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CALGARY NORMATIVE STUDY: STUDY DESIGN OF A PROSPECTIVE LONGITUDINAL STUDY TO CHARACTERIZE POTENTIAL QUANTITATIVE MR BIOMARKERS OVER THE ADULT LIFESPAN

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CALGARY NORMATIVE STUDY: STUDY DESIGN OF A PROSPECTIVE LONGITUDINAL STUDY TO CHARACTERIZE POTENTIAL QUANTITATIVE MR BIOMARKERS OVER THE ADULT LIFESPAN

ABSTRACT:

Introduction: A number of magnetic resonance (MR) imaging methods have been proposed to be useful, quantitative biomarkers of neurodegeneration in aging. The Calgary Normative Study (CNS) is an ongoing single-centre, prospective, longitudinal study that seeks to develop, test and assess quantitative MR methods as potential biomarkers. The CNS has three objectives: first and foremost, to evaluate and characterize the dependence of the selected quantitative neuroimaging biomarkers on age over the adult lifespan; secondly, to evaluate the precision, variability and repeatability of quantitative neuroimaging biomarkers as part of biomarker validation providing proof of-concept and proof-of-principle; and thirdly, provide a shared repository of normative data for comparison to various disease cohorts.

Methods and Analysis: Quantitative MR mapping of the brain including longitudinal relaxation time (T1), transverse relaxation time (T2), T2*, magnetic susceptibility (QSM), diffusion and perfusion measurements, as well as morphological assessments are performed. The Montreal Cognitive Assessment (MoCA) and a brief, self-report medical history will be collected. Mixed regression models will be used to characterize changes in quantitative MR biomarker measures over the adult lifespan. In this report on study design, we report interim prevalence and demographic information of recruitment from 28 May 2013 to 31 December 2018.

Ethics and Dissemination: Participants provide signed informed consent. Changes in quantitative MR biomarkers measured over the adult lifespan as well as estimates of

measurement variance and repeatability will be disseminated through peer-reviewed scientific publication.

STRENGTHS AND LIMITATIONS:

- Both cross-sectional and longitudinal quantitative MR data is being acquired in a large sample normal aging population to characterize changes in these potential neuroimaging biomarkers over the adult lifespan.
- Clinical and multiple quantitative imaging data are being collected and shared with the research community.
- Measures of repeatability and a process to update the imaging protocol are included in the study design.
- Associated self-reported medical history and cognitive assessments are limited

INTRODUCTION:

Quantitative imaging methods can be defined as "extraction and use of numerical or statistical features from medical images".[1] There are numerous examples where quantitative measures have improved detection of changes or monitoring of the brain.[2-4] Specifically, quantitative neuroimaging using MR is thought to be suitable for identifying potential biomarkers of cognitive impairment risk because it 1) may be more sensitive to early detection of pathological changes within otherwise normal appearing tissue,[5-9] 2) may be more directly related to underlying physiological processes of interest, 3) may have reduced variability across time and/or equipment platforms when compared to traditional, more qualitative, neuroimaging methods, 4) can provide less subjective interpretation, and 5) allows for a low risk, repeatable measurement.[1 10]

Numerous quantitative MR imaging biomarkers have been proposed to assess risk, progression and treatment of age-related neurodegenerative diseases and disorders including cerebral small vessel disease,[10] Alzheimer's disease,[3] Parkinson's disease,[11] multiple sclerosis,[12 13] and Huntington's disease.[14] These quantitative markers typically include measures of brain atrophy, white matter integrity, iron accumulation, and cerebral blood flow. Often, these measures can be determined using standard, vendor-provided MR sequences and freely available image processing packages. Less commonly, longitudinal (T1) and transverse (T2, T2*) relaxometry, vascular permeability and quantitative susceptibility mapping using more customized approaches have been used.

Development of quantitative imaging biomarkers requires appropriate validation and qualification as outlined by the Quantitative Imaging Biomarkers Alliance (QIBA) organized by the Radiological Society of North America.[15 16] An advantage of quantitative imaging is that the measurements obtained should be, in principle, independent of the specifics of acquisition. While brain atrophy, white matter hyperintensity volume, white matter integrity as measured by diffusion imaging metrics, number of cerebral microbleeds, number and volume of infarcts (including ischemic, hemorrhagic, and lacunar), relative increase in tissue iron have all been associated with one or more neurodegenerative processes, detailed validation of these quantitative biomarkers is relatively lacking.[16] Furthermore, quantitative methods like MR relaxometry, e.g., T1, T2, and T2* mapping, quantitative susceptibility mapping, and arterial spin labelling measurement of cerebral perfusion also require comprehensive evaluation or validation as potential quantitative neuroimaging biomarkers of neurodegenerative disease or pathology.

The Calgary Normative Study (CNS) is a single centre, prospective, longitudinal study with three objectives: 1) to evaluate and characterize the dependence of the selected quantitative neuroimaging biomarkers on age over the adult lifespan; 2) to evaluate the precision, variability and repeatability of quantitative neuroimaging biomarkers as part of biomarker validation providing both proof of-concept and proof-of-principle[16]; and 3) provide a shared repository of normative data for comparison to various disease cohorts. In developing this study, we developed a plan incorporate the ability to revise our data acquisition protocol so that we can refine and accommodate emerging quantitative techniques. We also aimed to complete all imaging procedures and other study evaluations within 2 hours. Our primary research interest is in cerebral small vessel disease and this guided our selection of potential quantitative neuroimaging biomarkers, though in both theory and practice they have proven to be of broad application in other neuroimaging studies. Here we describe the study design and methods used in the CNS and report on recruitment and data sharing. Reporting the specific short- and long-repeatability or changes in quantitative metrics over the adult lifespan are beyond the scope of this report and will be submitted as separate submissions for publication subsequently.

MATERIALS AND METHODS:

Participant Eligibility and Characterization:

This study was approved by the local research ethics board (REB). Study recruitment is ongoing; volunteers over 18 year of age are being recruited from the community, primarily through local poster advertisement and word-of-mouth. Interested individuals provided written informed consent and were screened for eligibility based on self-reported absence of significant neurological disease, psychiatric disorders, or contraindications for MR imaging at 3 T. Participants with uncertain contraindications (poorly characterized implant, *etc*.) were not

imaged and excluded from the study. Participants provided a brief medical history, completed a Montreal Cognitive Assessment (MoCA)[17], completed our institutional MR safety screening and underwent MR imaging.

Demographic and simplified medical information collected includes age, sex, ethnicity, handedness, years of education, smoking history, weight, hypertension or taking medication for the treatment of hypertension, dyslipidemia or taking medication for the treatment of high cholesterol, and presence of diabetes mellitus, and family history of stroke, dementia, Alzheimer's disease, or cardiovascular disease. Participants were also asked if they are willing to return for a follow-up visit and if they are willing to be contacted in the future for possible participation in other research studies. All data were de-identified and labelled with CNS-specific identification numbers.

The MoCA is a cognitive screening test designed to assist the detection of mild cognitive impairment, which was administered and scored by trained research personnel. If participants were not fluent in English, then alternate language versions of the MoCA (Mandarin and Spanish) were offered and were administered with the aid of a translator. Participants that scored <26 on the MoCA were considered screen failures for normal cognition.[17] However, these individuals still completed the study procedures, though we anticipate that their data may be excluded from some subsequent analyses. Participants were not informed of their MoCA scores because the MoCA alone is not sufficient to diagnose a clinical cognitive disorder such as MCI, and there will be false positive low scores. If concerns about a clinical cognitive disorder arose during the testing the participant was advised to consult their primary care practitioner.

We sought to enroll an approximately balanced distribution of men and women in each of six age categories: 18-29, 30-39, 40-49, 50-59, 60-69, 70+ years. We anticipate that recruitment of

eligible normal older participants would be more difficult due to the increased prevalence of stroke and or other significant neurological disease with age. For this reason, we decided to group individuals 70 years of age and older into a single age category, anticipating that we would have difficulty recruiting a sufficient number of individuals over 80 years of age.

