MarkVCID2 MRI Free Water (FW)
Biomarker Kit Protocol (no page limit)

1. Brief description of the biomarker kit

Cerebral small vessel disease (SVD), which refers to a group of pathological processes affecting the small blood vessels in the brain, including arteries, arterioles, venules, and capillaries, and is the leading cause of vascular cognitive impairment disease (VCID)[1]. Of particular interest in SVD is the free water (FW) diffusion MRI model. Extracellular FW represents the water molecules in the white matter tissue's extracellular space that are unimpeded by physical barriers like axon membranes and myelin. The remaining water molecules, those within or near cellular structures, reflect the architecture of white matter tracts. Diffusion-weighted imaging (DWI), a **non-invasive MRI sequence**, allows for the extraction of the proportion, refers to as the fraction, of FW in each white matter location. The FW kit proposes mean FW (mFW), which is defined as the average FW fraction within the white matter tissue, as a candidate imaging biomarker for direct incorporation into SVD-VCID clinical trials. Previous studies demonstrated associations between mFW and hemodynamic alteration in adult individuals[2], with inflammatory biomarkers in older individuals[3], with common imaging markers of cerebral SVD[4] and with clinical status in cerebral SVD[5]. mFW abnormal level has also been found to be predominantly driven by SVD[6] and detectable years before severe pathophysiological manifestations of SVD injury become evident[7, 8]. The results from the recently completed FW kit biological validation phase[9] indicate that mFW is associated with UDS3-EF (a composite measure of executive function[10]) in three MarkVCID sub-cohorts, regardless of vascular risk factors. This association was also replicated across five independent large data sources, including adult individuals (see Table 1). Furthermore, mFW has also been found to predict UDS3-EF decline over a 1.3-year period in the MarkVCID longitudinal cohort[9]. All these findings position **mFW as a highly promising sensitive biomarker of SVD in the context of VCID research**.

2. Summary of kit instrumental validation results

Seven MarkVCID sites participated to the instrumental validation of the FW kit[11]. The instrumental validation process evaluated the inter-rater reliability among sites (20 participants), test-retest repeatability (6 or 7 participants per site), and inter-scanner reproducibility (16 participants) across 3 types of MRI scanners with similar resolutions (Siemens Prisma, Siemens Trio and Philips Achieva) and, to enhance generalizability, one extra scanner with interpolated resolution (General Electric) using intra-class correlation coefficients (ICC).
The study revealed that **the FW kit demonstrates remarkable analytical performances** with excellent inter-rater reliability (ICC=0.997, confidence interval: [0.993; 0.999], p<0.001;), test-retest repeatability (0.995 [0.99; 0.997], p<0.001) and inter-scanner reproducibility (3 scanners: 0.96 [0.911; 0.985], p<0.001; 4 scanners: 0.964 [0.926; 0.986], p<0.001) of mFW measures[11].
Table 2 below contains intra-class coefficients of mFW between sites (inter-rater reliability study), sessions (test-retest repeatability study) and scanners (inter-scanner reproducibility study).

3. Protocol for image acquisition

To balance accuracy and scan time, the FW kit DWI protocol[12] uses a single-shell (b=1000 s/mm^2), 40-direction diffusion sequence with a voxel size of 2.0×2.0×2.0 mm^3 and six b=0 s/mm^2 volumes and a reverse polarity acquisition to estimate and correct image distortions in the DWI data. **Total scan duration is approximately 8.5 minutes**.

4. Additional data collection required for analysis

The FW kit uses **UDS3-EF, a validated executive function composite score** derived from the Uniform Data Set (v3.0)[10, 13]. The UDS3-EF is an item response theory-based composite score calculated from the following tests: Digit Span Backwards (total correct), Trail Making Test (TMT) parts A and B (correct lines per minute), lexical fluency (F and L words—total correct), and semantic fluency (animal and vegetable fluency—total correct). The FW kit also requires the following data: age, sex, education, diabetes, smoking and hypertension.

5. Protocol for image processing

Prerequisites

The FW kit requires a 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) with an installation of the FMRIB Software Library (FSL, https://www.fmrib.ox.ac.uk/fsl) and Python with the following python libraries: “matplotlib” and “dipy”. The current version of the kit uses Python 3.6.5.

