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

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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.

Keywords: Alzheimer's disease; cerebral small vessel disease; diffusion tensor imaging; free water imaging; white matter; biomarker

Abbreviations

AD	Alzheimer's disease
Aβ	amyloid-beta
DTI	diffusion tensor imaging
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
PET	positron emission tomography
P-tau	phosphorylated- tau_{181}
SUVR	standardised uptake value ratio
SVD	cerebral small vessel disease
T-tau	total tau
WMH	white matter hyperintensity

1. Introduction

Alzheimer's disease (AD) and cerebral small vessel disease (SVD) are the two leading causes of cognitive decline and dementia.[1] Altered white matter microstructure is considered a key finding in both conditions[2,3] and has consistently been associated with cognitive deficits.[4-6] 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.[7,8] 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), which can be detected both globally and regionally.[4,5] 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.

In memory clinic patients, AD and SVD often co-exist.[9] The extent to which each of these conditions contribute to diffusion MRI alterations is largely elusive. 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 MRI alterations.[10] As such, free water imaging might be able to disentangle the effects of AD and SVD.[11-14] Previous studies using DTI or free water imaging were limited by the lack of biomarker evidence of AD pathology or insufficient consideration of mixed pathology. Assessing the individual contributions of AD and SVD towards diffusion MRI alterations requires a systematic study covering the entire spectrum of "pure AD", mixed disease, and "pure SVD".

The uncertainty regarding the origin and interpretation of diffusion alterations in memory clinic patients impedes widespread implementation in research and clinical practice. Therefore, the aim of this study was to determine the effect of AD and SVD on diffusion MRI

in a memory clinic setting. We examined associations between biomarkers of AD, MRI markers of SVD, and diffusion measures from both conventional DTI and free water imaging. Six study samples (N=365 participants) were included to systematically cover the entire spectrum of AD, mixed disease, and SVD, and to account for both cerebrospinal fluid (CSF) and positron emission tomography (PET) markers. In addition to the common memory clinic setting with predominantly mixed disease, our analysis also included patient samples with pure, genetically-defined AD or SVD. This enabled us to examine effects of both diseases on diffusion measures without confounding pathology. Analyses were performed separately within each sample in order to validate results and address generalizability using the six independently recruited samples.

2. Methods

2.1 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 in 2.1.1-2.1.3. 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.

2.1.1 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$_{1-40}$ (Aβ 40), amyloid-beta$_{1-42}$ (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.[15]

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).

2.1.2 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.[16]

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.[17,18]

2.1.3 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).[19] 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.[5,20]

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.[4] 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.

2.2 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,[5] DELCODE,[21] ADNI,[22] UVCI,[23] SVCI,[17] and CADASIL.[11] 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.

2.3 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,[5] DELCODE,[15] ADNI (http://adni.loni.usc.edu), UVCI,[24] and SVCI.[18] 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.

2.4 Small vessel disease markers

We used an established total SVD score (ordinal variable)[25] 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.[25] Two trained raters (SF, NV) assessed these lesions according to the STRIVE consensus criteria:[2] WMHs were rated using the Fazekas scale,[26] 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.[27]

WMH volume was calculated from a previously described semi-automated segmentation pipeline.[4]

2.5 Statistical analyses

All statistical analyses were performed in R (version 3.5.1).[28] The statistical significance level was set at $\alpha < 0.05$.

Associations between AD biomarkers, SVD markers, age, 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).

To perform multivariable analysis in the presence of multicollinearity (i.e. intercorrelations among disease markers, **Supplementary Fig. 1**), we used random forest regressions (R package 'party'; version 1.3-2).[29] 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.[11] 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.[30] To address this possibility, we performed a post-hoc mediation analysis (R package 'lavaan'; version 0.6-4)[31] 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,[32] 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,[33] 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 2D optimization and $P < 0.05$, corrected for multiple comparisons.

3. Results

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

3.1 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 3.3).

3.2 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 and SVD marker 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).

3.3 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. As a further indication for the presence of cerebral amyloid angiopathy, most (8 out of 9) DIAN participants with cerebral microbleeds showed a strictly lobar distribution, and one participant had disseminated cortical superficial siderosis.

