MarkVCID Detailed Biomarker Kit Protocol
MRI Peak Skeletonized Mean Diffusivity

1. Executive Summary

Biomarker: MRI Peak Skeletonized Mean Diffusivity (PSMD)
Category: Susceptibility/Risk

What hypothesis is being testing in the individual site and multi-site analyses?

We hypothesize that higher PSMD values will be associated with lower performance in cognitive tasks typically affected in SVD and that are predictive of dementia, such as processing speed, executive function, and memory and fluency[1,2] as defined below.

Additionally, we also hypothesize that PSMD will correlate with cognitive performance independently of white matter hyperintensity (WMH) burden.

What aspects of SVD biology and outcome does the biomarker measure?

DTI has been shown to be more sensitive for the detection of small vessel disease compared with conventional MRI measures of the white matter. PSMD provides an index of mean diffusivity (MD) dispersion in the white matter skeleton, where MD is the extent of diffusion of water molecules in that voxel of tissue. Higher PSMD values indicate greater white matter microstructural damage.

Include a timeline for validation of the biomarker

We propose a timeline of 2.5 years for the validation of this biomarker across sites that have agreed to cross-validate this kit: UCSF/UCD/UCLA, JHU, UKY, USC, UNM, Rush, and CHARGE.

2. Brief Description of Biomarker Kit

Description of final biomarker that will address the hypothesis
PSMD, derived from Diffusion tensor imaging (DTI) data, is robust across MRI machines and DTI acquisition parameters, can be fully-automated, and has been shown to be more sensitive to SVD cross-sectionally and longitudinally than WMH burden or the presence of covert brain infarcts.[3]

Kit 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 a 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.

3. Participating sites

Six MarkVCID sites have agreed to cross-validate the PSMD kit (UCSF/UCD/UCLA, JHU, UKY, USC, UNM, Rush), in addition to legacy samples from CHARGE (Table).

MarkVCID Site Participation in Biomarker Validation

*Sites **marked** yes (**Y**) or no (**N**) if the site is providing data and/or performing analysis for each imaging kit AND providing samples and/or running assays for each fluid kit. BLUE indicates lead site.*

	Arteriolosclerosis based on WMH & DTI		Cerebrovascular Reactivity		Peak Skeletonized Mean Diffusivity		White Matter Hyperintensity		Longitudinal WMH from 3D FLAIR		Endothelial Signaling (mesoscale)		Endothelial Inflammation (exosomes)	
	Provide Data	Perform Analysis	Provide Data	Perform Analysis	Provide Data	Perform Analysis	Provide Data	Perform Analysis	Provide Data	Perform Analysis	Provide Samples	Run Assay	Provide Samples	Run Assay
UCSF/ UCD/UCLA	Y	Y	?	?	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
JHU	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y
UKY	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
USC	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	N
UNM	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Rush	Y	Y	N	N	Y	Y	Y	Y	Y	Y	Y**	N	Y**	N
CHARGE	Y	Y	Y*	Y	Y	Y	Y	Y	Y	Y	Y**	Y	Y**	Y

*Pending approval for prospective recruitment from UTSA site **Rush and CHARGE will provide frozen exosome legacy samples

4. Protocol for MRI acquisition

- MRI experiment protocol:

FOV	256
Number of Slices	80
TR (ms)	9800
TE (ms)	84
Base resolution	128
Spatial Resolution	2x2x2
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

- The diffusion protocol and gradients in TRIO and PRISMA format are provided with the Kit.

- This kit requires only one MRI scan at baseline. PSMD derived from baseline MRI will be related to baseline cognition in cross-sectional analyses and to change in cognition in longitudinal analyses.

- The instrumental validation requires PSMD derived from two MRI scans, one week apart, in six participants.

5. Additional data collection required for analysis

- Data collection should include age, sex, educational level, diabetes, smoking, and hypertension. The consortium has agreed to collect these variables at each site.

- For exclusions, we recommend not collecting data from participants with large artery infarcts or large hemorrhages at the MRI examination, as these may affect the calculation of PSMD.

- WMH Fazekas scores will be valuable to estimate the added contribution of PSMD above a classic MRI marker of SVD. The Fazekas score will be introduced as a covariate in the models to assess the added contribution to PSMD (see analytical plan).

6. Protocol for MRI analysis

Pre-processing

Pre-processing of DTI data will be performed using dcm2niix and FSL tools 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

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 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 approximatively 12 minutes with a standard desktop computer.

Instructions and scripts to launch the PSMD pipeline are provided as a separate zip file "scripts_PSMD_CONSORTIUM.zip".

7. Step-by-step analytic plan

7.1. Instrumental validation

A previous study assessing the reproducibility of PSMD in 7 CADASIL patients using 2 scanners with different field strengths (3T and 1.5T) found a high inter-scanner reproducibility (ICC = 0.948). Considering that 0.7 is the minimum acceptable ICC for a useful biomarker, we will require at least at least 30 subjects (6 per site in at least 5 sites) to achieve 90% power to detect a difference from the null hypothesis (ICC=0.7).

