MarkVCID2 MRI Peak Skeletonized Mean Diffusivity (PSMD)

Biomarker Kit Protocol

1. Brief description of the biomarker kit

Peak-width of Skeletonized Mean Diffusivity (PSMD) is a quantitative measure derived from Diffusion Tensor Imaging (DTI) sequences on MRI. PSMD provides an estimate of mean diffusivity (MD) dispersion in the white matter skeleton, where higher values indicate greater water dispersion and white matter microstructural damage.[1] This kit includes scripts for the generation of PSMD from diffusion sequences and a video tutorial.

PSMD is robust across MRI machines and DTI acquisition parameters, is fully automated, and has been shown to be more sensitive to SVD cross-sectionally and longitudinally than white matter hyperintensities (WMH) burden or the presence of covert brain infarcts. (*Manuscript under submission*)

Category 1: Susceptibility/Risk

Context of use 1: *Subject selection.* We hypothesize that PSMD is a suitable neuroimaging biomarker to sensitively and specifically identify or risk-stratify participants across the spectrum of cerebral small vessel disease (SVD) at an appropriate stage for inclusion in trials of vascular contributions to cognitive impairment and dementia (VCID).

Category 2: *Disease monitoring*

Context of use 2: *Study outcome.* We hypothesize that PSMD is a suitable neuroimaging biomarker to monitor the progression of cerebral SVD sensitively and efficiently, and therefore the progression of VCID.

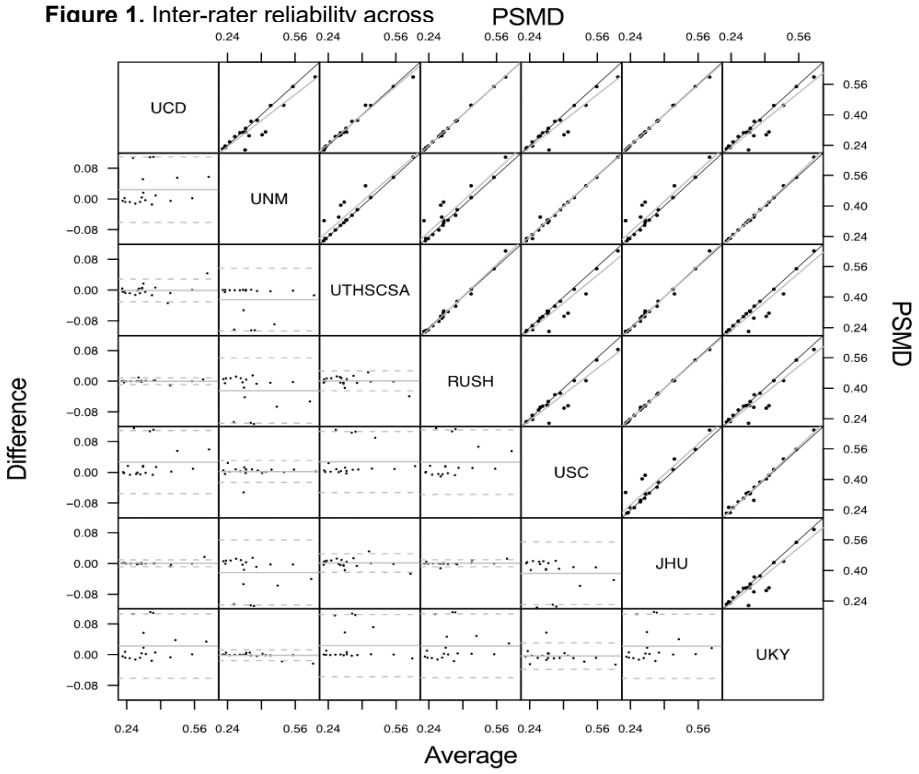
2. Summary of kit instrumental validation results (including instrumental validation citation)

A comprehensive instrumental validation analysis demonstrated that the PSMD kit has excellent inter-rater reliability, test-retest repeatability, and inter-site reproducibility.[2] The PSMD kit is fully automated and robust across MRI machines and DRI acquisition parameters.

2.1. Inter-rater reliability

Inter-rater reliability was evaluated using a dataset of 20 participants selected to cover the spectrum of SVD burden. Each site generated PSMD following the kit's instructions. After the removal of a single DTI scan with poor quality, we observed an average **ICC_{AA}=0.945 (CI: [0.897;0.976]), p<0.001** for agreement, exceeding the pre-specified goal of ICC>0.7 for reliability. **Figure 1** summarizes scatterplots and ICC across paired MarkVCID sites, with ICC ranging from 0.904-0.999.