In DELCODE, where simple regression analysis showed only weak effects of Aβ 42, none of the mediation analyses were significant (all $P > 0.136$).

3.4 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.[32] We addressed this aspect in a sensitivity analysis restricted to amyloid-positive individuals (Supplementary Tables 6-8, Supplementary Fig. 2). Simple linear regressions between tau and diffusion measures in amyloid-positive individuals were not significant, except for DIAN (n=46; p-tau and MDu, β_s=0.32, $R^2_{adj.}$=0.08, *P*=0.031; p-tau and FW, β_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,

β_s=0.35, $R^2_{adj.}$=0.10, *P*=0.017; WMH volume and FW, β_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 $P > 0.051$).

3.5 Regional tau is not associated with diffusion alterations

Tau is a localized pathology starting in the entorhinal cortex[33] and previous literature suggests localized effects of tau on white matter microstructure.[32,34,35] 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.

4. 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, diffusion MRI alterations are largely determined by SVD. Results were consistent across all memory clinic samples, illustrating the robustness of our findings. Our study facilitates the interpretation of diffusion MRI alterations and the development towards clinical application.

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.[30] Since the DIAN sample also included asymptomatic mutation carriers up to 15 years before estimated symptom onset, another explanation is that the association between Aβ and diffusion measures is strongest in early, preclinical AD. This view is supported by a recent study demonstrating an association

between Aβ and diffusion measures over the adult lifespan in cognitively healthy participants.[36] Overall, we conclude that while the effect of AD on diffusion measures is apparent in DIAN patients with pure and early AD, the presence of SVD in the memory clinic 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,[13,14,32,34,37-39] although some studies found no association.[40,41] Importantly, however, only one of these studies accounted for SVD. Hence, the effect of AD on diffusion alterations might have been overestimated. Only Strain and colleagues[34] 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, as well as for the interpretation of diffusion MRI alterations in clinical routine.

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,[32] 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.[42,43] 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.

By design, our memory clinic samples were heterogeneous, which in our view accurately reflects a real-life memory clinic setting. To study pure forms of AD and SVD, we included genetically defined samples. Furthermore, the sensitivity analysis in subgroups with amyloid-positive individuals allowed to study memory clinic patients who met the biological definition of AD. Although statistical power was reduced, the strong effect of SVD on diffusion measures was also confirmed in these subgroups.

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.[44] 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.[11] 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[12] and showed Aβ-related longitudinal changes.[14] 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.[12,14] Furthermore, multi-shell diffusion data, which would be necessary for more complex parametrization of the fluid compartments,[45-47] was not available in the study samples. This would have allowed to control for the effects of capillary blood flow (intravoxel incoherent motion) in the free water estimation.[47]

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.[48] 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. While we also included voxel-based analyses to identify regional associations, our study mostly focused on global, whole-brain averages of diffusion measures. Thus, we cannot exclude that analyses in specific subregions will yield different results. Because of limitations in the diffusion MRI acquisition protocols (no reversed phase-encoding, directions not sampled on entire sphere), we were not able to correct for susceptibility-induced distortions or to employ a more modern approach for correction of eddy current-induced distortions, motion, and outlier slices.[49] Finally, the lack of pathological confirmation of the presence and extent of AD and SVD pathology originates from the paucity of autopsy studies with high quality, standardized antemortem diffusion MRI.

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 indicate that our results might be 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 the effect of SVD on diffusion alterations largely exceeds the effect of AD. Our systematic analysis contributes to the interpretation of diffusion MRI in memory clinic patients and further advances its application in clinical practice. We 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.[50] 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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Declarations of Interest

None.

Supplementary Material

Supplementary Table 1. Diagnostic criteria in memory clinic samples

Supplementary Table 2. Diffusion parameters

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

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

Supplementary Table 5. Simple regression models in genetically-defined samples

Supplementary Table 6. Simple regression models in Alzheimer's disease focused samples in amyloid-positive individuals

Supplementary Table 7. Simple regression models in small vessel disease focused samples in amyloid-positive individuals

Supplementary Table 8. Simple regression models in genetically-defined samples in amyloid-positive individuals

Supplementary Table 9. DIAN consortium

Supplementary Table 10. DELCODE study group

Supplementary Figure 1. Correlation matrices

Supplementary Figure 2. Simple regression analyses in amyloid positive individuals.

