36	0.12	**0.001**
WMHvol	-0.35	0.11	**0.002**	0.45	0.20	**0.000**	-0.08	-0.01	0.484	0.42	0.17	**0.000**	0.47	0.21	**0.000**
SVD score	-0.18	0.02	0.113	0.16	0.01	0.157	-0.11	0.00	0.345	0.13	0.00	0.255	0.18	0.02	0.115
CADASIL (n = 68)															
Age	-0.51	0.25	**0.000**	0.56	0.30	**0.000**	-0.42	0.16	**0.000**	0.02	-0.01	0.888	0.52	0.26	**0.000**
Sex (f-m)	-0.19	-0.01	0.450	0.28	0.00	0.267	-0.03	-0.01	0.900	-0.47	0.04	0.064	0.25	0.00	0.322
WMHvol	-0.84	0.71	**0.000**	0.89	0.79	**0.000**	-0.71	0.49	**0.000**	0.39	0.14	**0.001**	0.87	0.76	**0.000**
SVD score	-0.55	0.29	**0.000**	0.54	0.28	**0.000**	-0.54	0.28	**0.000**	0.02	-0.01	0.878	0.52	0.26	**0.000**

$P < 0.05$ in bold. Abbreviation: β_s = standardised beta; FAu = uncorrected fractional anisotropy; FAt = free water corrected tissue compartment of fractional anisotropy; FW = free water content; MDu = uncorrected mean diffusivity; MDt = free water corrected tissue compartment of mean diffusivity; P-tau = phosphorylated-tau_{181}; $R^2_{adj.}$ = adjusted explained variance; SVD score = total small vessel disease score; T-tau = total tau; WMHvol = white matter hyperintensity volume.

Supplementary Table 6. DIAN consortium

Last Name	First	Affiliation
Allegri	Ricardo	FLENI Institute of Neurological Research (Fundacion para la Lucha contra las Enfermedades Neurologicas de la Infancia)
Bateman	Randy	Washington University in St. Louis School of Medicine
Bechara	Jacob	Neuroscience Research Australia
Benzinger	Tammie	Washington University in St. Louis School of Medicine
Berman	Sarah	University of Pittsburgh
Bodge	Courtney	Brown University-Butler Hospital
Brandon	Susan	Washington University in St. Louis School of Medicine
Brooks	William (Bill)	Neuroscience Research Australia
Buck	Jill	Indiana University
Buckles	Virginia	Washington University in St. Louis School of Medicine
Chea	Sochenda	Mayo Clinic Jacksonville
Chhatwal	Jasmeer	Brigham and Women's Hospital–Massachusetts General Hospital
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Cinco	Jake	University College London
Clifford	Jack	Mayo Clinic Jacksonville
Cruchaga	Carlos	Washington University in St. Louis School of Medicine
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Flynn	Gigi	Washington University in St. Louis School of Medicine
Fox	Nick	University College London
Franklin	Erin	Washington University in St. Louis School of Medicine
Fujii	Hisako	Osaka City University
Gant	Cortaiga	Washington University in St. Louis School of Medicine
Gardener	Samantha	Edith Cowan University, Perth
Ghetti	Bernardino	Indiana University
Goate	Alison	Icahn School of Medicine at Mount Sinai
Goldman	Jill	Columbia University
Gordon	Brian	Washington University in St. Louis School of Medicine
Graff-Radford	Neill	Mayo Clinic Jacksonville
Gray	Julia	Washington University in St. Louis School of Medicine
Groves	Alexander	Washington University in St. Louis School of Medicine
Hassenstab	Jason	Washington University in St. Louis School of Medicine
Hoechst- Swisher	Laura	Washington University in St. Louis School of Medicine
Holtzman	David	Washington University in St. Louis School of Medicine
Hornbeck	Russ	Washington University in St. Louis School of Medicine
Houeland DiBari	Siri	German Center for Neurodegenerative Diseases (DZNE) Munich
Ikeuchi	Takeshi	Niigata University
Ikonomovic	Snezana	University of Pittsburgh
Jerome	Gina	Washington University in St. Louis School of Medicine
Jucker	Mathias	German Center for Neurodegenerative Diseases (DZNE) Tubingen
Karch	Celeste	Washington University in St. Louis School of Medicine
Kasuga	Kensaku	Niigata University
Kawarabayashi	Takeshi	Hirosaki University
Klunk	William (Bill)	University of Pittsburgh
Koeppe	Robert	University of Michigan
Kuder-Buletta	Elke	German Center for Neurodegenerative Diseases (DZNE) Tubingen
Laske	Christoph	German Center for Neurodegenerative Diseases (DZNE) Tubingen
Lee	Jae-Hong	Asan Medical Center
Levin	Johannes	German Center for Neurodegenerative Diseases (DZNE) Munich
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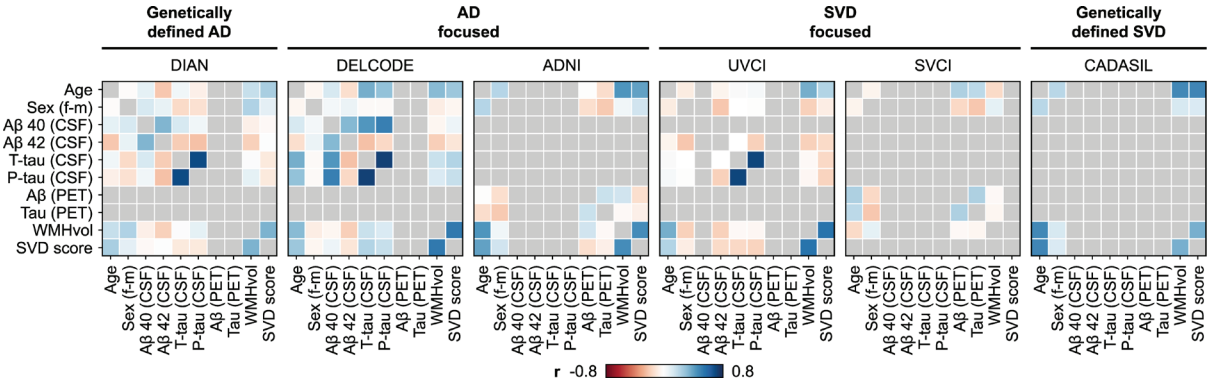

Supplementary Figure 1. Correlation matrices. Intercorrelations (multicollinearity) between AD biomarkers, SVD markers, age and sex. Grey boxes indicate "not available". Abbreviations: AD = Alzheimer's disease; P-tau = phosphorylated-tau181; SVD = small vessel disease; SVD score = total small vessel disease score; T-tau = total tau; WMHvol = white matter hyperintensity volume.

Supplementary Text 1. CSF and PET markers

CSF markers

Aβ 40, Aβ 42, t-tau, and p-tau CSF measurements were analyzed locally (within each study) with study specific assays for DIAN (Araque Caballero *et al.*, 2018), DELCODE (Jessen *et al.*, 2018), and UVCI (de Wilde *et al.*, 2017). For the subgroup analysis we used the following cut-offs for Aβ 42 (CSF) abnormality: < 496 pg/ml (DELCODE) (Jessen *et al.*, 2018) and < 640 pg/ml (UVCI) (Zwan *et al.*, 2014). For DIAN no study-specific cut-off was available, thus we applied the more restrictive DELCODE threshold (< 496 pg/ml).

PET markers

Aβ [^{18}F]-florbetapir (ADNI) or Aβ [^{18}F]-florbetaben (SVCI) and tau [^{18}F]AV-1451 PET measures were obtained. Details on PET acquisition and analysis are available for ADNI (http://adni.loni.usc.edu) and SVCI (Kim *et al.*, 2018). For ADNI, we used the freesurfer-derived global Aβ (PET) SUVR scores across the frontal, anterior-posterior cingulate, lateral-parietal, and lateral-temporal gray matter regions with whole cerebellum as the reference region (provided by the ADNI-PET Core). For SVCI we used locally calculated global Aβ PET SUVR scores across 25 cerebral cortex regions with cerebellar grey matter as the reference region (Kim *et al.*, 2018). For the subgroup analysis we used the following Aβ (PET) cut-offs for abnormality: Aβ [^{18}F]-florbetapir > 1.11 (ADNI) (Landau *et al.*, 2012) and Aβ [^{18}F]-florbetaben

> 1.45 (SVCI) (Bullich *et al.*, 2017). For both PET samples, we calculated an established global mean tau PET SUVR score (Maass *et al.*, 2017).