A nominal sample size of 20 men and 20 women was initially targeted for each age category, for a nominal CNS recruitment of 240. This sample size estimate was based on estimates of variance from previous work using voxel-based analysis of T2 changes in temporal lobe epilepsy.[2] The study was initially powered ($\alpha = 0.05$, $1\text{-}\beta = 0.8$) to detect T2 changes of 3.4 ms or greater. Larger or smaller sample sizes may be required depending on the quantitative measure, region, and analysis method chosen. To accommodate differences in power requirements, protocol revisions, and secondary analyses (eg sex differences), recruitment will continue after the nominal sample size has been achieved. Establishing better variance estimates for each quantitative imaging method is one of the objectives of the Calgary Normative Study.

MR Imaging Acquisition Protocol:

MR imaging is being completed at a single centre on a 3 T MR scanner (MR750, General Electric Healthcare, Waukesha, WI) using the vendor-supplied, 12-channel head, neck and spine coil. Standard procedure at our centre is to perform daily quality control scans and to follow the vendor recommended service guidelines.

Prior to setting up the initial MR protocol, we informally surveyed five local research teams with respect to their MR imaging protocols and typical outcome measures. This survey guided some of our protocol decisions and ensured that aspects of the MR acquisition parameters were similar to other studies in our centre. This similarity in some protocol acquisitions makes it feasible to share and leverage the data appropriately for comparison with disease cohorts.

The MR imaging protocol includes conventional structural 3D T1-weighted (3D-T1), T2-weighted fluid attenuated inversion recovery (FLAIR), as well as diffusion, pseudo-continuous arterial spin labelling (pcASL), resting state functional MR imaging (rs-fMRI) based on blood oxygen level dependent (BOLD) contrast, quantitative susceptibility mapping (QSM), and both T1 (qT1) and T2 (qT2) relaxometry sequences. Susceptibility-weighted imaging (SWI) results were derived from the acquired QSM image data. The quantitative neuroimaging metrics of interest generated from each sequence are summarized in Table 1. Details of the acquisition protocol are summarized in Table 2. The field of view was 240 mm for all sequences except 3D-T1 and QSM, which have a field of view of 256 mm.

Table 1: Acquired MR sequences, potential quantitative neuroimaging measurement outcomes, and associated physiological changes.

	Outcome	Associated Physiological Change(s)
3D-T1	structural brain volumes; infarct detection	atrophy
FLAIR	white matter hyperintensity volume, detection of lesions presumed vascular origin	inflammation, demyelination, gliosis, axonal loss
rs-fMRI	functional connectivity	network changes in brain regions with synchronous activity
DWI	FA, MD, RD, PSMD, structural connectivity	microstructural changes in white matter
T1 mapping	regional T1 relaxation values	change in local biochemical environment e.g., lipid, hemosiderin
T2 mapping	regional T2 relaxation values	diffuse inflammation, demyelination, gliosis, axonal loss
QSM	regional relative magnetic susceptibility values; regional R2* values; detection of cerebral microbleeds	iron concentration, bleeding
pcASL	cerebral blood perfusion (CBF)	tissue perfusion

Table 2A: MR acquisition parameters for Phase 1 (n=54)

Sequence Type	Slice Orient-ation	TR (ms)	TE (ms)	Flip Angle (°)	Acquisition Matrix	Reconstructed Resolution (mm)	Other Parameters	Acquisition Duration (min:s)
3D-T1	coronal	~7	~2.5	8	256 x 256	0.94 x 0.94 x 1.0	TI = 650 ms	5:03
FLAIR	axial	9000	148	90	256 x 256	0.94 x 0.94 x 3.0	TI = 2250 ms	4:50
rsFMRI	axial	2000	30	70	64 x 64	3.75 x 3.75 x 3.8	200 volumes	5:10
DWI	axial	9000	~80	90	80 x 80	0.94 x 0.94 x 3.0	b=1000 s/mm^2; 31 directions	6:01
qT1[18]	axial	15000	~24	40	160 x 160	1.0 x 1.0 x 4.0	8 echoes; B1 map included	8:09
qT2	coronal	3000	~8-128	90	256 x 128	0.94 x 0.94 x 4.0	16 echoes; echo spacing = 8 ms	9:48
QSM[19]	axial	30	~3-28	20	192 x 192	1.0 x 1.0 x 1.0	8 echoes; echo spacing = 3.4 ms	4:13
pcASL	axial	4899	~11	111	512 x 10	1.88 x 1.88 x 5.0	post label delay = 2025 ms; NEX = 1	6:55

Table 2B: MR acquisition parameters for Phase 2 (*n*=271)

Sequence Type	Slice Orient-ation	TR (ms)	TE (ms)	Flip Angle (°)	Acquisition Matrix	Reconstructed Resolution (mm)	Other Parameters	Acquisition Duration (min:s)
3D-T1	sagittal	~7	~2.5	8	256 x 256	0.94 x 0.94 x 1.0	TI = 650 ms	5:44
FLAIR	axial	9000	148	90	256 x 256	0.94 x 0.94 x 3.0	TI = 2250 ms	4:50
rsFMRI	axial	2000	30	70	64 x 64	3.75 x 3.75 x 3.8	200 volumes	5:10
DWI	axial	9000	~80	90	80 x 80	0.94 x 0.94 x 3.0	*b*=1000 s/mm^2; 31 directions	6:01
qT1[20]	axial	15000	~24	40	160 x 160	1.0 x 1.0 x 4.0	8 echoes; variable TI	5:35
qT2	axial	3000	~8 - 128	90	256 x 128	0.94 x 0.94 x 4.0	16 echoes; echo spacing = 8 ms	9:48
QSM[19]	axial	30	~3 - 28	20	192 x 192	1.0 x 1.0 x 1.0	8 echoes; echo spacing = 3.4 ms	3:58
pcASL	axial	4899	~11	111	512 x 8	1.79 x 1.79 x 3.5	post label delay = 2025 ms; NEX = 3	4:45

DTI— diffusion-weighted imaging; FLAIR—fluid attenuated inversion recovery; IR—inversion recovery; pcASL—pseudo-continuous arterial spin labelling; QSM—quantitative susceptibility mapping; rsFMRI—resting state functional magnetic resonance imaging; T1—spin-lattice relaxation time constant; T2—spin-spin relaxation time constant; TI—inversion time.

In designing this study, we recognized a need to evolve elements of the acquisition protocol, potentially in response to improved or new quantitative imaging techniques. The study was designed to incorporate distinct phases in order to accommodate protocol revisions. Revisions are proposed to and reviewed by the CNS protocol committee who evaluates the impact of the change, the number of participants completed in the current phase, and the number of overall protocol changes. The committee selects and recommends the appropriate timing of any proposed changes. This approach allows for a formal, orderly, well specified, evolution of the study data acquisition over time. To date, two study phases have been completed. The assigned CNS study identification number is used to help identify into which phase a subject is enrolled.

MR imaging was complete by trained staff (>15 years MR imaging research experience) or registered MR technicians. If any possible abnormalities in the images were noted, a neuroradiologist reviewed the images to determine if findings were of potential clinical significance, per the standard policy at our centre. Clinically significant findings as determined by the radiologist were reported to the participant by the radiologist following the procedure as outlined in our REB-approved ethics application.

Image Assessment and Processing:

Images were visually inspected for imaging artifacts like motion or spike noise, prior to any processing steps. The 3D-T1, FLAIR, diffusion-weighted and susceptibility-weighted images were reviewed more thoroughly to identify individuals with possible covert disease pathology associated with cerebral small vessel disease by an expert reader (FS) with >15 years of experience. Presence and number of lacunar stroke, cerebral microbleeds, recent subcortical infarcts, and scoring of white matter hyperintensities were recorded using the criteria for cerebral small vessel disease outlined in the STRIVE paper.[21] Other incidental findings were noted and

recorded. Standard and custom image processing pipelines will be used to generate and analyze the quantitative images and maps. These procedures are briefly summarized in Table 3.