Supplementary Text 1. CSF and PET markers

Supplementary Text 2. Processing of diffusion measures

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Figure legends

Figure 1. Study concept and participant selection flowchart. Samples cover the entire spectrum of AD, mixed disease, and SVD.

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.

Figure 2. Simple regression analyses. Simple linear regression analyses between diffusion measures and AD biomarkers or SVD markers. Standardized β is represented by color.

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.

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.

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.

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;
AD, Alzheimer's disease; CDR, clinical dementia rating; DTI, diffusion tensor imaging; FAu, uncorrected fractional anisotropy; 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 1

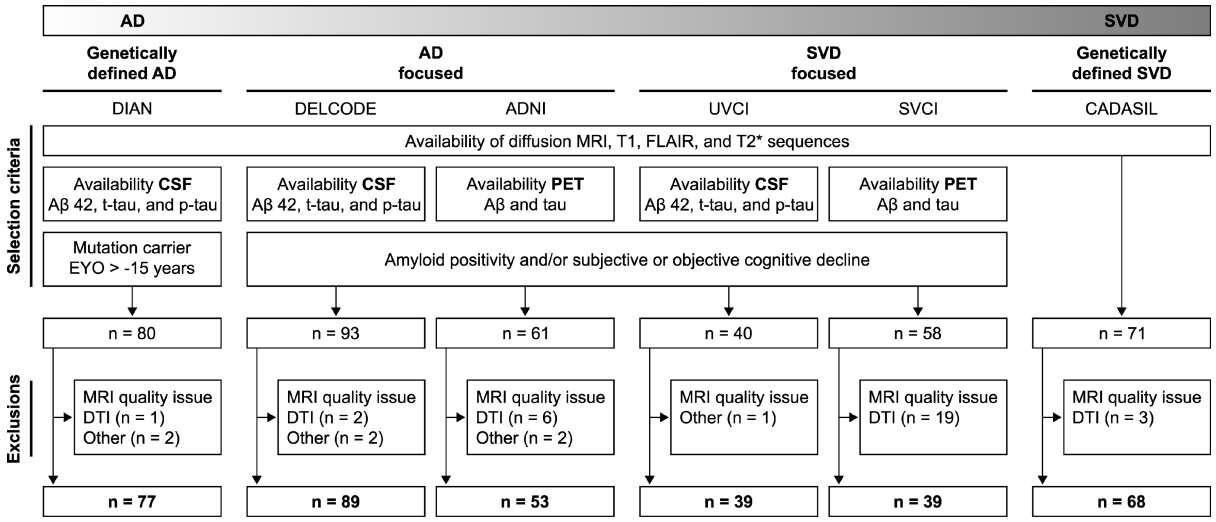

AD
SVD
Genetically defined AD
AD focused
SVD focused
Genetically defined SVD
DIAN
DELCODE
ADNI
UVCI
SVCI
CADASIL
Selection criteria
Availability of diffusion MRI, T1, FLAIR, and T2* sequences
Availability CSF Aβ 42, t-tau, and p-tau
Availability CSF Aβ 42, t-tau, and p-tau
Availability PET Aβ and tau
Availability CSF Aβ 42, t-tau, and p-tau
Availability PET Aβ and tau
Mutation carrier EYO > -15 years
Amyloid positivity and/or subjective or objective cognitive decline
n = 80
n = 93
n = 61
n = 40
n = 58
n = 71
Exclusions
MRI quality issue DTI (n = 1) Other (n = 2)
MRI quality issue DTI (n = 2) Other (n = 2)
MRI quality issue DTI (n = 6) Other (n = 2)
MRI quality issue Other (n = 1)
MRI quality issue DTI (n = 19)
MRI quality issue DTI (n = 3)
n = 77
n = 89
n = 53
n = 39
n = 39
n = 68