Supplementary Text 2. Processing of diffusion measures

All diffusion images were processed with the same pipeline. After visual inspection to exclude major artefacts, raw diffusion images were pre-processed using the MRtrix v3.0 package (http://www.mrtrix.org) and the Functional Magnetic Resonance Imaging of the Brain software library (FSL), v5.0.10 (Smith *et al.*, 2004). Noise and Gibbs ringing artefacts were removed ('dwidenoise', 'mrdegibbs' (Kellner *et al.*, 2016); MRtrix) and images were corrected for subject motion and eddy current induced distortions ('eddy_correct'; FSL). Conventional DTI measures, i.e. uncorrected fractional anisotropy (FAu) and mean diffusivity (MDu), as well as free water imaging measures, i.e. the free water corrected tissue measures, FAt and MDt, and the free water content (FW), were calculated as previously described (Duering *et al.*, 2018). Global and voxel-wise alterations of diffusion measures were assessed on the skeleton of main white matter tracts, which was calculated using the tract-based spatial statistics pipeline (Smith *et al.*, 2006) within FSL. For all samples, an FAt threshold ≥ 0.3 and a custom-made mask (Baykara *et al.*, 2016) were used to exclude areas prone to CSF contamination, a crucial aspect in patient samples with brain atrophy (Berlot *et al.*, 2014).

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2.2 Study 2: Application of diffusion MRI markers

The following section includes the research article entitled “Minor gait impairment despite white matter damage in pure small vessel disease”. The manuscript was published 2019 in *Annals of clinical and translational neurology* (Finsterwalder *et al.*, 2019).

Minor gait impairment despite white matter damage in pure small vessel disease

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2.2.1 Abstract

Objective: Gait impairment is common in patients with cerebral small vessel disease (SVD). However, gait studies in elderly SVD patients might be confounded by age-related comorbidities, such as polyneuropathy or sarcopenia. We therefore studied young patients with the genetically defined SVD CADASIL. Our aim was to examine the effects of pure SVD on single and dual task gait, and to investigate associations of gait performance with cognitive deficits and white matter alterations.

Methods: We investigated single task walking and calculatory, semantic, or motoric dual task costs in 39 CADASIL patients (mean age 50 ± 8) using a computerized walkway. We obtained 3 Tesla MRI and neuropsychological data on processing speed, the main cognitive deficit in CADASIL. Spatio-temporal gait parameters were standardized based on data from 192 healthy controls. Associations between white matter integrity, assessed by diffusion tensor imaging, and gait were analyzed using both a global marker and voxel-wise analysis.

Results: Compared to controls, CADASIL patients showed only mild single task gait impairment, and only in the rhythm domain. The semantic dual task additionally uncovered mild deficits in the pace domain. Processing speed was not associated with gait. White matter alterations were related to single task stride length but not to dual task performance.

Interpretation: Despite severe disease burden, gait performance in patients with pure small vessel disease was relatively preserved in single and dual tasks. Results suggest that age-related pathologies other than small vessel disease might play a role for gait impairment in elderly SVD patients.

2.2.2 Introduction

Gait impairment and cognitive deficits are common symptoms in cerebral small vessel disease (SVD) and a major cause of loss of independence (Román *et al.*, 2002; Chabriat *et al.*, 2009). For mobility capabilities, both symptoms bear a high risk for falls and fractures, specifically in the elderly (Bridenbaugh and Kressig, 2015). Senile, vascular gait impairment in sporadic SVD patients has been characterized by a reduction of gait velocity (Rosano *et al.*, 2006; Verghese *et al.*, 2007; de Laat *et al.*, 2010a; de Laat *et al.*, 2011; Smith *et al.*, 2015), reduction of stride length (Rosano *et al.*, 2006; de Laat *et al.*, 2010a; de Laat *et al.*, 2011), and increased double support times (Rosano *et al.*, 2006; de Laat *et al.*, 2010a). However, the etiology of gait impairment in SVD is still debated. The traditional view holds that lesions in strategic white matter tracts have a detrimental effect on supraspinal locomotor control (Rosano *et al.*, 2006; de Laat *et al.*, 2010a; Loos *et al.*, 2018; Van Der Holst *et al.*, 2018). This view is challenged by studies showing that gait control can also be affected by age-related instability due to degenerative musculoskeletal impairments e.g. joint problems, sarcopenia or polyneuropathy (Bridenbaugh and Kressig, 2015). A complementary view is that cognitive deficits are a major cause of gait disturbances and falls (Montero-Odasso *et al.*, 2012). The notion is based on experiments using cognitive dual-tasking, in which participants perform an attention-demanding task while walking (Bayot *et al.*, 2018). Gait performance deteriorates under this condition in healthy subjects (Theill *et al.*, 2011) and especially in cognitively impaired subjects (Al-Yahya *et al.*, 2011; Montero-Odasso *et al.*, 2012; Muir *et al.*, 2012; Doi *et al.*, 2014; Smith *et al.*, 2016). The underlying hypothesis is that walking, i.e. planning and execution of movements, postural control, motor coordination, and the secondary cognitive task compete for the same limited cognitive resources. While gait difficulties can be cognitively compensated during single task walking, this compensation mechanism is disrupted or limited by a secondary cognitive task. Thus, dual task walking can pronounce or even uncover gait deficits that are not obvious while walking only (Muir *et al.*, 2012; Bridenbaugh and Kressig, 2015).

Results of previous studies on gait impairment in SVD are based on sporadic SVD patients or individuals with mild cognitive impairment (MCI) aged 60 years or older (Rosano *et al.*, 2006; Verghese *et al.*, 2008; de Laat *et al.*, 2010a; de Laat *et al.*, 2011; Muir *et al.*, 2012; Loos *et al.*, 2018; Van Der Holst *et al.*, 2018). A potentially crucial limitation of these studies is the confounding by age-related co-pathologies, such as affected biomechanics, sarcopenia or disturbed sensory feedback (vision, proprioception). One approach to overcome these limitations is to explore the effect of dual-tasking on gait in a model disease of pure SVD

without confounding pathology. We therefore studied patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a genetically defined, pure form of SVD. CADASIL is characterized by an early disease onset between 35 and 50 years (Chabriat *et al.*, 2009). Conditions typically impacting on gait performance in elderly subjects, such as musculoskeletal constraints, joint abrasion, polyneuropathy, Alzheimer-type changes or other neurodegenerative pathology (Suttanon *et al.*, 2012), and normal-pressure hydrocephalus (Armand *et al.*, 2011) are uncommon in CADASIL patients.

SVD-related white matter alterations can be assessed using diffusion tensor imaging (DTI). DTI scalar measures are sensitive markers for SVD progression (Zeestraten *et al.*, 2016) and show a stronger association with gait decline than conventional SVD markers, such as white matter hyperintensities (WMH), lacunes, and microbleeds (Van Der Holst *et al.*, 2018).

The aim of the present study was to investigate the effect of pure SVD on single task and dual task walking. We hypothesized that (1) gait impairment in pure SVD would be most evident while dual task walking, (2) there is an association between processing speed, the main cognitive deficit in SVD, and gait, and (3) SVD-related white matter alterations (as assessed by DTI) are associated with gait performance.

To our knowledge this is the first study analyzing spatio-temporal gait data in patients with pure SVD. Here, we combine most recent methods of gait recording, diffusion tensor imaging and analysis.