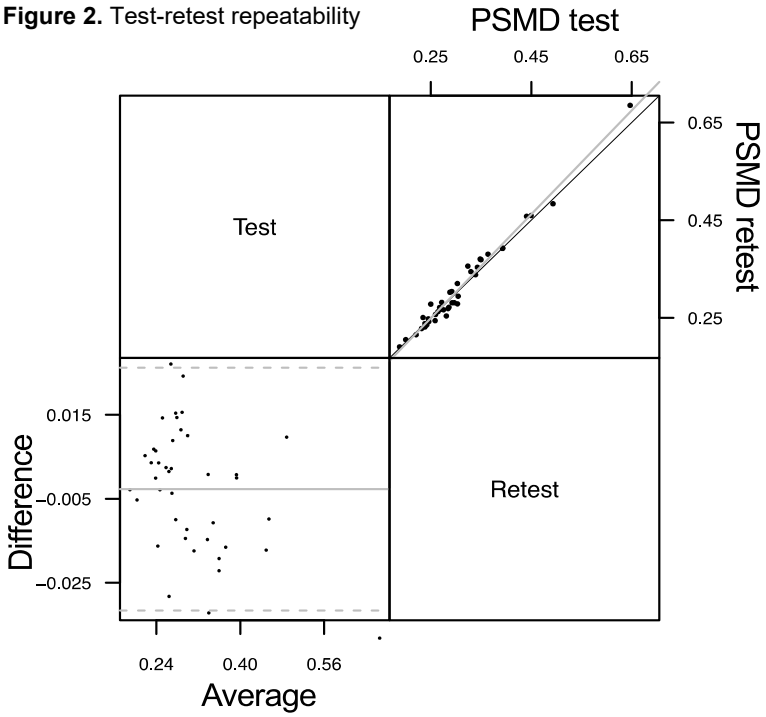
Figure 1. Inter-rater reliability across



2.2. Test-retest repeatability

Each site invited five to six participants to undergo a second brain MRI scan within 14 days of the initial visit, and generated PSMD values in this set of returning participants. We observed an average **ICC_{AA}=0.986 (CI: [0.974;0.993]), *p*<0.001** for agreement, exceeding the pre-specified goal of ICC>0.7 for repeatability. **Figure 2** presents the plotted PSMD test-retest values across sites.

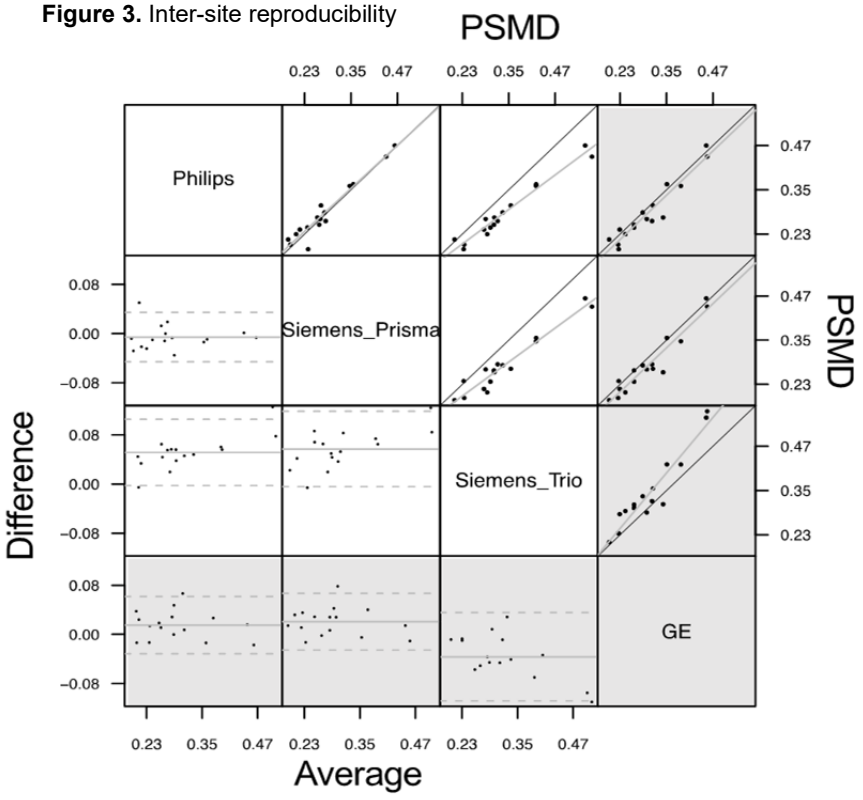
Figure 2. Test-retest repeatability



2.3. Inter-site reproducibility

The coordinating center (CHARGE) provided MarkVCID with a dataset of 20 participants across the spectrum of SVD burden who were imaged in different 3-Tesla scanners within a time span of 15 weeks. The main analysis included acquisitions in Philips Achieva, Siemens Trio, and Siemens Prisma scanners; images on a General Electric (GE) scanner were further assessed for generalizability. After the exclusion of four scans (for poor image quality, bad acquisition, or not being imaged in one of these machines), we observed an **ICC_C=0.954 (CI: [0.899; 0.982]), *p*<0.001** for consistency across the Philips Achieva, Siemens Trio, and Siemens Prisma scanners. The consistency for all scanners was also excellent **(ICC_C=0.948)** including the GE scanner). These estimates exceeded the pre-specified goal of ICC>0.7 for reproducibility. **Figure 3** describes the plotted PSMD inter-site values across sites.