Figure 2

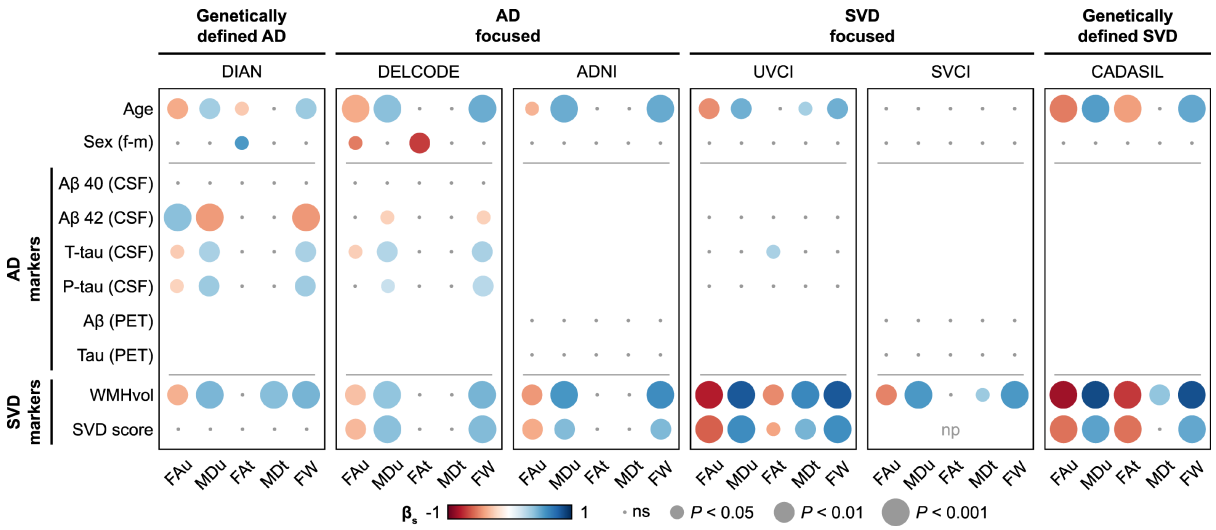
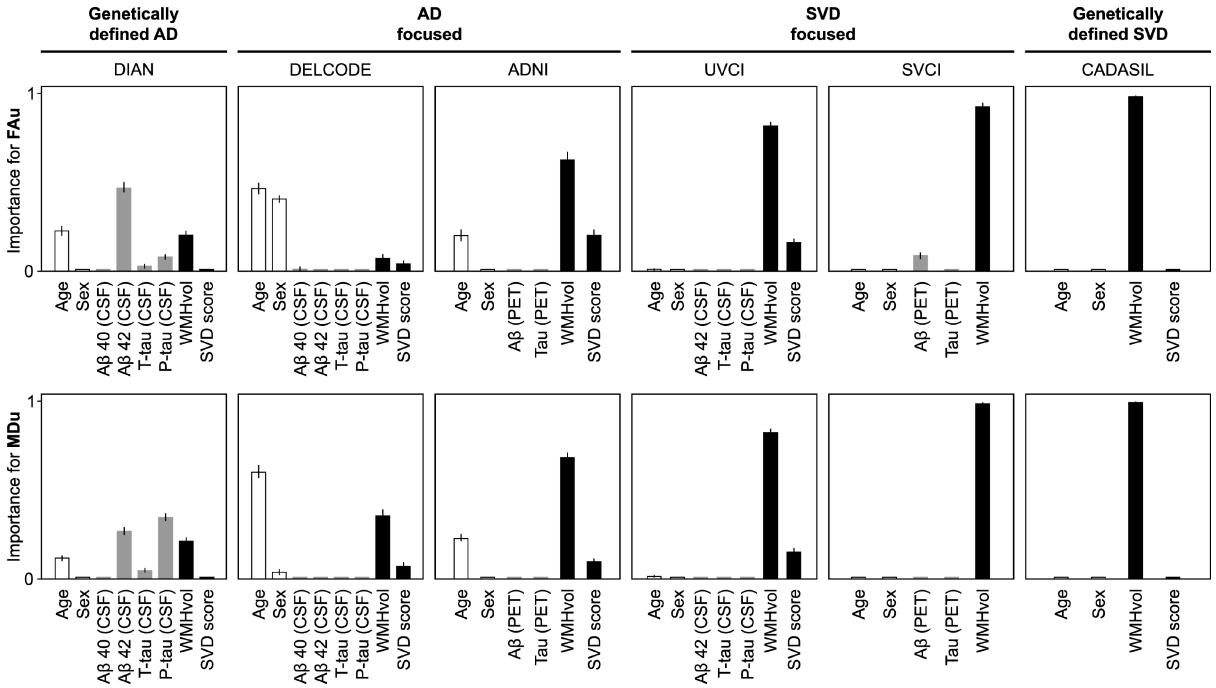