Table 3: Image processing pipelines and measurement outcomes.

Sequence Type	Processing Pipelines	Measurement Outcome
3D T1-weighted	Freesurfer version 6.0	whole brain and regional volumes cortical thickness
FLAIR	Cerebra-LET (CIPAC)	white matter hyperintensity volumes
rs-fMRI	FSL	default mode network connectivity
DWI	FSL, ExploreDTI	FA, MD, RD, PSMD, structural connectivity
qT1	CIPAC custom pipeline	mean regional T1 values, VBM
qT2	StimFit,[22]	mean regional T2 values, VBM
QSM	CIPAC custom pipeline	mean regional susceptibility values, mean regional T2* values, cerebral microbleed detection
pcASL	GE scanner generated CBF maps; BASIL (FSL)	mean regional CBF values

BASIL—Bayesian Inference for Arterial Spin Labeling; CBF—cerebral blood flow (mL/min/100 g tissue); Cerebra-LET—Cerebra Lesion Extraction Tool; CIPAC—Calgary Image Processing and Analysis Centre; DWI—diffusion-weighted imaging; FA—fractional anisotropy; MD—mean diffusivity; pcASL—pseudo-continuous arterial spin labelling; PSMD—peak skeletonized mean diffusivity; QSM—quantitative susceptibility mapping; RD—radial diffusivity; rs-fMRI—resting state functional magnetic resonance imaging; T1—spin-lattice relaxation time constant; T2—spin-spin relaxation time constant; VBM—voxel-based morphometry

Repeatability and Measurement Validation:

A subset of four participants was asked at the time of the first scan to undergo repeated scanning for estimates of precision, variance and repeatability of the proposed quantitative neuroimaging biomarkers. Additionally, these data were used to determine if changes in the MR scanner

software and hardware or in the MR acquisition parameters between protocol phases could be pooled or not depending on the sequence and processing pipelines used. Three additional time points were selected to include scanning before and after a MR system hardware and software upgrades. Not all sequences were acquired at all time points due to scanner and subject time availability. In these cases, a subset of data was acquired, which included 3D-T1, diffusion, T1 mapping, T2 mapping, ASL, QSM, but not FLAIR or rs-fMRI. Figure 1 illustrates the data acquisition timeline with scanner hardware or software changes.

Data Storage:

De-identified demographic, medical, and cognitive assessment data were entered and maintained in a database (REDCap; Vanderbilt University, Nashville TN). MR image data were organized in an Osirix database where images can be reviewed clinically, or the database can be queried for specific criteria. Approximately 17 000 DICOM image files are acquired per imaging session requiring 1.3 GB of disk space for storage without compression, 510 MB with loss-less compression. The exact number of images for each individual can vary depending on head size and the number of slices required to cover the whole brain and whether sequences to be repeated due to motion, for example.

Data Sharing, Ethics and Dissemination:

MR image data and demographic information are available upon request for academic purposes from qualified researchers. Investigators interested in accessing these data are required to complete a data sharing agreement available on request by emailing CalgaryNormStudy@ucalgary.ca. This brief, two-page form, requests a brief description of the study objectives and intended purpose, a list of MR sequences of interest, required medical information, and demographic information. The sharing agreement also requires that the study

requesting data has approval from the appropriate REB. Once the agreement is approved by the CNS leadership and requesting principal investigators, the selected data are provided through secure electronic transfer. All data are de-identified and labelled with CNS-specific identification numbers. Groups requesting data sharing are asked to use the data for the specific intended purpose and to acknowledge the CNS and the CNS funding agencies.

Characterization of the changes in quantitative MR biomarker measures over the adult lifespan as well as estimates of measurement variance and repeatability will be disseminated through peer-reviewed scientific publication.

Patient and Public Involvement:

This research was done without patient or public involvement in the study design and they were not consulted to develop relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

FINDINGS TO DATE:

Recruitment and Population Characteristics:

Study enrolment is ongoing. This report describes the first 55 months of recruitment between 28 May 2013 and 31 December 2018. Over this period 325 participants have consented, provided study data, and completed at least one MR imaging session. In Phase 1 of the study, 54 subjects were recruited. Data from Phase 1 were reviewed and the ASL, T1, and T2 mapping sequences were modified prior to Phase 2 of the study. In the second phase, 271 subjects were recruited. Protocol changes are detailed in the following section “Protocol Revision”.

Additionally, as of 31 December 2018, 114 participants have returned for a second scan session after 41± 5 months (mean ± SD). Of the recruited participants, 24 (7.4%) were not interested in

participating in other imaging studies and 33 (10.1%) were unsure or did not respond to the question prior to the first imaging session. Four individuals (1.2%) were no longer eligible for inclusion in the study after the baseline scan. Reasons for discontinuing participation in the study include existing diagnosis of multiple sclerosis (*n*=1) which was not disclosed until after the baseline MR scan, new diagnosis of mild cognitive impairment (*n*=1), new diagnosis of bipolar disorder (*n*=1) and multiple concussions (*n*=1).

The age and sex distributions of recruited participants are shown in Figure 2. Each age category has a nominal sample size of 40 individuals with nearly equal representation across the six age categories. More women than men (n=189 [58.1 %] versus n=136, respectively) have volunteered and this observation is consistent for each age category. Ninety percent of our participants were right-hand dominant. The distribution of represented ethnicities of the participants were 80.9% White, 16.6% Asian, 0.9% Hispanic, 0.3% First Nations, and 1.2% unknown or not reported.

Neuroimaging indications of covert cerebral small vessel disease are summarized for each age category in Table 4. Other incidental findings that were noted either at the time of the scan by the scan operator or during review of the images by a qualified reader include: cysts (4 pineal, 3 subcutaneous dermoid, 1 arachnoid, and 2 other), 2 subcutaneous nodules, 2 megacisterna magna, 1 pituitary lesion, 1 venous angioma, 1 meningioma/lipoma, 1 asymmetrical cerebellar tonsils, and 1 empty sella tuncica.

Examples of processed quantitative images and maps are shown in Figure 3.

Table 4: Study population prevalence of MR findings associated with cerebral small vessel disease per age category at baseline. Lacunar infarcts, cerebral microbleeds and Fazekas scores[23] of white matter hyperintensities are reported. The Fazekas score is a visual ordinal rating system for white matter hyperintensity severity. It ranges from 0-3, with higher scores indicating more WMH, and is scored separately for periventricular and subcortical regions. The mean ± standard deviation and maximum Fazekas scores for both periventricular and subcortical regions have been included. Summarized are 321 baseline scans; 4 scans were excluded due to poor scan quality or incomplete acquisition.