Figure 3. Inter-site reproducibility



3. Summary of kit biological validation results

We used data from the Framingham Heart Study (FHS) Offspring cohort for our power calculations. The partial correlation between log-PSMD and cognitive function, adjusting for age, sex, and educational level, was ρ=-0.21. To detect a partial correlation of that size with 80% power requires a sample size of n=175 to run this kit.

The clinical validation included 396 participants from MarkVCID-1. Existing data from three independent samples were used for replication. The first included the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE, N=6,172), which contributed data from four population-based cohorts: The Age Gene/Environment Susceptibility-Reykjavik (AGES), the Atherosclerosis Risk in Communities (ARIC) Study, and the Coronary Artery Risk Development in Young Adults (CARDIA) Study, and the FHS Third Generation and Omni 2 cohorts. The second and third samples included community-based samples from RUSH University (N=287) and the University of California Davis, Aging Diversity Cohort (UCD-ADC, N=435).

We related log-PSMD (derived from the kit protocol) to general cognitive function using linear regression models adjusting for age, sex, education, and total intracranial volume. A secondary model additionally adjusted for hypertension, diabetes, and smoking status. Finally, we investigated the contribution of PSMD to explain cognitive function above and beyond WMH by comparing the adjusted R-squared from a model including WMH as the independent variable (instead of PSMD) with the adjusted R-squared from another including both PSMD and WMH. This comparison was done in two cohorts representing the younger (FHS) and older (AGES) spectrum of participants included in the validation.

Higher PSMD was associated with lower general cognition in the MarkVCID-1 cohort independent of age, sex, intracranial volume, and education (Beta, [95% CI], -0.82 [-1.03, -0.61], p<0.001), and remained unchanged after additional adjustment for vascular risk factors (-0.87 [-1.09, -0.65], p<0.001). These findings were replicated in independent samples (**Table 1**).

Table 1. Association between PSMD and global cognition

	Model 1 **β [95% CI], *P* value**	**Model 2** **β [95% CI], *P* value**
MarkVCID-1 (n=396)	-0.8 [-1.2, -0.4], <0.001	-0.9 [-1.3, -0.4], <0.001
CHARGE (n=6156)	-1.0 [-1.1, -0.8], <0.001	-0.9 [-1.1, -0.8], <0.001
RUSH (n=287)	-1.5 [-2.0, -0.9], <0.001	-1.4 [-2.0, -0.8], <0.001
UCD-ADC-1* (n=388)	-0.8 [-1.1, -0.5], <0.001	-0.8 [-1.1, -0.5], <0.001
UCD-ADC-2* (n=47)	-1.0 [-1.8, -0.3], 0.010	-1.2 [-2.0, -0.3], 0.009

Model 1 is adjusted for age, age^2, sex, education level, and intracranial volume.

Model 2 is additionally adjusted for vascular risk factors: hypertension, diabetes, and smoking status.

*Samples differ based on the DTI protocol and cognitive batteries

Compared to WMH, PSMD explained an additional 0.2% to 2.51% of the variance in cognitive function in FHS (average age, 48 ±9 years) and in AGES (average age, 76 ±5 years), respectively.

This comprehensive validation work suggests PSMD is a robust and more sensitive neuroimaging marker of SVD, making it suitable for multi-site studies of VCID. (Manuscript under submission)

4. Protocol for image (MRI acquisition)

4.1. MRI protocol: The full MarkVCID neuroimaging protocol has been previously described.[3]

To balance scan time and accuracy, the DTI protocol uses a single-shell (b = 1000 s/mm2) and 40-direction diffusion sequence with a voxel size of 2.0 × 2.0 × 2.0 mm3 and six b = 0 s/mm2 (**Table 2**). Reverse polarity data are used to correct for image distortions.