Genetically defined AD
AD focused
SVD focused
Genetically defined SVD
DIAN
DELCODE
ADNI
UVCI
SVCI
CADASIL
Age
Sex (f-m)
Aβ 40 (CSF)
Aβ 42 (CSF)
T-tau (CSF)
P-tau (CSF)
Aβ (PET)
Tau (PET)
WMHvol
SVD score
AD markers
SVD markers
np
FAu MDu FAt MDt FW
β_s -1 1
ns
P < 0.05
P < 0.01
P < 0.001

Figure 3


Genetically defined AD
AD focused
SVD focused
Genetically defined SVD
DIAN
DELCODE
ADNI
UVCI
SVCI
CADASIL
Importance for FAu
Importance for MDu
1
0
Age
Sex
Aβ 40 (CSF)
Aβ 42 (CSF)
T-tau (CSF)
P-tau (CSF)
Aβ 42 (CSF)
Aβ (PET)
Tau (PET)
WMHvol
SVD score

Highlights

- Large-scale, systematic analysis of diffusion MRI in a memory clinic setting
- Strong effect of small vessel disease on diffusion MRI
- Measures from diffusion tensor imaging and free water imaging
- Consistent results across multiple memory clinic samples
- Regional tau PET is not associated with diffusion MRI alterations

Research in context

1. Systematic review: Diffusion MRI is widely used to assess white matter microstructure in both Alzheimer's disease (AD) and small vessel disease (SVD). Although AD and SVD frequently co-occur, the vast majority of studies did not consider mixed disease and the individual contributions of these conditions to diffusion MRI alterations have not yet been investigated systematically (as reviewed using Pubmed).

2. Interpretation: SVD more than AD determines diffusion alterations in a memory clinic setting, even in samples where AD was the clinically predominant disease. Our results validate diffusion measures as markers for SVD.

3. Future directions: Future studies and clinical applications of diffusion MRI need to consider the strong effect of SVD. A more complex parameterization of the fluid compartments, e.g. by neurite orientation dispersion and density imaging or a multi-shell model for free water imaging, may further increase the sensitivity in earlier or even asymptomatic stages of SVD.

Supplementary Table 1. Diagnostic criteria in memory clinic samples

	AD focused		SVD focused	
	DELCODE[a]	ADNI, phase 3[b]	UVCI[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

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[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 neurology. 2018;75(8):999-1007.

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 Alzheimer's disease; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease 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

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. 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; 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. 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); 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 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. 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; 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. Simple regression models in Alzheimer's disease focused samples in amyloid-positive individuals