Age (y)	Chronic Lacunar Infarcts		Cerebral Microbleeds		White Matter Hyperintensities (Fazekas Score)			
	Preval-ence	Average per Person	Preval-ence	Average per Person	Perivent Preval-ence	Perivent; max score	Subcort Preval-ence	Subcort; max score
18 - 29	1/69 (1.4%)	1.0	1/69 (1.4%)	1.0	19/67 (28.4%)	0.25 ± 0.45; 1	13/67 (19.4%)	0.37 ± 0.45; 2
30 - 39	0/54 (0%)	0.0	0/54 (0%)	0.0	28/56 (50.0%)	0.47 ± 0.50; 1	25/56 (44.6%)	0.55 ± 0.50; 1
40 – 49	0/44 (0%)	0.0	2/44 (4.5%)	1.0	31/46 (67.4%)	0.64 ±0.47; 1	27/46 (57.4%)	0.74 ± 0.50; 1
50 – 59	2/50 (4.0%)	1.0	2/50 (4.0%)	2.0	41/48 (85.4%)	0.83 ± 0.39; 2	35/48 (72.9%)	0.88 ± 0.51; 2
60 – 69	6/63 (9.5%)	1.3	2/63 (3.2%)	1.0	51/62 (82.3%)	0.96 ± 0.55; 3	49/62 (79.0%)	1.12 ± 0.56; 2
≥ 70	9/43 (20.9%)	1.2	8/43 (18.6%)	1.5	42/42 (100.0%)	1.30 ± 0.62; 3	41/42 (97.6%)	1.24 ± 0.54; 3

Perivent—periventricular white matter; Subcort—subcortical white matter

Protocol Revisions:

In the first phase of the study (Table 2A), 3D-T1 images and qT2 were acquired with coronal slice orientation for consistency with other existing research protocols at our centre. Driven

equilibrium single observation of T1 (DESPOT1) method[18] was implemented. QSM acquisition used unipolar gradients.[19] After 54 participants were recruited and imaged, the data were reviewed and it was decided to modify the protocol prior to commencing the second phase (Table 2B). Phase 2 protocol adjustments included changing the slice orientation for the 3D-T1 and qT2, implementing a different approach for qT1, and improving the resolution of ASL. We found the DESPOT1 approach provided inconsistent T1 maps with values outside those reported in the literature. A T1 mapping method that is independent of B1 (and thus did not require a B1 map) was developed for brain T1 mapping[20]. The T2 mapping slice orientation was changed from coronal to axial to harmonize the protocol with newer ongoing studies within our centre. To improve the resolution and reduce through-plane blurring, the acquisition matrix and slice thickness of the ASL sequence were changed. The number of excitations was increased to maintain a similar signal to noise ratio.

Repeatability and Measurement Validation:

To assess short- and long-term repeatability a subset of 1 female and 3 male participants were scanned serially (Figure 1). The mean age (± SD) age of these subjects at time of first scan was 36.6 ± 11.7 years. These four participants have been scanned a total 12 times: 3 scans at each time point (baseline, 18, 23, and 39 months).

Significant changes to our scanner occurred at 1) 9 months into the study (software upgrade version DV22.0 to DV24.0 R01, which included modification of a vendor provided multi-echo spin echo sequence for qT2), 2) 21 months (software and hardware upgrade DV24.0 to DV25 R01), and 3) 33 months (head coil replacement). Other standard service and maintenance changes (repair or replacement of cables, gradient amplifiers, coils, etc.) occurred and are noted in Figure 1. All reported times are from start of the CNS in May 2013. Subsequent analysis of

the key quantitative measurements will provide estimates of repeatability, suitable to improve study power calculations.

Data Sharing:

A subset of the imaging and other data has been shared with eight investigators at the University of Calgary. Internal sharing of data was and remains the principle anticipated target group of the CNS Study; however, we also have had opportunity to share data with three investigative teams external to our institution (University of Alberta, Canada; State University of Campinas, Brazil; and University Medical Centre Utrecht, Netherlands). Data equivalent of over 1400 scans have been shared; data from some participants has been shared more than once. The research area of the investigators accessing the CNS data range from depression, inflammatory bowel disease, machine learning, migraine, epilepsy, and stroke. In most cases, only a portion of the acquired data was requested. The most frequently requested imaging data in descending order of demand were 3D-T1, rs-fMRI, FLAIR, pcASL, QSM and qT2.

DISCUSSION:

This report demonstrates a successful recruitment strategy for a prospective, community-recruited quantitative MR study and describes the flexible design and data collection. The data collected will provide estimates of variability and repeatability of each quantitative method and provide data essential for the validation of potential biomarkers of neurodegeneration associated with aging. A distinctive aspect of this study is that it has been designed to incorporate both typical clinical MR sequences and multiple, research-based, quantitative biomarkers including MR relaxometry, diffusion imaging, quantitative susceptibility mapping and cerebral perfusion within a 60-minute scan time. While there are a number of studies that have evaluated one or

two of these methods, there are very few that have incorporated this extensive multimodal approach within an imaging session.[10]

Recruitment and Population Characteristics:

The prevalence of neuroimaging findings indicative of covert cerebrovascular small vessels disease in participants in the 70+ age category was up to 20.9%. Similar prevalence of covert lacunar infarcts of presumed vascular origin, white matter hyperintensities and cerebral microbleeds in older community-dwelling populations have been reported previously.[24] We may need to consider increasing our recruitment numbers in the older age categories to maintain a balanced design of healthy individuals over the adult lifespan. The decision on whether to oversample the older participants is dependent on the definition of what is "normal" aging. Should individuals with possible evidence of cerebral small vessel disease be excluded from the analyses? This question raises some controversy and is as yet unresolved. Given the limited self-reported medical history collected in this study, it will be difficult to determine a definitive diagnosis of cerebral small vessel disease, or other potential confounds like cardiovascular disease.

MR Protocol:

Typically, quantitative MR relaxometry, susceptibility, diffusion and perfusion methods are not all incorporated into either clinical or research studies. This may be due to the additional time required for data acquisition or the expertise and time required for data processing to generate quantitative maps. MR fingerprinting (MRF) has been proposed to overcome a number of these limitation, specifically addressing the longer acquisition times. This emerging approach to quantitative imaging simultaneously generates T1, T2, T2*, and proton density maps from a single acquisition with an approximate scan duration of as little as 16 s.[25 26] MRF uses voxel-

wise pattern matching to a dictionary of MR signal evolution to estimate the MR tissue characteristics. While MRF methods overcome the time limitation for acquisition, these methods are still evolving and have limited availability.

The study design has taken into consideration the evolution of quantitative imaging techniques. Ongoing changes in the MR acquisition protocol can be implemented once an adequate number of participants in each age category have been acquired. The number of participants required can be determined for each quantitative imaging technique. An important outcome of this study will be to establish the measurement variability for each of these quantitative metrics. In practice, a limited number of changes are implemented at any given time. One limitation of this approach is the additional time required for repeatability measures prior to implementation of any intended changes to the MR acquisition protocol.

Repeatability and Measurement Validation:

These repeatability assessments were co-ordinated with selected scheduled changes in the MR operating system. These included major software and hardware upgrades and after the MR system main magnetic field was ramped down for renovations to the MR suite. However, we did not capture repeatability measurements after all unscheduled system changes, including repairs to or replacement of the 12-channel head, neck and spine coil. While this may be a limitation of the repeatability measurements ability to determine the impact of these changes, our MR system is regularly maintained and met the manufacturer specification and operation tolerances after each of these changes. Regular weekly quality assurance and manufacturer service to maintain the MR system within manufacturer specifications is performed, which should mitigate any systems changes.

Another limitation of the repeatability assessment is the limited number and long-term availability of selected participants for evaluation of repeatability and variability. Two participants have recently moved from the local area and new participants have been recruited for future repeatability measures. For purposes of repeatability assessment, we have generally assumed that no significantly physiological changes have occurred in the brains of these participants over either the short or long term. However, if changes were to occur within a given participant, we should be able to identify them as we have nominally three measurements at each time point. These three independent measurements are usually completed within 3 weeks for each individual.

Data Sharing:

Including conventional MR sequences in a purportedly healthy adult population along with research-based sequences, has generated greater interest in data sharing with other local and national studies. It also allows evaluation of these research-based sequences with the context of clinical findings that may help to better understand changes leading to traditional clinical findings. While this study does not match the size or extent of other larger MR initiatives, such as the UK Biobank[27], we have been able to successfully leverage the data by providing normal control data for other studies.

Conclusions:

Over 55 months from study inception in May 2013, the acquisition protocol has undergone one revision, data for four repeatability time points have been collected, 42-month follow-up data has been collected, and the data has been shared effectively. Our study sample includes ethnically diverse people represented in our local community.[28] Data collection and analyses remain

ongoing. Preparation of detailed reports on repeatability of the quantitative measures, cross-sectional, and longitudinal changes over the adult lifespan are underway.