Each site (UCSF/UCD/UCLA, JHU, UKY, USC, UNM, Rush, CHARGE) will randomly select six participants to undergo brain MRI imaging twice in the same scanner, no more than one week apart from the initial MRI, for repeated PSMD determination. We recommend imaging the same participant at the same time of day and weekday (i.e. Monday at 11am for initial MRI, and Monday at 11am of the following week for the second MRI).

- **Inter-rater reliability / same-scan reliability:**
  Baseline brain MRI scans from six participants selected for instrumental validation will be shared with other sites for inter-rater reliability. Each site will

estimate PSMD measures for 36 participants from other sites and share results. We anticipate the same scan reliability will be close to 1 since PSMD estimation is largely automated.

- **Test-retest reliability / same subject repeatability:**
  Each site will calculate ICC between PSMD measures derived from MRI scans at baseline and one-week later for the six participants selected for instrumental validation at their own sites.

7.2. PSMD measurement

- Follow the MarkVCID MRI-DTI protocol for image acquisition (see section 4).
- Generate individual-level PSMD estimates with the script provided with the kit (see kit components). Six returning participants at each site will have two PSMD estimates, one week apart, for instrumental validation.
- Once the data is available, the analysis plan below can be performed at each site (UCSF/ UCD/UCLA, UKY, USC, UNM, Rush, CHARGE). We can perform PSMD analysis for sites electing not to perform their own analysis for this kit (JHU).

7.3. Exclusions for main analysis

- Exclusion of participants with large artery infarcts or hemorrhages on MRI is recommended, as these may affect the estimation of PSMD.
- Exclude participants with dementia at baseline (limit analyses to cognitively normal and MCI participants)

7.4. Definition of cognitive domains: Each site is collecting the same neuropsychological measures at baseline and follow-up visits.

- The following measures will be used to create a composite measure of general cognitive function:
  - **Craft Story 21 Recall (Immediate):** Total story units recalled or verbatim scoring [0-44] after 20 minutes of first administration.
  - **Craft Story 21 Recall (Delayed):** Total story units recalled or verbatim scoring [0-44] after 20 minutes of first administration.
  - **Number Span Test Forward:** Number of correct trials, [0-14].
  - **Number Span Test Backward:** Number of correct trials, [0-14].
  - **Category Fluency – Animals**: Total number of animals named in 60 seconds, [0-77]
  - **Phonemic Fluency –Words beginning with F**: Number of F-words repeated in 1 minute [0-15]
  - **Trail Making Test A:** Total number of seconds to complete test, [0-150].
  - **Trail Making Test B:** Total number of seconds to complete test, [0-300].
  - **Multilingual Naming Test (MINT)**: Total correct without any cues (Un-cued) [0-32]

- Natural log-transform variables with skewed distributions.

- Reverse sign for Trail Making Test A and Trail Making Test B to indicate higher scores represent better performance.

- Standardize individual scores for each one of the previous cognitive measures:
  z-score $_{T1}$ = (individual cognitive score at T_1 – sample mean for the cognitive test at T_1) / sample standard deviation for the cognitive test at T_1

- Create a composite cognitive score at baseline (T1) by averaging individual z-scores $_{T1}$.

7.5. Analysis: Cross-sectional association between PSMD and cognition:

- Log-transform PSMD (in our experience this measure has a skewed distribution)

- Use linear regression models to regress PSMD (predictor) on general cognitive function (outcome), adjusting for age, sex, and education. In a second model, additionally adjust for, diabetes, smoking and hypertension.

  Model 1: Cognition ~ f(PSMD + age + sex + education)
  Model 2: Cognition ~ f(PSMD + age + sex + education + diabetes + smoking + hypertension)

7.6. Optional analysis: Assessing the added contribution of PSMD: We propose an additional analysis to assess the contribution of PSMD to explain cognitive performance in addition to a classic SVD marker such as WMH.

- Use linear regression models to regress Fazekas score (predictor) on the cognitive score (outcome), adjusting for baseline covariates.

- Run another model to regress Fazekas score at baseline (predictor) on general cognitive score at baseline (outcome), adjusting for baseline PSMD and covariates.

- Compare models 1a and 2a, 1b and 2b (e.g. using R-square) to estimate the additional variance in cognitive performance explained by PSMD.

  Model 1a: Cognition ~ f(Fazekas + age + sex + education)
  Model 1b: Cognition ~ f(Fazekas + PSMD + age + sex + education)

  Model 2a: Cognition ~ f(Fazekas + age + sex + education + diabetes + smoking + hypertension)
  Model 2b: Cognition ~ f(Fazekas + PSMD + age + sex + education + diabetes + smoking + hypertension)

8. Sample size calculation (*individual site* level)

In the Framingham Heart Study, we created a composite cognitive score following the analytical plan described in section 7. The composite variable included Logical Memory – immediate recall, Logical Memory – delayed recall, Digit Span Forward, Digit Span Backward, Category Fluency (animals), Phonemic Fluency (FAS), Trail Making Test A, Trail Making Test B, and the Boston Naming Test.