Downloading and installation instructions

- Download the FW kit (scripts_FW_CONSORTIUM.zip) at https://markvcid.partners.org/markvcid2-protocols-resources
- Move this file to a more permanent location
- Unzip the file. It will create a new directory named scripts_FW_CONSORTIUM
- Open the *MAIN_script_FW.sh* file located in the scripts_FW_CONSORTIUM folder. Three paths at the beginning of the script should be modified and set for the user's system environment (see below)

```
# MODIFY FOLLOWING DIRECTORIES
# FSL_PATH IS THE MAIN FSL PATH AND SHOULD CONTAIN BIN/, CONFIG/, LIB/... SUBDIRECTORIES)
# PYTHON_EXEC IS THE PATH FOR THE PYTHON EXECUTABLE
set FWMRN_PATH = /data/home/maillard/scripts_FW_CONSORTIUM/
set BINFSL_PATH = /data/condorWorkspace/fsl/
set PYTHON_EXEC = /usr/bin/python
```

  - FWMRN_PATH: full path to the scripts_FW_CONSORTIUM/ directory
  - BINFSL_PATH: full path to the FSL main directory
  - PYTHON_EXEC: full path to the python executable

Before running the script

Check that:

- the MAIN_script_FW.sh script is executable. This can be achieved by running the following command: chmod +x MAIN_script_FW.sh
- The 4D DWI volume and brain mask have the NIfTI compressed format (i.e. *nii.gz* extension)
- The format of bval and bvec files is compatible with FSL

How to run the script

- Enter the full path to *MAIN_script_FW.sh* with the following inputs: subject's 4D DWI volume, brain mask, b-vector and b-values files and the output directory. If the output directory doesn't exist, it will be automatically created by the script. See example below:

```
$ /FWscriptdir/MAIN_script_FW.sh /subjectdir/data.nii.gz /subjectdir/brain.nii.gz /subjectdir/file.bval /subjectdir/file.bvec /subjectdir/outputdir/
```

FW script | 4D DWI volume | Brain mask | bval file | bvec file | Output dir

Output files

- The script will generate a file, named *summary.txt*, located in the output directory and that contains the mFW measure. Computation time takes approximatively 4 minutes with a standard desktop computer.
- The output directory contains other useful files including:
  - fwc_wls_dti_FA.nii.gz: FW-corrected FA image in native space
  - fwc_wls_dti_FA_warp.nii.gz: FW-corrected FA image in FSL template space
  - wls_dti_FA.nii.gz: FA image in native space (no FW correction)
  - wls_dti_FW.nii.gz: FW image in native space
  - wls_dti_FW_warp.nii.gz: FW image in FSL template space
  - nat2std.mat: affine transformation matrix
  - nat2std_warp.nii.gz: warp-field coefficients image

Pre-processing tips (not part of the kit)

Pre-processing of DWI data can be performed using dcm2niix and FSL tools[14] and includes the following steps: (1) conversion from DICOM to NIfTI files using dcm2niix; (2) correction for susceptibility-induced distortions using *topup* from FSL; (3) brain extraction using *bet* from FSL; and (4) correction for eddy current using *eddy* from FSL

What does this script do exactly?

The model considers two co-existing compartments per voxel: one compartment is a free-water compartment, which models isotropic diffusion with a diffusion coefficient of water at body temperature (37 °C) fixed to 3 ×

10^{-3} mm^2/s[15]. The free-water fraction is expected to predominantly highlight water molecules in the extracellular space. The second compartment is the tissue compartment, which accounts for all other molecules, i.e., all intra- and extracellular molecules that are hindered or restricted by tissue membranes[16]. The script contains the following steps: 1) the tissue compartment is modeled by a diffusion tensor characterizing the “tissue” molecules, as well as the fractional volume of the free-water compartment in each voxel, resulting in the FW fraction map, 2) the individual FA map obtained from *dtifit* is linearly and non-linearly registered to the standard FSL FA template space (FMRIB 1-mm FA template) using linear and nonlinear transformations, 3) the resulting transformation parameters are applied to the FW map, 4) a white matter (WM) mask is defined by thresholding the FSL FA template at a value of 0.3 to reduce cerebrospinal fluid partial volume contamination[17], 5) an overall measure of mean FW is computed by superimposing the WM mask onto the individual coregistered FW fraction map and averaging values within these WM voxels and 6) an overall measure of mean FW fraction mFW) is stored in a text file.