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FIGURE CAPTIONS

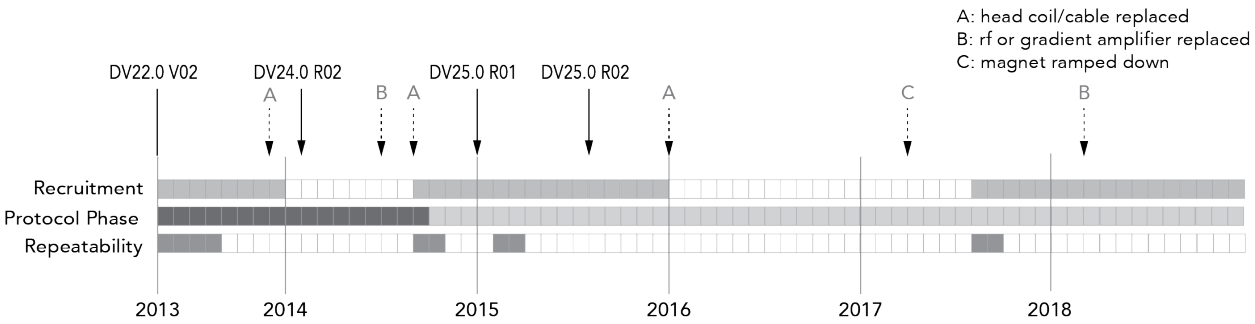
Figure 1: Timeline of MR protocols, recruitment phases, and scanner hardware and software upgrades. The filled squares, each representing one month, indicate when participants were actively recruited and scanned, when the MR acquisition protocol was revised and when repeatability measurements were completed. The solid arrows indicate version changes to the scanner software with the version number indicated above each arrow. The update between DV24.0 and DV25.0 included installation of some additional hardware. The dashed arrows indicate hardware repair or changes.

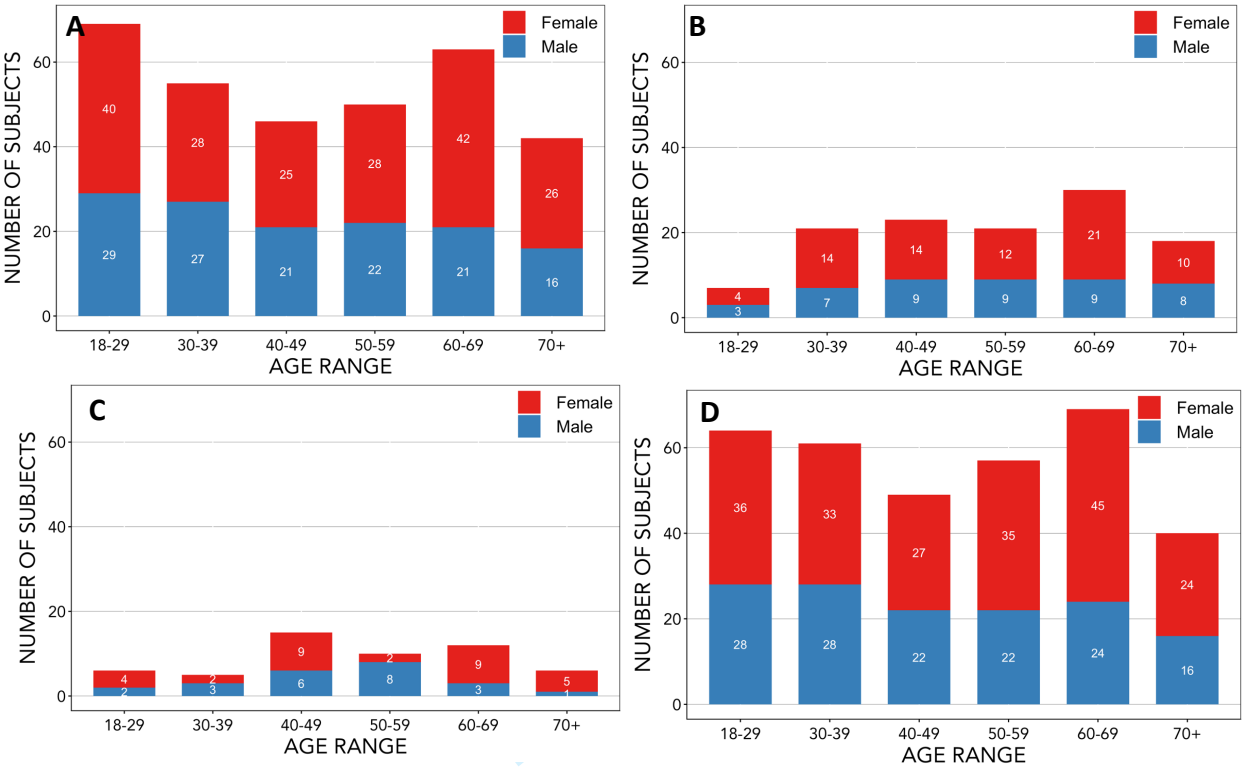
Figure 2A-D: Distribution of age at study entry and sex of participants recruited up to December 2018. The age and sex distribution of participants has been provided for A) all baseline MR scans completed B) follow-up MR scans completed C) participants whose scans were completed with Phase 1 MR acquisition protocol and D) participants whose scans were completed with Phase 2 MR acquisition protocol. Phase 1 MR acquisition protocol was used only for baseline scans; Phase 2 MR acquisition protocol was used for some baseline and all follow-up scans. The exact number of participants for each sex in a given age category are indicated in the appropriate portion of the bar representing that category.

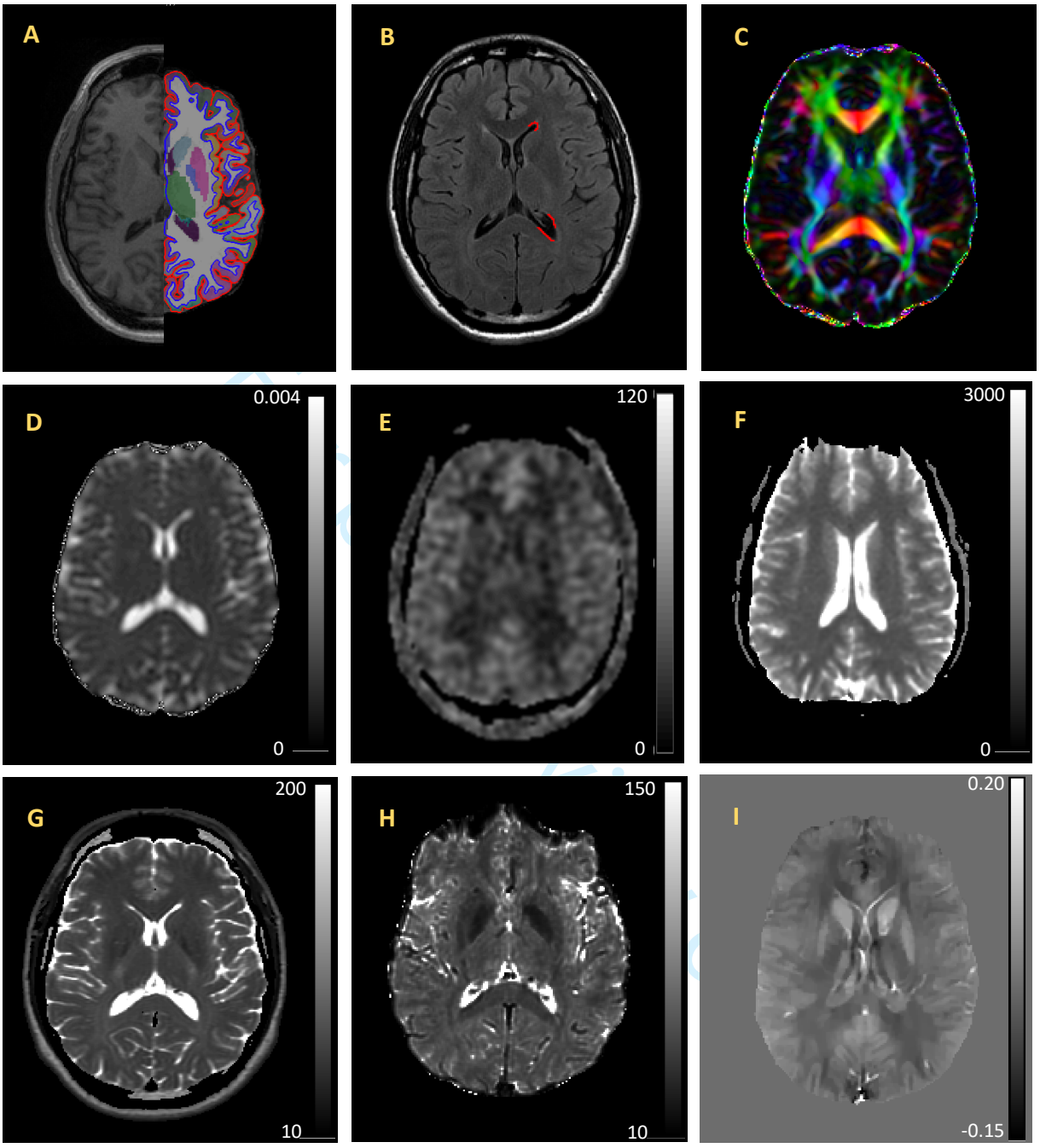
Figure 3: Example quantitative imaging maps and associated processing overlays. A) 3D-T1 image shown with FreeSurfer segmentation and parcellation results overlay on the left hemisphere. B) FLAIR with WMH mask (red) on the left hemisphere C) FA color map indicating the primary diffusion direction of white matter tracts (red indicates left/right; green indicates anterior/posterior; and blue indicates inferior/superior). D) mean diffusivity (MD) map with voxel intensity values are in mm^2/s. E) Cerebral blood flow map. Voxel intensity values are in mm/min/100g tissue. F) Quantitative T1 map. Voxel intensity values are in ms. G) T2 map Voxel intensity values are in ms. H) T2* map. Voxel intensity values are in ms. I) Quantitative susceptibility map. Voxel intensity values are in parts per million (ppm). All images are from the same individual and are shown in radiological orientation.