Table 2. DTI parameters	
FOV	256
Number of Slices	80
TR (ms)	9800
TE (ms)	84
Base Resolution	128
Spatial Resolution	2×2×2
Phase Partial Fourier	(6/8)
PAT MODE	GRAPPA
Accel. Factor PE	2
IPAT Slice or MB	1
Echo Spacing	0.7
BW	1628
Diffusion Directions	40
b0	0
n(b0)	5
b1	1000
n(b1)	40
PE Mode	AP and repeat PA
Distortion Correction	topup/eddy
Total Diffusion Volumes	45

4.2. Components

Hardware:

- Computer with Linux or Mac OS X
- For Windows, a Linux virtual machine is needed (e.g., the NeuroDebian Virtual Machine (http://neuro.debian.net/vm.html))

Software:

- **Mandatory**: An installation of the FMRIB Software Library (FSL, https://www.fmrib.ox.ac.uk/fsl): The PSMD pipeline has been tested with FSL versions 5.0.6 and 5.0.9, but other FSL versions may also work. FSL is a free tool. See https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Licence for license details.
- **Optional**: dcm2niix (https://github.com/rordenlab/dcm2niix). This tool is designed to convert neuroimaging data from the DICOM format to the NIfTI format. This software is open source. The bulk of the code is covered by the BSD license. Some units are either public domain or use the MIT license.

Pre-processing of DTI data:

- Conversion from DICOM to NIfTI files using dcm2niix
- Correction for susceptibility-induced distortions using TOPUP
- Correction for eddy current and using eddy
- Tensor fitting using dtifit

PSMD pipeline: The following steps will be performed through the PSMD tool

- Skeletonization using the FA image and the FSL-TBSS pipeline with the standard FMRIB skeleton as the target
- Projection of MD data onto the skeleton
- Masking of the skeleton in order to focus the analysis on the cerebral hemispheres and to exclude areas with frequent CSF contamination
- Histogram analysis with calculation of peak-width
- PSMD calculation will take approximately 12 minutes with a standard desktop computer.
- The pipeline will generate the following output metrics, including PSMD, in a text file

  - Output files:
    - TOTAL_METRICS_Skel.csv Includes metrics only
    - **TOTAL_METRICS_Skel_header.csv** Includes headers and metrics

  - Format:

Header	Description
NAME	Subject ID
mean_skel_MD_LH_RH	MD mean
sd_skel_MD_LH_RH	MD SD
Pw90S_skel_MD_LH_RH	**PSMD metric**
mean_skel_FA_LH_RH	FA mean
sd_skel_FA_LH_RH	FA SD
mean_skel_AD_LH_RH	AD mean
sd_skel_AD_LH_RH	AD SD
mean_skel_RD_LH_RH	RD mean
sd_skel_RD_LH_RH	RD SD

- Instructions and scripts to launch the PSMD pipeline are provided as a separate zip file "scripts_PSMD_CONSORTIUM.zip"
- A video tutorial is available at https://drive.google.com/file/d/1Uh6vAZqIczN55bs0qjo_TkeCHYWVT-ea/view
- We recommend the following exclusions, as they may affect the estimation of PSMD:
  - Large artery infarcts or hemorrhages on MRI
  - Incidental abnormalities at the time of MRI (i.e., tumor, MS)

5. Additional data collection required for analysis

- Age, sex, sex^2, and intracranial volume may be needed for model adjustments[4]
- Large stroke or other incidental abnormalities at the time of MRI (for exclusions)

6. Protocol for image processing

Pre-processing

Pre-processing of DTI data will be performed using dcm2niix and FSL tools[5] and includes the following steps:

- Conversion from DICOM to nifty files using dcm2niix
- Correction for Susceptibility-induced Distortions using TOPUP
- Correction for eddy current and using eddy
- Tensor fitting using dtifit

Additional information and examples can be found at: https://github.com/miac-research/psmd

PSMD pipeline

The following steps will be performed through the PSMD tool:

- Skeletonization using the FA image and the FSL-TBSS pipeline with the standard FMRIB skeleton as the target
- Projection of MD data onto the skeleton
- Masking of the skeleton in order to focus the analysis on the cerebral hemispheres and to exclude areas with frequent cerebral spinal fluid (CSF) contamination
- Histogram analysis for the calculation of peak-width (PSMD as is the difference between the 95th and 5th percentile of voxel-based MD values within the MD skeleton)

References

1. Baykara, E. *et al.* A novel imaging marker for small vessel disease based on skeletonization of white matter tracts and diffusion histograms. *Ann Neurol* **80**, 581-92 (2016).
2. Maillard, P. *et al.* Instrumental validation of free water, peak-width of skeletonized mean diffusivity, and white matter hyperintensities: MarkVCID neuroimaging kits. *Alzheimers Dement (Amst)* **14**, e12261 (2022).
3. Lu, H. *et al.* MarkVCID cerebral small vessel consortium: II. Neuroimaging protocols. *Alzheimers Dement* **17**, 716-725 (2021).
4. Beaudet, G. *et al.* Age-Related Changes of Peak Width Skeletonized Mean Diffusivity (PSMD) Across the Adult Lifespan: A Multi-Cohort Study. *Front Psychiatry* **11**, 342 (2020).
5. Jenkinson, M., Beckmann, C.F., Behrens, T.E., Woolrich, M.W. & Smith, S.M. Fsl. *Neuroimage* **62**, 782-90 (2012).