2.2.3 Methods

Subjects

We included 39 CADASIL patients from an ongoing, prospective single-center study in Munich, Germany. CADASIL was confirmed by either molecular genetic testing (sequencing of the *NOTCH3* gene) or by ultrastructural analysis of a skin biopsy (detection of pathognomonic granular osmiophilic material in vessel walls). Inclusion criteria were age ≤ 70 years, absence of focal neurological deficits (e.g. paresis after stroke), absence of signs for polyneuropathy, available data for gait, neuropsychological testing, and MRI. All examinations were performed within two consecutive days. 192 age- and sex-matched healthy controls were recruited from local staff or by advertisement. In a standardized interview, none of the controls reported any auditory, vestibular, neurologic, cardio-vascular or orthopedic disorders. A short

physical examination was performed to exclude impairments in motor and sensory functions, coordination, balance, orientation, and short-term memory. All study participants had normal or corrected-to-normal vision. Leg length was measured in all subjects to be used as covariable in the statistical analysis. The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee. Written informed consent was obtained from all subjects.

Quantitative gait assessment

Spatio-temporal gait performance was assessed using the electronic, pressure-sensitive GAITRite® carpet (CIR Systems, Havertown, USA) with a length of 670 cm and a sampling rate of 120 Hz. It was recorded under four different conditions similar to previously used experimental protocols (Beauchet *et al.*, 2005; Theill *et al.*, 2011; Montero-Odasso *et al.*, 2012; Muir *et al.*, 2012). Four trials were performed in each condition to increase the number of recorded gait cycles and thereby improve the reliability of gait parameters (König *et al.*, 2014; Perera *et al.*, 2016). In the first condition, subjects were asked to walk over the carpet with preferred speed (condition 1, single task). In the remaining three conditions, subjects were asked to perform dual tasks. In the first dual task condition, subjects performed a calculatory cognitive task while walking (condition 2, calculatory dual task), i.e. serial 7 task. Using this task, we tested the effect of a secondary, working memory task on gait (Lee and Kang, 2002). Next, subjects performed a semantic cognitive task while walking (condition 3, semantic dual task), i.e. a verbal fluency task. This task was used to test the effect of a semantic memory task on gait (Weiss *et al.*, 2003). Finally, subjects performed a motoric, control task while walking (condition 4, motoric dual task), i.e. carrying an empty tray. Subjects were asked to prioritize the secondary task during walking.

Each walk was started 150 cm in front of the carpet and continued for 150 cm beyond it in order to record steady-state locomotion. Gait parameters were recorded as the mean of the four trials within each condition. We selected eight gait parameters that have been reported to correlate with cognitive deficits (Verghese *et al.*, 2007) and/or neuroimaging aspects of SVD (de Laat *et al.*, 2010b). These parameters can be assigned to three different domains (Verghese *et al.*, 2008). Parameters assigned to the pace domain were (1) velocity (cm/s), (2) cadence (steps/min), and (3) stride length (cm). Parameters assigned to the rhythm domain were (4) double support phase (% of gait cycle when both feet simultaneously have ground contact) and (5) swing phase (% of gait cycle when one foot is in the air). Parameters assigned to the variability domain were (6) stride time variability (%), (7) stride length variability (%), and (8)

base of support variability (%). Variability was calculated as the coefficient of variation in percentage (CV = [standard deviation of parameter/mean of parameter] × 100). It represents the magnitude of stride-to-stride fluctuations within one gait parameter, with less variability suggesting higher gait automaticity and stability (Hausdorff, 2005). All gait parameters were calculated with respect to the left leg side.

For analysis, we used single task walking performance and dual task costs, i.e. the relative difference between dual task walking and single task walking (dual task costs = ([dual task walking – single task walking]/single task walking) × 100). We assessed dual task costs in order to examine performance alterations under dual task walking in relation to single task walking.

Gait parameters in the single task and dual task costs were standardized. We transformed raw data into z-scores by calculating means and standard deviations of 192 healthy controls (tested with the same gait protocol at our institution) separately for males and females in age ranges of 20-39, 40-59, and 60-79 years. We used z-scores as an intuitive measure for effect size of differences between CADASIL patients and healthy controls. Negative z-values represent worse performance compared to controls. A z-value of 0 represents no difference between CADASIL patients and controls, i.e. norm performance.

Neuropsychological assessment

The Trail Making Test (TMT) is a paper-pencil test on executive functions, specifically mental flexibility and processing speed (Kortte *et al.*, 2002). In TMT matrix A participants are asked to connect numbers presented at different locations on the sheet of paper from 1 to 25 in increasing order as quickly as possible. In TMT matrix B numbers and letters have to be connected alternately in increasing order. TMT raw test scores were transformed into age- and education-corrected z-scores based on normative data from the literature (Tombaugh, 2004). We pre-specified processing speed for cognitive function analysis, because it is the most prominently and often only affected cognitive domain in SVD (Peters *et al.*, 2005). More specifically, we used a previously established compound score of processing speed (mean z-score of TMT A and B), which has been shown to highly correlate with white matter alterations in SVD (Duering *et al.*, 2011; Zieren *et al.*, 2013; Baykara *et al.*, 2016).

Magnetic resonance imaging

MRI scans of all CADASIL patients were acquired on a single 3.0 T Magnetom Verio scanner (Siemens Healthineers, Erlangen, Germany). The MRI protocol included 1 mm isotropic 3D-

T1, 1 mm isotropic 3D fluid-attenuated inversion recovery (FLAIR), 2D-T2 and diffusion MRI sequences (30 diffusion directions; b-value 1000 s/mm^2, 2 mm isotropic). Complete details on sequence parameters have been described previously (Duering *et al.*, 2018).

The following SVD lesions were quantified according to the STRIVE consensus criteria (Wardlaw *et al.*, 2013) to enable a better interpretation of sample characteristics: WMH volume, lacune volume, and brain volume. Processing pipelines have been described previously (Duering *et al.*, 2011; Tuladhar *et al.*, 2015). All volumes were normalized for head size by the intracranial volume.

Diffusion tensor imaging

We used DTI to study the effect of white matter alterations on gait. DTI is a sensitive technique to characterize white matter microstructure by quantifying water diffusion in brain tissue (Nucifora *et al.*, 2007). In SVD, the magnitude of diffusion in brain tissue is increased (increase in mean diffusivity, MD). To extract DTI measures, we performed the following processing steps:

After visual inspection to exclude major artefacts, diffusion data were pre-processed using MRtrix v3.0 package (http://www.mrtrix.org) and the Functional Magnetic Resonance Imaging of the Brain software library (FSL), v5.0.10 (Smith *et al.*, 2004). After noise and Gibbs ringing artefacts removal using ‘dwidenoise’ (Veraart *et al.*, 2016) and ‘mrdegibbs’ (Kellner *et al.*, 2016) (MRtrix), images were corrected for subject motion and eddy-currents (‘eddy_correct’; FSL). Diffusion tensors and scalar diffusion measures were estimated using ‘dtifit’ (FSL).

We analyzed the effect of both global and regional white matter alterations on gait. As a global measure for SVD-related white matter alterations, we calculated the peak width of skeletonized mean diffusivity (PSMD) (Baykara *et al.*, 2016). PSMD is a fully automated SVD burden marker and sensitively captures global alterations in white matter integrity. We pre-specified PSMD as a marker for global white matter alterations, because it highly correlates with processing speed (Duering *et al.*, 2011; Zieren *et al.*, 2013) and outperforms other MRI based markers (such as WMH volume, lacune volume, and brain volume) in explaining clinical deficits (Baykara *et al.*, 2016). PSMD was calculated with a publicly available script (http://www.psmd-marker.com).

To analyze regional white matter alterations, we calculated voxel-wise MD values within major white matter tracts. We used the tract-based spatial statistics (TBSS) pipeline (Smith *et al.*,

2006) within FSL with standard parameters and a fractional anisotropy standard template in Montreal Neurological Institute (MNI) space, provided by FSL. Rigorous checks were performed at each step of the pipeline. Finally, a custom mask was applied to exclude regions close to cerebrospinal fluid in order to avoid partial volume effects.

Statistical Analyses

Statistical analyses were performed in R (v3.4.1) (R Core Team, 2013). Wilcoxon signed-rank tests against zero were used to examine whether z-scores (representing differences between CADASIL and healthy controls) were significantly different from zero, i.e. from norm performance. We used non-parametric testing due to presence of non-normally distributed values in patients.

The association between processing speed or global white matter alterations (assessed by PSMD) and gait performance was evaluated by multiple, linear regression models corrected for patients' leg lengths. Gait parameters were used as dependent variables. Gait parameters, processing speed scores, and PSMD values were power transformed in case of non-normal distribution. *P*-values of multiple regressions and Wilcoxon signed-rank tests against zero were Bonferroni-corrected. Statistical significance level was set at $\alpha_{corr.} < 0.05$.