The partial correlation between log-PSMD and the composite cognitive score, 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 around n=175. Therefore, **we recommend a minimum sample size of n=175 participants per site** to be adequately powered to run this kit.

9. Plan for longitudinal data collection analysis

- Covariates (baseline): age, sex, educational level, diabetes, smoking, and hypertension

- Predictors (baseline): PSMD
- Outcome measures (**longitudinal**): Change in composite measure of general cognitive function. Follow the same approach to calculate a composite variable of general cognitive function at follow up but this time using the baseline sample mean and standard deviation when computing the z-scores:

  z-score $_{T2}$ = (individual cognitive score at T_2 – sample mean for the cognitive test at T_1) / sample standard deviation for the cognitive test at T_1

- Create a composite cognitive change score (T2) by averaging individual z-scores $_{T2}$.

- Use linear regression models to assess the association between PSMD (baseline) and change in general cognitive score adjusting for covariates.

  Model 1: *Change* in cognition ~ f(PSMD + age + sex + education)
  Model 2: *Change* in cognition ~ f(PSMD + age + sex + education + diabetes + smoking + hypertension)

10. Plan for reporting outcomes

Please report the information in the table below per participant:

Variable	Description
ID	Participant ID
MRI date (baseline)	Date of baseline MRI
PSMD (baseline)	Computed PSMD at baseline
Fazekas score (baseline)	Fazekas score at baseline
Health status (baseline)	Cognitively normal=0, MCI=1, Demented=2
Cognitive evaluation date (baseline)	Date of cognitive evaluation visit date at baseline
Craft Story 21 Recall – Immediate (baseline)	Total story units recalled, verbatim scoring [0-44]
Craft Story 21 Recall – Delayed (baseline)	Total story units recalled, verbatim scoring [0-44] after 20 minutes of first administration
Number Span Test Forward (baseline)	Number of correct trials [0-14]
Number Span Test Backward (baseline)	Number of correct trials [0-14]
Category Fluency – Animals (baseline)	Total number of animals named in 60 seconds [0-77]
Phonemic Fluency –Words beginning with F (baseline)	Number of correct F-words generated in 1 minute [0-15]
Trail Making Test A (baseline)	Total number of seconds to complete [0-150]
Trail Making Test B (baseline)	Total number of seconds to complete [0-300]
Multilingual Naming Test (MINT) (baseline)	Total correct without any cues [0-32]
Composite cognitive score (baseline)	Average z-score $_{T1}$ (see section 7.4)
Age (baseline)	Age of the participant at MRI
Sex	Sex
Education	Participant's educational level
Diabetes (baseline)	Diabetes (yes/no)
Smoking (baseline)	Current smoking (yes/no)
Hypertension (baseline)	Hypertension (yes/no)
Health status (follow-up)	Cognitively normal=0, MCI=1, Demented=2
Cognitive evaluation date (follow-up)	Date of cognitive evaluation visit date at last follow-up
Craft Story 21 Recall – Immediate (last follow-up)	Total story units recalled, verbatim scoring [0-44]
Craft Story 21 Recall – Delayed (last follow-up)	Total story units recalled, verbatim scoring [0-44] after 20 minutes of first administration
Number Span Test Forward (last follow-up)	Number of correct trials [0-14]
Number Span Test Backward (last follow-up)	Number of correct trials [0-14]
Category Fluency – Animals (last follow-up)	Total number of animals named in 60 seconds [0-77]
Phonemic Fluency – Words beginning with F (last follow-up)	Number of correct F-words generated in 1 minute [0-15]
Trail Making Test A (last follow-up)	Total number of seconds to complete [0-150]
Trail Making Test B (last follow-up)	Total number of seconds to complete [0-300]
Multilingual Naming Test (MINT) (last follow-up)	Total correct without any cues [0-32]
Composite cognitive change score (from last follow-up)	Average z-score $_{T2}$ (see outcome measure in section 9)

11. Plan for sharing data, samples/images, protocols

- We plan to share all necessary PSMD Kit components (package, manuals, template tables) with cross-validating sites. This and additional information will also be shared through a dedicated PSMD folder at the MarkVCID portal.

- Biomarker and outcome data collected in CHARGE participating cohorts through previously funded projects will be shared with other UH2/UH3 investigators as mutually decided by the SVD Biomarker consortium. New data generated in the UH3 phase will also be shared with participating sites.

References

1 Elias, M. F. *et al.* The preclinical phase of alzheimer disease: A 22-year prospective study of the Framingham Cohort. *Arch Neurol* **57**, 808-813 (2000).

2 Blacker, D. *et al.* Neuropsychological measures in normal individuals that predict subsequent cognitive decline. *Arch Neurol* **64**, 862-871, doi:10.1001/archneur.64.6.862 (2007).

3 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**, 581-592, doi:10.1002/ana.24758 (2016).

Appendix 1: MarkVCID Comprehensive Dataset: Clinical Measures
click here:
https://markvcid.partners.org/system/files/external/protocols/Clinical_Data_Measures_7.12.19_ext_investigators.pdf