Table 1 Effect of mFW on executive function composite score in the different cohorts

Cohort	Model M1			Model M1 +VRF		
	Beta	SE	Pvalue	Beta	SE	Pvalue
MarkVCID cohorts						
Cohort 1	-7.417	1.279	<0.001	-7.325	1.315	<0.001
Cohort 2	-8.927	1.807	<0.001	-8.585	1.891	<0.001
Cohort 3	-4.027	1.789	0.026	-3.425	1.864	0.068
Legacy cohorts						
UCSF	-9.87	1.76	<0.0001	-10.63	2.55	<0.0001
UCD ADRC	-6.18	0.603	<0.0001	-6.16	0.605	<0.0001
RUSH	-1.905	0.69	0.00597	-2.07	0.66	0.0018
ARIC	-1.24	0.304	<0.0001	-1.08	0.32	0.0007
FHS (Offspring and Gen3 combined)	-4.06	0.51	<0.0001	-3.80	0.51	<0.0001
Offspring	-5.17	0.74	<0.0001	-5.03	0.75	<0.0001
Gen3	-1.62	0.77	0.036	-1.09	0.79	0.16

mFW: mean free water; VRF: vascular risk factors including diabetes, smoking and hypertension status. Beta: regression coefficient; SE: standard error.
Model M1 included baseline EFC as the dependent variable and mFW as the independent variable, adjusting for age, sex and education. Results for FHS sub-cohorts (Offspring and Gen3) are reported with italic font.
RUSH used log_{10}-transformed FW measures to normalize distribution

Table 2 Intra-class coefficients of mean FW fraction (mFW) between sites (inter-rater reliability study), sessions (test-retest repeatability study) and scanners (inter-scanner reproducibility study)

Inter-rater	UCD-UNM	1 ([1; 1], p<0.001)
	UCD-UTHSCSA	0.993 ([0.933; 0.998], p<0.001)
	UCD-RUSH	0.998 ([0.981; 1], p<0.001)
	UCD-USC	0.999 ([0.996; 1], p<0.001)
	UCD-UKY	1 ([1; 1], p<0.001)
	UNM-UTHSCSA	0.992 ([0.932; 0.998], p<0.001)
	UNM-RUSH	0.999 ([0.984; 1], p<0.001)
	UNM-USC	0.999 ([0.994; 1], p<0.001)
	UNM-UKY	1 ([1; 1], p<0.001)
	UTHSCSA-RUSH	0.986 ([0.804; 0.997], p<0.001)
	UTHSCSA-USC	0.994 ([0.977; 0.998], p<0.001)
	UTHSCSA-UKY	0.992 ([0.941; 0.998], p<0.001)
	RUSH-USC	0.996 ([0.934; 0.999], p<0.001)
	RUSH-UKY	0.998 ([0.985; 0.999], p<0.001)
	USC-UKY	0.999 ([0.996; 1], p<0.001)
	UCD-JHU	1 ([1; 1], p<0.001)
	UNM-JHU	1 ([1; 1], p<0.001)
	UTHSCSA-JHU	0.992 ([0.929; 0.998], p<0.001)
	RUSH-JHU	0.999 ([0.984; 1], p<0.001)
	USC-JHU	0.999 ([0.993; 1], p<0.001)
	UKY-JHU	1 ([0.999; 1], p<0.001)
Test-retest		0.995 ([0.99; 0.997], p<0.001)
Inter-scanner	Philips-Siemens_Prisma	0.977 ([0.936; 0.992], p<0.001)
	Philips-Siemens_Trio	0.945 ([0.85; 0.98], p<0.001)
	Siemens_Prisma-Siemens_Trio	0.958 ([0.883; 0.985], p<0.001)
	Philips-GE	0.966 ([0.905; 0.988], p<0.001)
	Siemens_Prisma-GE	0.992 ([0.978; 0.997], p<0.001)
	Siemens_Trio-GE	0.949 ([0.861; 0.982], p<0.001)

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