Regional associations between white matter alterations (voxel-wise MD values as independent variables) and gait parameters (dependent variables) were performed using permutation test theory with a standard general linear model ('randomise'; FSL). All linear models were corrected for leg length. The number of permutations was set at 5000. Significant voxels within the skeletonized MD maps were identified using threshold-free cluster enhancement with $P < 0.05$, corrected for multiple comparisons.

Table 1. Sample characteristics

	CADASIL n = 39
Demographic characteristics	
Age, years mean (SD), [min, max]	50.0 (8.1) [32.0, 62.0]
Education, years, mean (SD)	10.8 (1.6)
Female, No. [%]	27 [69]
Cognitive scores	
TMT-A[a] median (IQR), [min, max]	-0.22 (1.31) [-8.62, 1.29]
TMT-B[a], median (IQR), [min, max]	-0.43* (2.58) [-12.66, 1.72]
Processing speed[a], median (IQR), [min, max]	-0.55** (2.06) [-10.64, 1.36]
Verbal fluency[a] median (IQR), [min, max]	0.20 (1.39) [-1.83, 2.61]
MMSE median (IQR), [min, max]	30 (1) [27, 30]
Imaging characteristics	
PSMD, 10^{-4} mm^2/s median (IQR), [min, max]	4.54 (2.32) [2.67, 9.21]
Normalized WMHV, %, median (IQR), [min, max]	4.40 (6.04) [0.09, 22.84]
Normalized LV, % median (IQR), [min, max]	0.01 (0.06) [0.00, 0.25]
BPF, median (IQR), [min, max]	0.80 (0.06) [0.70, 0.87]

[a]Age- and education-adjusted z-scores; ** $P_{corr.} < 0.01$, * $P_{corr.} < 0.05$; Wilcoxon signed-rank tests against zero. Abbreviations: BPF = brain parenchymal fraction; IQR = interquartile range; LV = lacune volume; MMSE = Mini-Mental State Examination; PSMD = peak width of skeletonized mean diffusivity; TMT = Trail Making Test; WMHV = white matter hyperintensity volume.

2.2.4 Results

Sample characteristics

Sample characteristics are provided in **Table 1**. CADASIL patients showed a high WMH lesion load (**Fig. 1**). Raw values of single task gait performance and dual task costs for CADASIL patients as well as for healthy controls are depicted in **Table 2**.

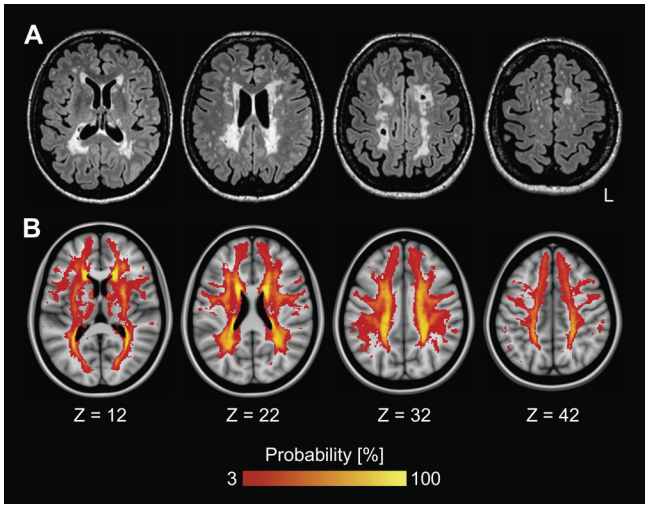

Figure 1. White matter hyperintensities in pure SVD. White matter hyperintensities on fluid-attenuated inversion recovery (FLAIR) images. (A) Subject with median lesion load. (B) Lesion frequency map superimposed onto the Montreal Neurological Institute 152 standard brain template. Abbreviation: L = left.

Table 2. Raw values of single task walking and dual task costs in CADASIL and healthy controls

	CADASIL (n=39)				Healthy controls (n=192)			
	Single task	Dual task costs			Single task	Dual task costs		
		calculatory	semantic	motoric		calculatory	semantic	motoric
Pace								
Vel[§] *cm/s*	115.0 (47.7) [56.8, 186.4]	-16.6 (24.8) [-59.3, 24.2]	-17.4 (18.4) [-61.3, 14.8]	-4.7 (19.5) [-47.6, 30.2]	113.6 (23.3) [74.5, 166.9]	-10.4 (17.2) [-39.7, 7.4]	-7.6 (16.8) [-33.0, 16.4]	3.9 (13.8) [-27.4, 27.5]
Cad[§] *steps/min*	111.5 (15.6) [82.2, 138.1]	-8.7 (13.2) [-44.1, 13.9]	-9.3 (15.8) [-41.4, 4.7]	-0.2 (11.6) [-25.6, 15.7]	112.0 (13.0) [88.0, 132.4]	-5.3 (11.1) [-42.6, 9.9]	-5.0 (13.3) [-25.3, 8.1]	4.0 (7.7) [-12.4, 17.0]
SLen[§] *cm*	127.8 (26.9) [73.0, 181.5]	-7.6 (10.5) [-38.2, 13.6]	-6.5 (12.5) [-33.7, 21.7]	-2.5 (10.9) [-30.7, 13.0]	125.0 (16.0) [98.2, 157.2]	-3.6 (10.7) [-20.4, 5.0]	0.5 (8.8) [-13.6, 9.4]	0.9 (9.2) [-18.0, 10.6]
Rhythm								
DSupp[§] *%*	24.4 (5.7) [17.6, 34.4]	14.0 (18.4) [-12.1, 77.4]	11.6 (14.5) [-4.1, 47.2]	3.3 (12.8) [-10.1, 40.2]	21.3 (8.0) [13.0, 29.1]	5.0 (13.4) [-8.6, 912.8]	4.7 (13.9) [-9.7, 45.2]	-1.9 (12.9) [-12.3, 13.6]
Swing[§] *%*	37.8 (2.4) [31.1, 40.4]	-3.7 (4.9) [-30.3, 4.7]	-2.5 (6.3) [-21.7, 3.5]	-1.3 (4.0) [-17.8, 8.1]	39.1 (5.1) [35.0, 43.8]	-1.4 (3.4) [-16.9, 5.2]	-0.2 (4.8) [-8.0, 6.2]	0.4 (4.0) [-7.4, 6.4]
Variability								
STime[§] CV *%*	2.0 (1.4) [0.6, 7.8]	54.1 (147.4) [-78.3, 1005.0]	47.9 (165.6) [-83.5, 750.0]	-5.6 (62.3) [-67.4, 231.5]	1.7 (0.9) [0.8, 5.0]	94.3 (129.8) [-65.5, 1186]	6.4 (15.1) [-8.1, 32.2]	2.6 (61.1) [-75.8, 252.8]
SLen[§] CV *%*	2.0 (1.6) [0.6, 10.7]	91.1 (93.3) [-51.1, 503.8]	81.0 (126.2) [-40.6, 425.2]	11.6 (97.6) [-63.0, 351.4]	2.3 (1.3) [0.8, 6.1]	38.1 (110.2) [-50.6, 330.3]	27.8 (71.5) [-56.5, 219.0]	-6.5 (65.3) [-69.1, 127.4]
BoS[§] CV *%*	18.5 (11.0) [6.7, 57.0]	6.1 (63.2) [-60.2, 192.3]	-0.1 (74.1) [-66.8, 410.5]	-6.2 (71.5) [-56.0, 261.5]	20.1 (14.5) [6.8, 80.6]	-13.3 (98.3) [-94.2, 261.0]	-24.8 (67.2) [-148.8, 233.3]	-7.6 (64.7) [-77.5, 152.7]

[§]Median (interquartile range) [min, max]. Abbreviations: BoS CV = base of support variability; Cad = cadence; DSupp = double support; SLen = stride length; STime CV = stride time variability; SLen CV = stride length variability; Swing = swing phase; Vel = velocity.