AUTHORS' CONTRIBUTIONS:

Cheryl R McCreary	conceptualizaion, investigation, data collection and curation, formal analyses, writing original manuscript draft
Marina Salluzzi,	conceptualization, investigation, data collection and curation, analyses, review and editing manuscript
Linda B Andersen	conceptualization, data collection and curation, review and editing manuscript
David Gobbi	methodology, review and editing manuscript
M Louis Lauzon	methodology, review and editing manuscript
Feryal Saad	investigation, review and editing manuscript
Eric E Smith	methodology, investigation, review and editing manuscript
Richard Frayne	conceptualization, funding acquisition, review and editing manuscript







STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5-14
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-14
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-14
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	NA
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	14
		(b) Give reasons for non-participation at each stage	15
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	14-16
		(b) Indicate number of participants with missing data for each variable of interest	15
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	18-22
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18-20
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	21
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	22

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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CALGARY NORMATIVE STUDY: STUDY DESIGN OF A PROSPECTIVE LONGITUDINAL STUDY TO CHARACTERIZE POTENTIAL QUANTITATIVE MR BIOMARKERS OF NEURODEGENERATION OVER THE ADULT LIFESPAN

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Primary Subject Heading:	Neurology
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Keywords:	Dementia < NEUROLOGY, Neuroradiology < RADIOLOGY & IMAGING, Magnetic resonance imaging < RADIOLOGY & IMAGING

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CALGARY NORMATIVE STUDY: STUDY DESIGN OF A PROSPECTIVE LONGITUDINAL STUDY TO CHARACTERIZE POTENTIAL QUANTITATIVE MR BIOMARKERS OF NEURODEGENERATION OVER THE ADULT LIFESPAN

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Keywords: quantitative MR, relaxometry, susceptibility, adult lifespan, study design

Running Title: Calgary Normative Study Design

CALGARY NORMATIVE STUDY: STUDY DESIGN OF A PROSPECTIVE LONGITUDINAL STUDY TO CHARACTERIZE POTENTIAL QUANTITATIVE MR BIOMARKERS OF NEURODEGENERATION OVER THE ADULT LIFESPAN

ABSTRACT:

Introduction: A number of magnetic resonance (MR) imaging methods have been proposed to be useful, quantitative biomarkers of neurodegeneration in aging. The Calgary Normative Study (CNS) is an ongoing single-centre, prospective, longitudinal study that seeks to develop, test and assess quantitative MR methods as potential biomarkers of neurodegeneration. The CNS has three objectives: first and foremost, to evaluate and characterize the dependence of the selected quantitative neuroimaging biomarkers on age over the adult lifespan; secondly, to evaluate the precision, variability and repeatability of quantitative neuroimaging biomarkers as part of biomarker validation providing proof of-concept and proof-of-principle; and thirdly, provide a shared repository of normative data for comparison to various disease cohorts.

Methods and Analysis: Quantitative MR mapping of the brain including longitudinal relaxation time (T1), transverse relaxation time (T2), T2*, magnetic susceptibility (QSM), diffusion and perfusion measurements, as well as morphological assessments are performed. The Montreal Cognitive Assessment (MoCA) and a brief, self-report medical history will be collected. Mixed regression models will be used to characterize changes in quantitative MR biomarker measures over the adult lifespan. In this report we describe the on study design, strategies to recruit and perform changes to the acquisition protocol from inception to 31 December 2018, planned statistical approach and data sharing procedures for the study.

Ethics and Dissemination: Participants provide signed informed consent. Changes in quantitative MR biomarkers measured over the adult lifespan as well as estimates of

measurement variance and repeatability will be disseminated through peer-reviewed scientific publication.

STRENGTHS AND LIMITATIONS:

- Both cross-sectional and longitudinal quantitative MR data are being acquired in a large sample normal aging population to characterize changes in these potential neuroimaging biomarkers over the adult lifespan.
- Clinical and multiple quantitative imaging data are being collected and shared with the research community.
- Measures of repeatability and a process to update the imaging protocol are included in the study design.
- Associated self-reported medical history and cognitive assessments are limited

INTRODUCTION:

Quantitative imaging methods can be defined as "extraction and use of numerical or statistical features from medical images".[1] There are numerous examples where quantitative measures have improved detection of changes or monitoring of the brain.[2-4] Specifically, quantitative neuroimaging using MR is thought to be suitable for identifying potential biomarkers of cognitive impairment risk because it 1) may be more sensitive to early detection of pathological changes within otherwise normal appearing tissue,[5-9] 2) may be more directly related to underlying physiological processes of interest, 3) may have reduced variability across time and/or equipment platforms when compared to traditional, more qualitative, neuroimaging methods, 4) can provide less subjective interpretation, and 5) allows for a low risk, repeatable measurement.[1 10]

Numerous quantitative MR imaging biomarkers have been proposed to assess risk, progression and treatment of age-related neurodegenerative diseases and disorders including cerebral small vessel disease,[10] Alzheimer's disease,[3] Parkinson's disease,[11] multiple sclerosis,[12 13] and Huntington's disease.[14] These quantitative markers typically include measures of brain atrophy, white matter integrity, iron accumulation, and cerebral blood flow. Often, these measures can be determined using standard, vendor-provided MR sequences and freely available image processing packages. Less commonly, longitudinal (T1) and transverse (T2, T2*) relaxometry, vascular permeability and quantitative susceptibility mapping using more customized approaches have been used.