	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=44)															
Age	-0.44	0.17	**0.003**	0.43	0.17	**0.003**	-0.31	0.08	**0.040**	0.18	0.01	0.246	0.49	0.22	**0.001**
Sex (f-m)	-0.88	0.18	**0.003**	0.48	0.04	0.114	-0.99	0.23	**0.001**	0.03	-0.02	0.929	0.64	0.08	**0.033**
Aβ 40 (CSF)	-0.03	-0.02	0.827	0.04	-0.02	0.784	-0.01	-0.02	0.966	0.06	-0.02	0.716	0.06	-0.02	0.722
Aβ 42 (CSF)	0.04	-0.02	0.813	0.00	-0.02	0.998	0.00	-0.02	0.981	0.06	-0.02	0.719	-0.05	-0.02	0.742
T-tau (CSF)	-0.17	0.00	0.277	0.15	0.00	0.323	-0.16	0.00	0.309	0.07	-0.02	0.641	0.18	0.01	0.234
P-tau (CSF)	-0.16	0.00	0.307	0.15	0.00	0.319	-0.10	-0.01	0.504	0.08	-0.02	0.608	0.20	0.02	0.203
WMHvol	-0.28	0.05	0.070	0.35	0.11	**0.018**	-0.09	-0.01	0.546	0.13	-0.01	0.406	0.40	0.14	**0.007**
SVD score	-0.26	0.05	0.085	0.32	0.08	**0.033**	-0.17	0.00	0.279	0.13	-0.01	0.384	0.34	0.10	**0.022**
ADNI (n=37)															
Age	-0.35	0.10	**0.032**	0.54	0.27	**0.001**	0.12	-0.01	0.485	0.03	-0.03	0.853	0.53	0.26	**0.001**
Sex (f-m)	0.10	-0.03	0.778	0.24	-0.01	0.483	0.57	0.05	0.088	0.12	-0.02	0.716	0.18	-0.02	0.588
Aβ (PET)	-0.09	-0.02	0.614	0.12	-0.01	0.495	0.05	-0.03	0.782	0.10	-0.02	0.566	0.12	-0.01	0.492
Tau (PET)	-0.06	-0.02	0.708	0.01	-0.03	0.935	-0.19	0.01	0.271	0.24	0.03	0.156	-0.04	-0.03	0.807
WMHvol	-0.49	0.22	**0.002**	0.58	0.32	**0.000**	-0.01	-0.03	0.946	0.09	-0.02	0.612	0.63	0.38	**0.000**
SVD score	-0.30	0.06	0.074	0.43	0.17	**0.007**	0.07	-0.02	0.663	0.29	0.06	0.080	0.42	0.15	**0.010**

$P < 0.05$ in bold. 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; 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 7. Simple regression models in small vessel disease focused samples in amyloid-positive individuals

	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=22)															
Age	-0.33	0.07	0.129	0.35	0.08	0.105	-0.23	0.01	0.305	0.24	0.01	0.281	0.37	0.09	0.091
Sex (f-m)	0.34	0.07	0.121	-0.33	0.07	0.130	0.28	0.03	0.205	-0.42	0.13	0.054	-0.36	0.09	0.101
Aβ 42 (CSF)	-0.05	-0.05	0.827	-0.05	-0.05	0.833	-0.13	-0.03	0.568	0.01	-0.05	0.954	-0.04	-0.05	0.870
T-tau (CSF)	0.32	0.06	0.151	-0.24	0.01	0.292	0.31	0.05	0.159	-0.09	-0.04	0.687	-0.22	0.00	0.336
P-tau (CSF)	0.31	0.05	0.163	-0.24	0.01	0.278	0.28	0.03	0.205	0.01	-0.05	0.981	-0.23	0.00	0.310
WMHvol	-0.73	0.51	**0.000**	0.79	0.61	**0.000**	-0.45	0.16	**0.036**	0.54	0.26	**0.010**	0.81	0.63	**0.000**
SVD score	-0.47	0.18	**0.028**	0.51	0.22	**0.016**	-0.29	0.04	0.191	0.42	0.14	0.051	0.50	0.22	**0.017**
SVCI (n=19)															
Age	-0.18	-0.02	0.456	0.16	-0.03	0.522	-0.11	-0.05	0.646	0.18	-0.02	0.454	0.17	-0.03	0.482
Sex (f-m)	0.66	0.01	0.305	-0.50	-0.02	0.441	0.78	0.03	0.227	0.55	-0.01	0.395	-0.57	-0.01	0.383
Aβ (PET)	-0.11	-0.05	0.657	0.20	-0.02	0.409	0.11	-0.05	0.664	0.30	0.04	0.215	0.19	-0.02	0.433
Tau (PET)	0.02	-0.06	0.925	-0.04	-0.06	0.870	0.03	-0.06	0.898	0.16	-0.03	0.513	-0.05	-0.06	0.853
WMHvol	-0.54	0.25	**0.016**	0.58	0.30	**0.009**	-0.43	0.14	0.065	0.44	0.14	0.063	0.57	0.28	**0.011**
SVD score	np	np	np	np	np	np	np	np	np	np	np	np	np	np	np