Moderate single task gait changes in the rhythm domain

Fig. 2 shows the gait profile of CADASIL patients during single task walking. CADASIL patients performed worse than controls in the rhythm domain, i.e. prolonged double support (z-score median = -1.00; $P_{corr.} = 2.9\times10^{-7}$) and shorter swing phase (z-score median = -0.94; $P_{corr.} = 7.4\times10^{-9}$). Of note, effect sizes were only modest with about 1 standard deviation. Other domains than the rhythm domain were not affected.

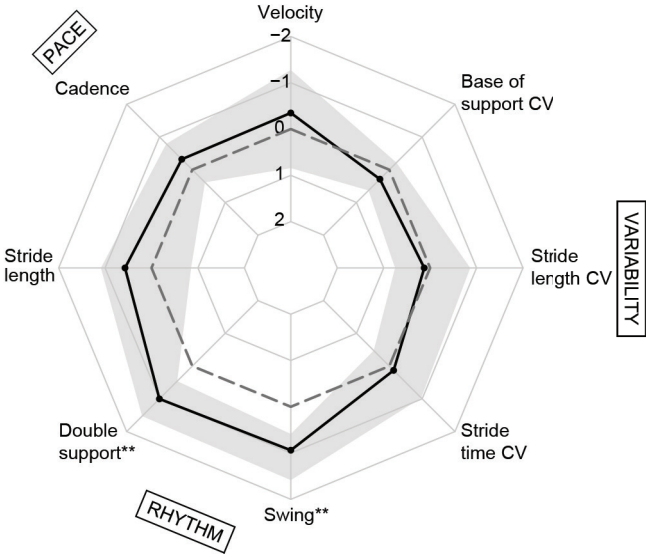

Figure 2. Single task walking. Median z-values in CADASIL (solid black line) and interquartile ranges (grey) for single task walking parameters. Negative values represent worse performance compared to healthy controls (dashed norm line). $^{**}P_{corr.} < 0.01$, Wilcoxon signed-rank tests against zero. Abbreviation: CV = coefficient of variation.

Moderate increase in semantic dual task costs in the rhythm and pace domain

The effects of the calculatory, semantic, and motoric task on gait performance were assessed by dual task costs (**Fig. 3**). Gait performance was predominantly changed by the semantic task. More specifically, semantic dual task walking pronounced deficits in the rhythm domain, which had already been affected in single task walking (i.e. prolonged double support, z-score median = -0.27; $P_{corr.} = 0.002$, and swing phase, z-score median = -0.34; $P_{corr.} = 0.005$). In addition, the semantic task uncovered deficits in the pace domain (i.e. reduced gait velocity, z-score median = -0.88; $P_{corr.} = 2.2\times10^{-5}$, cadence, z-score median = -0.46; $P_{corr.} = 0.002$, and stride length, z-score median = -0.80; $P_{corr.} = 3.3\times10^{-4}$). The calculatory task affected swing phase only (z-score median = -0.42; $P_{corr.} = 0.020$). Again, effect sizes for dual task worsening were only moderate

(less than 1 standard deviation of the performance in healthy subjects). We did not find a dual-tasking effect in the variability domain. As expected, the motoric task, which has been used as a control task, did not worsen gait.

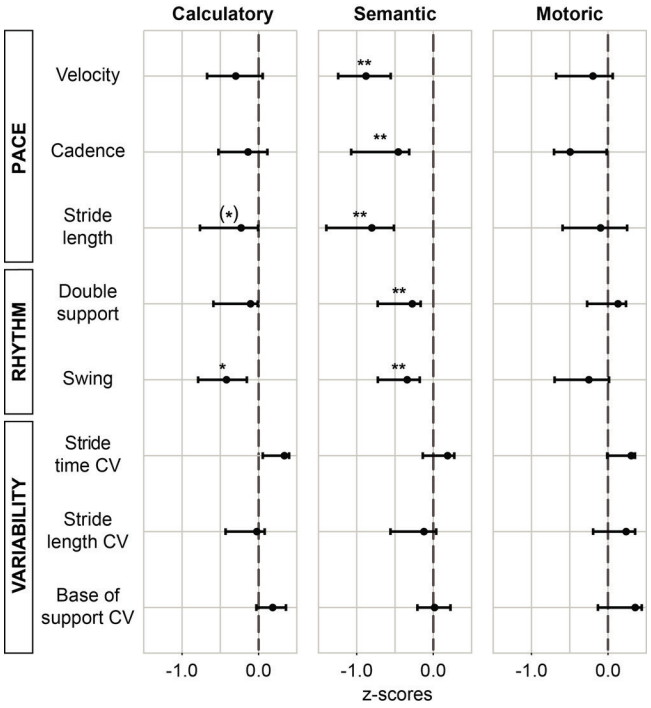

Figure 3. Dual task costs. Median z-values (dots) of different dual task costs in CADASIL patients. Negative values represent higher costs compared to healthy controls (dashed norm line). $^{**}P_{corr.} < 0.01$, $^{*}P_{corr.} < 0.05$, (*) $P_{uncorr.} < 0.05$; Wilcoxon signed-rank tests against zero; bars depict the 95% confidence interval. Abbreviation: CV = coefficient of variation.

Processing speed is not related to single task walking or dual task costs

Compared with healthy controls, CADASIL patients performed significantly worse in speed-dependent cognitive tests (**Table 1**). Also, processing speed was significantly associated with global white matter alterations (PSMD) ($\beta = -0.78$, $R^2_{adj.} = 15.3\%$; $P_{corr.} = 0.008$). To investigate whether processing speed impacts on gait performance, we examined associations with single task walking or dual task costs. There was no significant association with any single task parameter or dual task costs (all $P_{uncorr.} > 0.051$, all $P_{corr.} > 0.410$).

Global white matter alterations are associated with single task stride length

Finally, we examined the impact of SVD-related white matter alterations on gait performance. First, we assessed the relationship between global white matter alteration (PSMD) and gait parameters. In the single task, higher PSMD was associated with shorter stride length (i.e. β = -0.21, $R^2_{adj.}$ = 18.0%; $P_{corr.}$ = 0.030) (**Supplementary Table 1**). The association between PSMD and single task velocity was marginally significant (β = -0.18, $R^2_{adj.}$ = 13.7%; $P_{corr.}$ = 0.090). There was no association with any other single task parameter or dual task costs (all $P_{corr.}$ > 0.220).

Regional effects of white matter alterations (MD) on gait performance were assessed using voxel-wise regression analyses. Higher MD values in the entire white matter skeleton were associated with shorter stride length and slower gait velocity in the single task (**Fig. 4**). Infratentorial white matter regions did not show significant voxels.

Significant associations were also found for single task cadence, double support, stride time variability, and stride length variability. For these gait parameters, instead of the entire skeleton being significant, we found smaller significant clusters (**Fig. 4**). Still, for these smaller clusters there was no clear preference for specific white matter tracts, as individual clusters were distributed over the entire white matter. No significant voxels were found for swing phase or base of support. Importantly, similar to the global analysis using PSMD, no significant regional associations were found for dual task costs.

2.2.5 Discussion

We investigated the effect of pure, genetically defined SVD on gait while walking only (single task walking) and while performing a secondary cognitive or motoric task (dual task walking). We found that (1) despite severe brain lesions, single task gait performance in CADASIL patients was relatively preserved, with minor deficits only in the rhythm domain. (2) The semantic dual task aggravated gait rhythm deficits and uncovered pace deficits. (3) Cognitive impairment, i.e. processing speed deficits, in pure SVD did not worsen gait and (4) global white matter alterations affected single task stride length only.