Development of quantitative imaging biomarkers requires appropriate validation and qualification as outlined by the Quantitative Imaging Biomarkers Alliance (QIBA) organized by the Radiological Society of North America.[15 16] An advantage of quantitative imaging is that the

measurements obtained should be, in principle, independent of the specifics of acquisition. While brain atrophy, white matter hyperintensity volume, white matter integrity as measured by diffusion imaging metrics, number of cerebral microbleeds, number and volume of infarcts (including ischemic, hemorrhagic, and lacunar), relative increase in tissue iron have all been associated with one or more neurodegenerative processes, detailed validation of these quantitative biomarkers is relatively lacking.[16] Furthermore, quantitative methods like MR relaxometry, e.g., T1, T2, and T2* mapping, quantitative susceptibility mapping, and arterial spin labelling measurement of cerebral perfusion also require comprehensive evaluation or validation as potential quantitative neuroimaging biomarkers of neurodegenerative disease or pathology.

The Calgary Normative Study (CNS) is a single centre, prospective, longitudinal study with three objectives: 1) to evaluate and characterize the dependence of the selected quantitative neuroimaging biomarkers on age over the adult lifespan; 2) to evaluate the precision, variability and repeatability of quantitative neuroimaging biomarkers as part of biomarker validation providing both proof of-concept and proof-of-principle[16]; and 3) provide a shared repository of normative data for comparison to various disease cohorts. In developing this study, we developed a plan incorporate the ability to revise our data acquisition protocol so that we can refine and accommodate emerging quantitative techniques. We also aimed to complete all imaging procedures and other study evaluations within 2 hours. Our primary research interest is in cerebral small vessel disease and this guided our selection of potential quantitative neuroimaging biomarkers, though in both theory and practice they have proven to be of broad application in other neuroimaging studies. Here we describe the study design and methods used in the CNS and report on recruitment and data sharing. Reporting the specific short- and long-

repeatability or changes in quantitative metrics over the adult lifespan are beyond the scope of this report and will be submitted as separate submissions for publication subsequently.

MATERIALS AND METHODS:

Participant Eligibility and Characterization:

This study was approved by the local research ethics board (REB). Study recruitment is ongoing; volunteers over 18 year of age are being recruited from the community, primarily through local poster advertisement and word-of-mouth. Interested individuals provided written informed consent and were screened for eligibility based on self-reported absence of significant neurological disease, psychiatric disorders, or contraindications for MR imaging at 3 T. Participants with uncertain contraindications (poorly characterized implant, *etc*.) were not imaged and excluded from the study. Participants provided a brief medical history, completed a Montreal Cognitive Assessment (MoCA)[17], completed our institutional MR safety screening and underwent MR imaging.

Demographic and simplified medical information collected includes age, sex, ethnicity, handedness, years of education, smoking history, weight, hypertension or taking medication for the treatment of hypertension, dyslipidemia or taking medication for the treatment of high cholesterol, and presence of diabetes mellitus, and family history of stroke, dementia, Alzheimer's disease, or cardiovascular disease. Participants were also asked if they are willing to return for a follow-up visit and if they are willing to be contacted in the future for possible participation in other research studies. All data were de-identified and labelled with CNS-specific identification numbers.

The MoCA is a cognitive screening test designed to assist the detection of mild cognitive impairment, which was administered and scored by trained research personnel. If participants were not fluent in English, then alternate language versions of the MoCA (Mandarin and Spanish) were offered and were administered with the aid of a translator. Participants that scored <26 on the MoCA were considered screen failures for normal cognition.[17] However, these individuals still completed the study procedures, though we anticipate that their data may be excluded from some subsequent analyses. Participants were not informed of their MoCA scores because the MoCA alone is not sufficient to diagnose a clinical cognitive disorder such as MCI, and there will be false positive low scores. If concerns about a clinical cognitive disorder arose during the testing the participant was advised to consult their primary care practitioner.

We sought to enroll an approximately balanced distribution of men and women in each of six age categories: 18-29, 30-39, 40-49, 50-59, 60-69, 70+ years. We anticipate that recruitment of eligible normal older participants would be more difficult due to the increased prevalence of stroke and or other significant neurological disease with age. For this reason, we decided to group individuals 70 years of age and older into a single age category, anticipating that we would have difficulty recruiting a sufficient number of individuals over 80 years of age (Figure 1).

A nominal sample size of 20 men and 20 women was initially targeted for each age category, for a nominal CNS recruitment of 240. This sample size estimate was based on estimates of variance from previous work using voxel-based analysis of T2 changes in temporal lobe epilepsy.[2] The study was initially powered ($\alpha = 0.05$, $1\text{-}\beta = 0.8$) to detect T2 changes of 3.4 ms or greater. Larger or smaller sample sizes may be required depending on the quantitative measure, region, and analysis method chosen. To accommodate differences in power requirements, protocol revisions, and secondary analyses (eg sex differences), recruitment will

continue after the nominal sample size has been achieved. Establishing better variance estimates for each quantitative imaging method is one of the objectives of the Calgary Normative Study.

MR Imaging Acquisition Protocol:

MR imaging is being completed at a single centre on a 3 T MR scanner (MR750, General Electric Healthcare, Waukesha, WI) using the vendor-supplied, 12-channel head, neck and spine coil. Standard procedure at our centre is to perform daily quality control scans and to follow the vendor recommended service guidelines.

Prior to setting up the initial MR protocol, we informally surveyed five local research teams with respect to their MR imaging protocols and typical outcome measures. This survey guided some of our protocol decisions and ensured that aspects of the MR acquisition parameters were similar to other studies in our centre. This similarity in some protocol acquisitions makes it feasible to share and leverage the data appropriately for comparison with disease cohorts.

The MR imaging protocol includes conventional structural 3D T1-weighted (3D-T1), T2-weighted fluid attenuated inversion recovery (FLAIR), as well as diffusion, pseudo-continuous arterial spin labelling (pcASL), resting state functional MR imaging (rs-fMRI) based on blood oxygen level dependent (BOLD) contrast, quantitative susceptibility mapping (QSM), and both T1 (qT1) and T2 (qT2) relaxometry sequences. Susceptibility-weighted imaging (SWI) results were derived from the acquired QSM image data. The quantitative neuroimaging metrics of interest generated from each sequence are summarized in Table 1. Details of the acquisition protocol are summarized in Table 2. The field of view was 240 mm for all sequences except 3D-T1 and QSM, which have a field of view of 256 mm.

Table 1: Acquired MR sequences, potential quantitative neuroimaging measurement outcomes, and associated physiological changes.

	Outcome	Associated Physiological Change(s)
3D-T1	structural brain volumes; infarct detection	atrophy
FLAIR	white matter hyperintensity volume, detection of lesions presumed vascular origin	inflammation, demyelination, gliosis, axonal loss
rs-fMRI	functional connectivity	network changes in brain regions with synchronous activity
DWI	FA, MD, RD, PSMD, structural connectivity	microstructural changes in white matter
T1 mapping	regional T1 relaxation values	change in local biochemical environment e.g., lipid, hemosiderin
T2 mapping	regional T2 relaxation values	diffuse inflammation, demyelination, gliosis, axonal loss
QSM	regional relative magnetic susceptibility values; regional R2* values; detection of cerebral microbleeds	iron concentration, bleeding
pcASL	cerebral blood perfusion (CBF)	tissue perfusion

Table 2A: MR acquisition parameters for Phase 1 (*n*=54)