P < 0.05 in bold. 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); 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 8. Simple regression models in genetically-defined samples in amyloid-positive individuals

	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=46)															
Age	-0.10	-0.01	0.495	0.08	-0.02	0.579	-0.11	-0.01	0.483	0.07	-0.02	0.620	0.09	-0.02	0.571
Sex (f-m)	0.32	0.00	0.284	-0.09	-0.02	0.768	0.71	0.11	**0.015**	0.37	0.01	0.212	-0.09	-0.02	0.769
Aβ 40 (CSF)	0.01	-0.02	0.940	-0.01	-0.02	0.966	0.04	-0.02	0.806	-0.04	-0.02	0.808	0.00	-0.02	0.998
Aβ 42 (CSF)	0.27	0.05	0.074	-0.23	0.03	0.127	0.22	0.02	0.150	-0.20	0.02	0.183	-0.24	0.04	0.111
T-tau (CSF)	-0.24	0.03	0.114	0.26	0.04	0.086	-0.16	0.00	0.284	0.05	-0.02	0.730	0.24	0.04	0.102
P-tau (CSF)	-0.25	0.04	0.090	0.32	0.08	**0.031**	-0.08	-0.02	0.609	0.13	-0.01	0.401	0.31	0.07	**0.038**
WMHvol	-0.28	0.06	0.056	0.35	0.10	**0.017**	0.02	-0.02	0.919	0.39	0.13	**0.008**	0.37	0.12	**0.011**
SVD score	-0.03	-0.02	0.861	0.03	-0.02	0.854	0.00	-0.02	0.985	0.13	-0.01	0.405	0.04	-0.02	0.810
CADASIL															
Age	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
Sex (f-m)	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
WMHvol	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
SVD score	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na

P < 0.05 in bold. 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; na, not available; 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 9. 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
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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
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Hornbeck	Russ	Washington University in St. Louis School of Medicine
Houeland DiBari	Siri	German Center for Neurodegenerative Diseases (DZNE) Munich
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Kasuga	Kensaku	Niigata University
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Laske	Christoph	German Center for Neurodegnerative Diseases (DZNE) Tubingen
Lee	Jae-Hong	Asan Medical Center
Levin	Johannes	German Center for Neurodegnerative Diseases (DZNE) Munich
Martins	Ralph	Edith Cowan University
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Supplementary Table 10. DELCODE study group