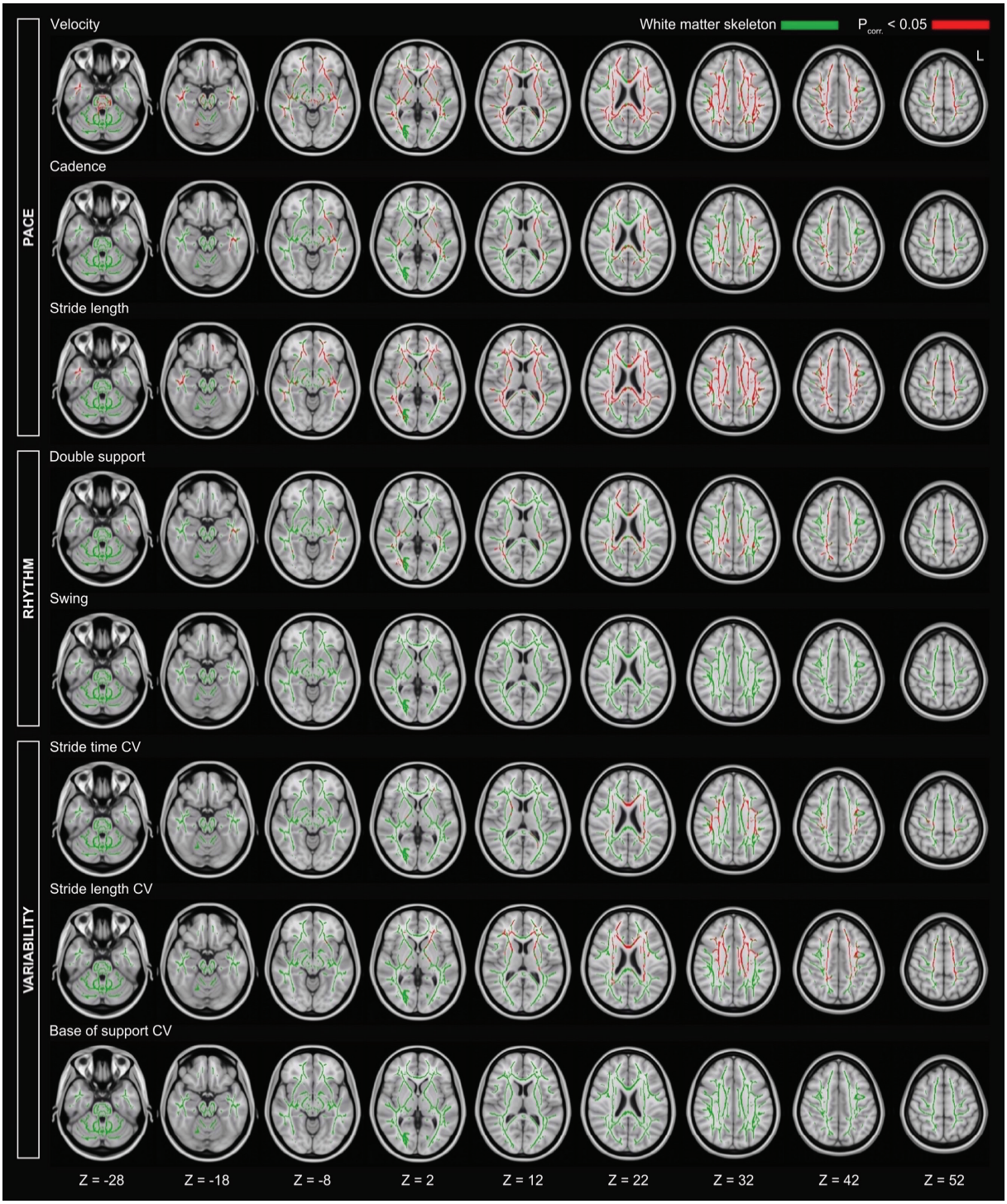

Figure 4. Voxel-wise associations between mean diffusivity (MD) and single task walking. Axial slices of the white matter skeleton (green) superimposed onto the Montreal Neurological Institute 152 standard brain template. Depicted are significant associations (red) after correction for multiple comparisons. Abbreviations: CV = coefficient of variation; L = left.

Gait performance of pure SVD patients with severe white matter alterations differed only slightly from that of healthy controls, i.e. around one standard deviation in the rhythm domain.

Dual task walking, which has been used to uncover or pronounce gait deficits (Bridenbaugh and Kressig, 2015) caused a moderate deterioration of the rhythm features and unmasked gait abnormalities in the pace domain that were not present during single task walking. Interestingly, we did not find an association between cognitive performance (i.e. processing speed) during neuropsychological testing and gait performance in our sample. Our results thus suggest that severe SVD alone and its effect on cognition might only play a minor role in causing gait impairment. In elderly, sporadic SVD patients, the combination with other age-related pathologies might be decisive for gait decline. One might speculate that joint problems, sarcopenia and reduced sensory input are therefore more promising targets for prevention and rehabilitation of gait deficits in the elderly (Bridenbaugh and Kressig, 2015). For instance, treatment for sarcopenia could include physical exercise, balance training, and protein supplementation to support muscle gain (Naseeb and Volpe, 2017).

Affected gait domains in pure SVD

Although only moderate, differences in gait performance between pure SVD patients and healthy controls were detectable in the rhythm domain while single task walking. Changes in gait rhythm indicate difficulties in keeping balance and have been shown to correlate with SVD markers, like WMH (Rosano *et al.*, 2006).

Semantic dual-tasking pronounced gait deficits in the rhythm domain and additionally uncovered deficits in the pace domain, suggesting that brain networks that control rhythm and pace are interlinked with networks for the performance of the verbal fluency task. Control of gait rhythm and pace and the semantic task seem to compete for the same cognitive resources resulting in higher dual task costs. This is in line with previous studies showing that verbal fluency dual tasks resulted in reduction in gait velocity in community-dwelling older adults (Smith *et al.*, 2015), in individuals with MCI and Alzheimer's disease dementia (Muir *et al.*, 2012).

However, we did not find an effect of single or dual task walking on the variability domain in pure SVD patients indicating steady gait performance in all conditions. Gait variability has been described as a sensitive marker of dynamic gait stability and is an established parameter in fall risk assessment (Montero-Odasso *et al.*, 2012). It seems that our sample of pure SVD patients was able to engage enough cognitive resources to compensate increasing variability from stride to stride, even while cognitive dual-tasking.

Ultimately, comparing affected gait domains or variables between studies with different cohorts is difficult, not only because of age differences and accompanying comorbidities in the study samples, but also because of the number and kind of examined gait variables (i.e. only velocity in most studies), and differences in secondary cognitive or motor tasks while walking. An agreement of standardized dual task methodologies is crucial in future research to further study gait impairment in SVD or neurodegenerative diseases.

Differential effects of secondary, cognitive tasks

In our sample of relatively young, pure SVD patients, the semantic (verbal fluency) dual task worsened gait more than the calculatory (serial 7) dual task. Some studies investigated the effect of type and complexity of the secondary tasks on dual task walking (Beauchet *et al.*, 2005; Montero-Odasso *et al.*, 2012; Muir *et al.*, 2012; Walshe *et al.*, 2015). Contrary to our results, it has been shown that the serial 7 task generates greater cognitive load than verbal fluency tasks in frail, older adults and subjects with MCI and Alzheimer's disease, resulting in worse gait performance in the calculatory than the semantic task. A possible explanation for this difference with previous studies might be the typical cognitive profile in SVD, with deficits predominantly in processing speed. Verbal fluency is a semantic memory task imposing substantial demands upon processing speed during retrieval from semantic long-term memory.

Effect of processing speed on gait

Other than expected, we did not find an association between processing speed deficits and single task gait or dual task costs in pure SVD. Cognitive deficits, beside white matter alterations, are thought to be an important factor for gait disturbances, e.g. as shown in frail older adults, individuals with MCI, and demented patients while single task and dual task walking (Theill *et al.*, 2011; Donoghue *et al.*, 2012; Martin *et al.*, 2012; Montero-Odasso *et al.*, 2012; Muir *et al.*, 2012; Doi *et al.*, 2014; Ghanavati *et al.*, 2018). Yet, the effect of cognitive impairment on single and dual task gait has not been examined specifically in SVD. Our results in pure SVD patients suggest that cognitive deficits related to SVD do not worsen gait. Of note, none of our subjects was demented and thus we cannot exclude a detrimental effect of cognition on gait in late disease stages. Generalizability of existing study results about the relation between cognition and gait is limited, as tests used to measure processing speed or executive function vary between studies. Also, examined samples are considerably older than ours and the presence of age-related pathologies was not always systematically assessed or excluded.

The possibility remains that in previous studies associations between cognitive deficits and gait were at least in part driven by age-related comorbidities.

Effect of white matter alterations on gait

Correlation analyses revealed significant associations between SVD-related white matter alterations measured by DTI and reduced stride length and marginally velocity in pure SVD patients. The same parameters were affected in sporadic SVD patients with strategic brain lesions related to gait deficits (de Laat *et al.*, 2010a; Van Der Holst *et al.*, 2018). Using the voxel-wise analysis, we found no indication for regional effects or spatial heterogeneity. Instead, we found a rather global effect of supratentorial white matter alterations on pace parameters, i.e. on stride length and velocity, but not on cadence. In line with a study by de Laat *et al.* (2010a) only few voxels with higher MD were related to a lower cadence, suggesting that the control of cadence is less affected by white matter alterations than other pace parameters. Thus, white matter alterations might predominantly influence spatial characteristics of stepping like stride length. Temporal pace maker regions in the locomotor network, such as the cerebellar locomotor regions, do not appear to be affected in pure SVD.

Limitations

Some limitations need to be considered. First, we did not obtain MRI in our control group, therefore subclinical SVD cannot be excluded in our clinically healthy sample. Second, while the examination of eight different spatio-temporal gait variables allowed precise description of gait performance, correcting for multiple comparisons is accompanied by a loss of statistical power. Third, our results are based on cross-sectional data, which does not allow to draw conclusions on causality. While our sample of pure SVD patients was relatively small, it enabled to detect subtle differences between groups and provided the unique opportunity to study the effects of pure SVD.

Conclusion

Despite severe brain lesions in genetically defined, pure SVD patients, gait performance was relatively preserved. Differences between pure SVD patients and healthy controls in single task walking and dual task costs were only moderate. Neither processing speed performance nor white matter alterations were associated with dual task costs. We speculate that other age-related morbidities affecting the brain or other relevant organ systems, such as neurodegeneration, pharmacotherapy, sarcopenia, musculoskeletal disease, or polyneuropathy

contribute towards gait impairment in elderly people. These factors should be considered in future research, as well as in new strategies for intervention and rehabilitation.

2.2.6 Acknowledgements

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2.2.7 Conflict of interest

We report no relevant conflict of interest. MD reports personal fees from Bayer Vital GmbH and from Pfizer Pharma GmbH outside the submitted work.

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2.2.9 Supplementary material

Supplementary Table 1. Linear regressions with global white matter alterations (PSMD) in the single task

Regressor **Single task**	**β**	$P_{uncorr.}$	$P_{corr.}$	$R^2_{adj.}$ **[%]**
Pace				
Vel	-0.176	0.01	0.09	13.7
Cad	-0.148	0.08	0.65	5.5
SLen	-0.207	0.00	0.03*	18.0
Rhythm				
DSupp	-0.185	0.08	0.62	5.7
Swing	-0.190	0.33	1.00	0.0
Variability				
STime CV	-0.273	0.03	0.24	9.7
SLen CV	-0.192	0.04	0.22	8.5
BoS CV	-0.027	0.84	1.00	0.0

*$P_{corr.} < 0.05$; Linear regressions corrected for leg length. Abbreviations: BoS CV = base of support variability; Cad = cadence; DSupp = double support; SLen = stride length; SLen CV = stride length variability; STime CV = stride time variability; Swing = swing phase; Vel = velocity.

3. GENERAL DISCUSSION

The current thesis aimed at, first, clarifying the contribution of SVD and AD to diffusion MRI alterations (Study 1), and, second, applying diffusion measures as SVD markers to study gait impairment in pure SVD (Study 2).

In the following sections, main findings in Study 1 and 2 and their key implications within and across studies are summarized. Directions for future research with a focus on advanced diffusion MRI are pointed out.

3.1 Main findings

This thesis comprises two articles, on the validation (Study 1) and application (Study 2) of diffusion MRI markers of SVD in gait research: Study 1: "Small vessel disease rather than Alzheimer's disease determines diffusion MRI alterations in memory clinic patients" and Study 2: "Minor gait impairment despite white matter damage in pure small vessel disease".

3.1.1 Diffusion measures as valid markers for cerebral small vessel disease

The major findings in Study 1 are that, first, diffusion alterations (free water uncorrected DTI measures and FW) were consistently associated with SVD markers, even in samples with AD as the clinically predominant disease. The effect of AD biomarkers on diffusion measures was considerably smaller and outweighed by SVD. Second, differential effects of SVD and AD markers on measures from free water imaging were not observable. Results were remarkably consistent across memory clinic samples, indicating high generalizability of the findings. Our findings validate diffusion measures as markers of SVD.

3.1.2 Minor gait impairment despite severe white matter alterations in pure cerebral small vessel disease

The major findings of Study 2 are that, first, despite severe white matter alterations, gait impairment was only mild in pure SVD patients and, second, cognitive deficits were not associated with gait performance in our sample. Our findings indicate that the clinical notion of isolated white matter alterations being a major factor of gait impairment should be reconsidered.

3.2 Key implications

The main results of Study 1 and 2 have important implications for both clinical practice and research on SVD and gait impairment.

3.2.1 The value of diffusion MRI markers of cerebral small vessel disease

Despite the wide use of diffusion measures as markers of subtle white matter alterations, their underlying pathology has so far been largely unknown. The results of Study 1 indicate that diffusion measures are more sensitive for SVD pathology than for AD pathology. This finding underlines the value of diffusion measures as markers of SVD and their superiority compared to conventional MRI markers.

Diffusion MRI markers outperform conventional MRI markers of SVD, such as WMHs and lacunes, (see section 1.2) for several reasons. First, diffusion MRI markers provide a more detailed evaluation of the underlying white matter changes by detecting even subtle and gradual alterations invisible on conventional MRI. In contrast, conventional MRI markers typically coarsely dichotomize tissue into 'normal' and 'abnormal' (ter Telgte *et al.*, 2018). Second, diffusion MRI markers show higher associations with SVD-related clinical deficits, such as reduced processing speed, than conventional MRI markers (Tuladhar *et al.*, 2015; Baykara *et al.*, 2016). Third, in contrast to conventional MRI markers, diffusion markers are robust and can be calculated fully automated (Konieczny *et al.*, 2020). Therefore, diffusion measures should be used in studies to characterize SVD burden.

The results of Study 1 imply that clinicians should always consider SVD as the origin of diffusion alterations in patients with mixed disease. Being aware of the underlying pathology of diffusion alterations may improve diagnosis, disease monitoring, prognosis, and may allow the application of potential therapeutic interventions to reduce dementia incidence, for instance, through control of vascular risk factors (Satizabal *et al.*, 2016; Iadecola *et al.*, 2019). Similarly, researchers using diffusion MRI markers may now draw conclusions not only on the effects of white matter alterations in general, but also on the vascular rather than neurodegenerative etiology. More specifically, studies investigating associations between diffusion MRI markers and clinical deficits may shed more light on the relation between SVD-related white matter alterations and symptoms, as e.g. shown in Study 2 on gait impairment in SVD. In clinical trials, researchers may now effectively stratify populations according to SVD burden based on diffusion MRI markers.

3.2.2 The need to account for multiple age-related pathologies when studying gait impairment in the elderly

Results from Study 2 in pure SVD patients (CADASIL) suggest that SVD-related white matter alterations, in the absence of comorbidities, may not be as important for gait decline as previously thought (de Laat *et al.*, 2010; Rosario *et al.*, 2016; Pinter *et al.*, 2017; Loos *et al.*, 2018; Van Der Holst *et al.*, 2018). Instead, other age-related pathologies on the brain, or on relevant organ systems, independent of SVD, may be crucial for gait disturbances in the elderly. For instance, sarcopenia is considered a key factor for the reduction of physical performance including reduced gait speed (Cruz-Jentoft *et al.*, 2019; Keller, 2019). Despite being very common in the elderly, sarcopenia is frequently underreported and underdiagnosed (Keller, 2019). Furthermore, several studies show that polyneuropathy affects locomotion (Erdmann *et al.*, 2007; Hoffman *et al.*, 2015; Hanewinckel *et al.*, 2016). In a large population-based sample, elderly subjects who were diagnosed with definite or probable polyneuropathy differed around one standard deviation in global gait performance, i.e. average across several gait parameters, from subjects without polyneuropathy (Hanewinckel *et al.*, 2016). Participants with definite polyneuropathy were more likely to fall resulting in injury. Importantly, similar to sarcopenia, polyneuropathy is often overlooked. About half of the participants with polyneuropathy were newly diagnosed in the aforementioned study (Hanewinckel *et al.*, 2016). Thus, undetected comorbidities may affect gait in sporadic SVD.