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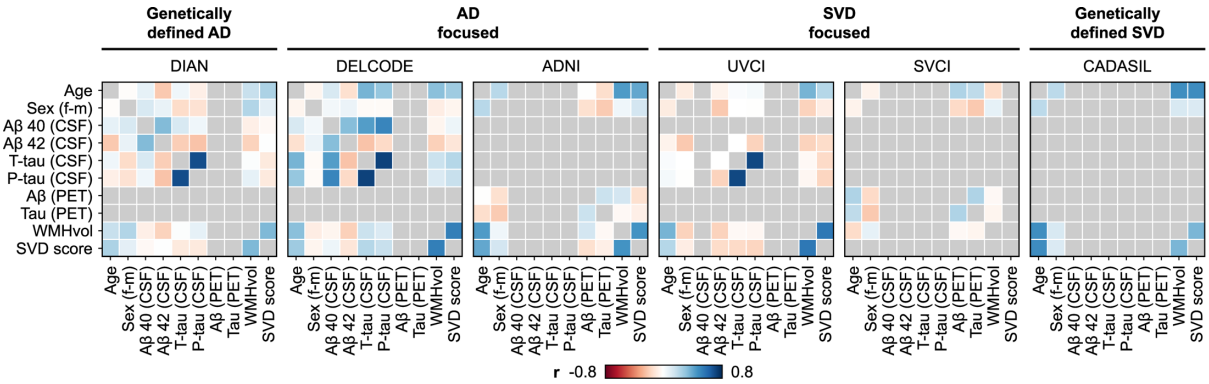
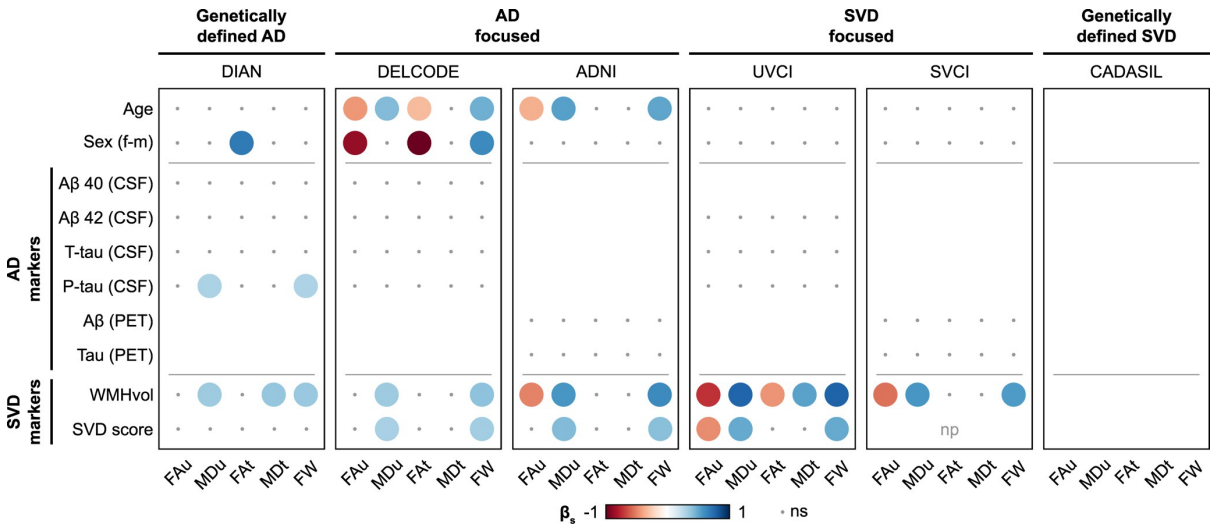


Supplementary Figure 1. Correlation matrices. Intercorrelations (multicollinearity) between AD biomarkers, SVD markers, age, and sex. Grey boxes indicate "not available". AD, Alzheimer's disease; P-tau, phosphorylated-tau$_{181}$; SVD, small vessel disease; SVD score, total small vessel disease score; T-tau, total tau; WMHvol, white matter hyperintensity volume.



Supplementary Figure 2. Simple regression analyses in amyloid-positive individuals.

Simple linear regression analyses between diffusion measures and AD biomarkers or SVD markers in amyloid-positive individuals (sensitivity analysis). Standardized β is represented by color.

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.

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,[1] DELCODE,[2] and UVCI.[3] For the subgroup analysis we used the following cut-offs for Aβ 42 (CSF) abnormality: < 496 pg/ml (DELCODE)[2] and < 640 pg/ml (UVCI).[4] 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.[5] 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.[5] For the subgroup analysis we used the following Aβ (PET) cut-offs for abnormality: Aβ [^{18}F]-florbetapir > 1.11 (ADNI)[6] and Aβ [^{18}F]-florbetaben > 1.45 (SVCI).[7] For both PET samples, we calculated an established global mean tau PET SUVR score.[8]

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.[9] Noise and Gibbs ringing artefacts were removed ('dwidenoise', 'mrdegibbs';[10] 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.[11] 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[12] within FSL. For all samples, an FAt threshold ≥ 0.3 and a custom-made mask[13] were used to exclude areas prone to CSF contamination, a crucial aspect in patient samples with brain atrophy.[14]

The number of diffusion MRI scans excluded from analysis are reported in Figure 1. Main reasons for exclusion were a cropped field-of-view, uncorrectable motion artefacts and uncorrectable registration errors within the tract-based spatial statistics pipeline.

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