Complementary, the interaction between SVD and age-related comorbidities may be decisive for gait performance. Gait disturbances related to infratentorial or peripheral constraints, e.g. sarcopenia or polyneuropathy, may not manifest, if intact white matter networks allow for supratentorial compensation (Schmid *et al.*, 2013). Yet, this compensation mechanism may be disrupted by SVD or neurodegenerative disease resulting in pathologic gait. This supratentorial compensation theory would explain why other studies found an effect of SVD on gait performance in elderly subjects, while we observed no such effect in pure SVD, in the absence of comorbidities. Gait impairment in sporadic SVD patients may be due to disrupted compensation of comorbidities and not primary due to SVD-related white matter alterations.

Taken together, studies indicate that aging affects the integrity of the central and peripheral nervous system, which is required for normal gait. Therefore, researchers should be aware of multiple confounding factors of aging and their possible interaction with SVD when studying gait impairment in the elderly.

3.3 Future directions

Future studies on diffusion MRI markers and gait impairment may focus on methodological improvements as suggested in the following sections.

3.3.1 Advanced diffusion MRI

Diffusion MRI markers of SVD may be further improved by more elaborated MRI acquisition, such as multi-shell diffusion imaging, i.e. using more than one diffusion weight, and more advanced diffusion modelling than DTI or free water imaging (Nir *et al.*, 2019). Advanced diffusion models include e.g. diffusion kurtosis imaging (DKI) and neurite orientation dispersion and density imaging (NODDI). DKI quantifies the deviation of diffusion processes from normal distribution. Less normally distributed diffusion indicates more complex white matter structure and thus higher tissue integrity. NODDI is a three-compartment model, similar to the free water model, but includes compartments of restricted extracellular and intracellular water, besides the free water compartment. Only recently, our group has shown that measures from multi-shell DKI and NODDI outperformed DTI measures in explaining cognitive deficits in SVD (Konieczny *et al.*, 2020). Importantly, these advanced diffusion measures are thought to be more sensitive to earliest subtle white matter alterations than simple DTI measures facilitating the detection of the clinically highly relevant group of early stage SVD patients (Konieczny *et al.*, 2020). It should be noted that increasing model complexity requires higher computational resources than simple DTI measures. Nevertheless, future studies could investigate the effect of SVD and AD on advanced diffusion measures and explore differential effects on the diffusion signal.

As described above, diffusion measures are common MRI markers for focal white matter alterations in the brain tissue. However, diffusion-based measures can also be obtained within large-scale structural brain networks, which may better capture disease burden than focal markers. Diffusion-based measures reflect brain network integrity, i.e. integrity of white matter tracts connecting different brain areas. In order to calculate network markers, first structural networks need to be constructed using tractography based on DTI or more elaborated methods such as constrained spherical deconvolution (Tournier *et al.*, 2004; Jeurissen *et al.*, 2014). Once networks are constructed, graph analysis allows for quantification of network properties (Hagmann *et al.*, 2007). Graph analysis conceptualizes the brain as a network, consisting of nodes (brain regions) and edges (connections between brain regions). From nodes and edges, various network measures can be calculated. For instance, several studies in SVD patients

reported decreased network efficiency, i.e. less efficient parallel information transfer in the whole network, and a decrease in the number of connections and strength of connectivity (Lawrence *et al.*, 2014; Tang *et al.*, 2015; Tuladhar *et al.*, 2016; Tuladhar *et al.*, 2017). Furthermore, a recent study reported that disorganizations of highly interconnected regions within structural networks contribute to cognitive impairment in SVD (Tuladhar *et al.*, 2017). To date, diffusion-based measures of structural network integrity are not specific for SVD, but may also be related to network alterations in e.g. AD (Reijmer *et al.*, 2015). Therefore, future studies should further investigate measures from structural network connectivity as markers for SVD.

3.3.2 New strategies in studying gait

Results from gait studies in the elderly can barely be compared, not only due to deficits in accounting for age-related instability and comorbidities, but also due to wide variations in gait protocols and gait analysis. This lack of consistency hampers progress in understanding the mechanisms of gait impairment and possible intervention strategies. Therefore, future studies should include comprehensive clinical examinations (identifying co-pathologies), agree on the kind of gait assessment (automatized and/or observational), specify gait parameters (quantitative and/or qualitative), and harmonize possible dual-task protocols (type of dual-task, control condition). Also, statistical analyses should include confounding factors of aging. Standards in gait assessment may facilitate comparability and interpretation of results.

Our results on gait performance in pure SVD patients challenge the view that isolated white matter alterations in pure SVD, in the absence of comorbidities, are a major cause of gait impairment. By studying relatively young CADASIL patients, we were able to minimize confounding factors associated with aging and comorbidities. However, the effect of SVD on gait performance should be confirmed in younger sporadic SVD patients (less than 60 years). Furthermore, future studies should investigate possible interactions between SVD and age-related comorbidities and explore whether SVD disrupts supratentorial compensation of e.g. sarcopenia (see 3.2.2 for the supratentorial compensation theory).

3.4 Conclusion

Diffusion measures (free water uncorrected DTI measures and FW) are valid markers for SVD-related white matter alterations. Advanced diffusion MRI and diffusion-based network analysis may further improve sensitivity and accuracy of markers for SVD.

Diffusion MRI markers provide new insight into the effect of SVD burden on gait impairment: Severe white matter alterations as assessed by diffusion MRI markers had only mild effect on gait performance in pure SVD, in the absence of comorbidities. Harmonization of gait studies may further clarify the role of SVD in gait impairment.

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AFFIDAVIT

I hereby confirm that the dissertation “Diffusion Imaging Markers of Cerebral Small Vessel Disease: Validation and Application” is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation “Diffusion Imaging Markers of Cerebral Small Vessel Disease: Validation and Application” selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Munich, April 7, 2020 Sofia Finsterwalder

DECLARATION OF AUTHOR CONTRIBUTIONS

Study 1: Small vessel disease rather than Alzheimer's disease determines diffusion MRI alterations in memory clinic patients

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S.F. and N.V. shared first authorship. S.F., N.V., and R.S., G.J.B., M.Du. contributed to the study concept and design. S.F., N.V., B.G., M.A.A.C., N.A.W., N.F., M.K.G., M.J.K., H.L.K., M.E., J.L., O.P., M.Di., G.J.B., and M.Du. contributed to the acquisition and analysis or interpretation of the data. C.M.K., N.R.G-R., S.S., H.O., R.F.A., and J.P.C. contributed to acquisition and analysis of the DIAN data. F.J., E.D., L.D., C.M., O.P., E.I.I., J.P., E.J.S., A.Sc., K.F., K.B., D.J., S.J.T., I.K., C.L., M.B., M.T.H., F.B., A.Sp., N.R., B.E-W., and K.S. contributed to acquisition and analysis of the DELCODE data. S.W.S., Y.K., D.L.N., H.J.K., and H.J. contributed to acquisition and analysis of the SVCI data. S.F., N.V., and M.Du. contributed to drafting the text and preparing the figures and tables.

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Study 2: Minor gait impairment despite white matter damage in pure small vessel disease

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S.F. is the first author of this study. S.F. contributed to the design and execution of data analysis, wrote the first draft, and prepared figures. M.W. contributed to the conception of the research project, the preparation of statistical analysis, and revised the manuscript. B.G. contributed to data analysis, preparation of figures, and revised the manuscript. A.D. contributed to the execution of data analysis. M.J.K. contributed to imaging data analysis and revised the manuscript. Re.S. contributed to the interpretation of the data and revised the manuscript. Ro.S. contributed to the conception, organization and execution of the research project, and revised the manuscript. M.D. contributed to the conception, organization, and execution of the research project, conceptualized statistical analysis, and revised the manuscript.

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