Sequence Type	Slice Orient-ation	TR (ms)	TE (ms)	Flip Angle (°)	Acquisition Matrix	Reconstructed Resolution (mm)	Other Parameters	Acquisition Duration (min:s)
3D-T1	coronal	~7	~2.5	8	256 x 256	0.94 x 0.94 x 1.0	TI = 650 ms	5:03
FLAIR	axial	9000	148	90	256 x 256	0.94 x 0.94 x 3.0	TI = 2250 ms	4:50
rsFMRI	axial	2000	30	70	64 x 64	3.75 x 3.75 x 3.8	200 volumes	5:10
DWI	axial	9000	~80	90	80 x 80	0.94 x 0.94 x 3.0	*b*=1000 s/mm^2; 31 directions	6:01
qT1[18]	axial	15000	~24	40	160 x 160	1.0 x 1.0 x 4.0	8 echoes; B1 map included	8:09
qT2	coronal	3000	~8-128	90	256 x 128	0.94 x 0.94 x 4.0	16 echoes; echo spacing = 8 ms	9:48
QSM[19]	axial	30	~3-28	20	192 x 192	1.0 x 1.0 x 1.0	8 echoes; echo spacing = 3.4 ms	4:13
pcASL	axial	4899	~11	111	512 x 10	1.88 x 1.88 x 5.0	post label delay = 2025 ms; NEX = 1	6:55

Table 2B: MR acquisition parameters for Phase 2 (*n*=271)

Sequence Type	Slice Orient-ation	TR (ms)	TE (ms)	Flip Angle (°)	Acquisition Matrix	Reconstructed Resolution (mm)	Other Parameters	Acquisition Duration (min:s)
3D-T1	sagittal	~7	~2.5	8	256 x 256	0.94 x 0.94 x 1.0	TI = 650 ms	5:44
FLAIR	axial	9000	148	90	256 x 256	0.94 x 0.94 x 3.0	TI = 2250 ms	4:50
rsFMRI	axial	2000	30	70	64 x 64	3.75 x 3.75 x 3.8	200 volumes	5:10
DWI	axial	9000	~80	90	80 x 80	0.94 x 0.94 x 3.0	*b*=1000 s/mm^2; 31 directions	6:01
qT1[20]	axial	15000	~24	40	160 x 160	1.0 x 1.0 x 4.0	8 echoes; variable TI	5:35
qT2	axial	3000	~8 - 128	90	256 x 128	0.94 x 0.94 x 4.0	16 echoes; echo spacing = 8 ms	9:48
QSM[19]	axial	30	~3 - 28	20	192 x 192	1.0 x 1.0 x 1.0	8 echoes; echo spacing = 3.4 ms	3:58
pcASL	axial	4899	~11	111	512 x 8	1.79 x 1.79 x 3.5	post label delay = 2025 ms; NEX = 3	4:45

DTI— diffusion-weighted imaging; FLAIR—fluid attenuated inversion recovery; IR—inversion recovery; pcASL—pseudo-continuous arterial spin labelling; QSM—quantitative susceptibility mapping; rsFMRI—resting state functional magnetic resonance imaging; T1—spin-lattice relaxation time constant; T2—spin-spin relaxation time constant; TI—inversion time.

In designing this study, we recognized a need to evolve elements of the acquisition protocol, potentially in response to improved or new quantitative imaging techniques. The study was designed to incorporate distinct phases in order to accommodate protocol revisions. Revisions are proposed to and reviewed by the CNS protocol committee who evaluates the impact of the change, the number of participants completed in the current phase, and the number of overall protocol changes. The committee selects and recommends the appropriate timing of any proposed changes. This approach allows for a formal, orderly, well specified, evolution of the study data acquisition over time.

To date, two study phases have been completed. The assigned CNS study identification number is used to help identify into which phase a subject is enrolled. In the first phase of the study (Table 2A), 3D-T1 images and qT2 were acquired with coronal slice orientation for consistency with other existing research protocols at our centre. Driven equilibrium single observation of T1 (DESPOT1) method[18] was implemented. QSM acquisition used unipolar gradients.[19] After 54 participants were recruited and imaged, the data were reviewed and it was decided to modify the protocol. A Phase 2 protocol (Table 2B) was implemented with changes in slice orientation for the 3D-T1 and qT2, approach for qT1, and resolution of ASL. We found the DESPOT1 approach provided inconsistent T1 maps with values outside those reported in the literature. A T1 mapping method that is independent of B1 (and thus did not require a B1 map) was developed for brain T1 mapping[20]. The T2 mapping slice orientation was changed from coronal to axial to harmonize the protocol with newer ongoing studies within our centre. To improve the resolution and reduce through-plane blurring, the acquisition matrix and slice thickness of the ASL sequence were changed. The number of excitations was increased to maintain a similar signal to noise ratio.

MR imaging was complete by trained staff (>15 years MR imaging research experience) or registered MR technicians. If any possible abnormalities in the images were noted, a neuroradiologist reviewed the images to determine if findings were of potential clinical significance, per the standard policy at our centre. Clinically significant findings as determined by the radiologist were reported to the participant by the radiologist following the procedure as outlined in our REB-approved ethics application.

Image Assessment and Processing:

Images were visually inspected for imaging artifacts like motion or spike noise, prior to any processing steps. The 3D-T1, FLAIR, diffusion-weighted and susceptibility-weighted images were reviewed more thoroughly to identify individuals with possible covert disease pathology associated with cerebral small vessel disease by an expert reader (FS) with >15 years of experience. Presence and number of lacunar stroke, cerebral microbleeds, recent subcortical infarcts, and scoring of white matter hyperintensities were recorded using the criteria for cerebral small vessel disease outlined in the STRIVE paper.[21] Other incidental findings were noted and recorded. Standard and custom image processing pipelines will be used to generate and analyze the quantitative images and maps. These procedures are briefly summarized in Table 3 with example images and maps shown in Figure 2.

Table 3: Image processing pipelines and measurement outcomes.

Sequence Type	Processing Pipelines	Measurement Outcome
3D T1-weighted	Freesurfer version 6.0	whole brain and regional volumes cortical thickness
FLAIR	Cerebra-LET (CIPAC)	white matter hyperintensity volumes
rs-fMRI	FSL	default mode network connectivity
DWI	FSL, ExploreDTI	FA, MD, RD, PSMD, structural connectivity
qT1	CIPAC custom pipeline	mean regional T1 values, VBM
qT2	StimFit,[22]	mean regional T2 values, VBM
QSM	CIPAC custom pipeline	mean regional susceptibility values, mean regional T2* values, cerebral microbleed detection
pcASL	GE scanner generated CBF maps; BASIL (FSL)	mean regional CBF values

BASIL—Bayesian Inference for Arterial Spin Labeling; CBF—cerebral blood flow (mL/min/100 g tissue); Cerebra-LET—Cerebra Lesion Extraction Tool; CIPAC—Calgary Image Processing and Analysis Centre; DWI—diffusion-weighted imaging; FA—fractional anisotropy; MD—mean diffusivity; pcASL—pseudo-continuous arterial spin labelling; PSMD—peak skeletonized mean diffusivity; QSM—quantitative susceptibility mapping; RD—radial diffusivity; rs-fMRI—resting state functional magnetic resonance imaging; T1—spin-lattice relaxation time constant; T2—spin-spin relaxation time constant; VBM—voxel-based morphometry

Repeatability and Measurement Validation:

A subset of four participants was asked at the time of the first scan to undergo repeated scanning for estimates of precision, variance and repeatability of the proposed quantitative neuroimaging biomarkers. Additionally, these data were used to determine if changes in the MR scanner software and hardware or in the MR acquisition parameters between protocol phases could be

pooled or not depending on the sequence and processing pipelines used. Three additional time points were selected to include scanning before and after a MR system hardware and software upgrades. Not all sequences were acquired at all time points due to scanner and subject availability. In these cases, a subset of data was acquired, which included 3D-T1, diffusion, T1 mapping, T2 mapping, ASL, QSM, but not FLAIR or rs-fMRI. Figure 3 illustrates the data acquisition timeline with scanner hardware or software changes.

Statistical Analyses:

From the repeatability data, the within-subject variance, minimum detectable difference, and repeatability will be estimated at select regions-of-interest from repeated scans at each time point. Within-subject variance will include the possible impact of MR system changes on these repeated measures across time points. Changes in quantitative neuroimaging outcomes over the adult lifespan will be assessed using linear mixed effects models. Linear and quadratic functions with age will be considered to determine the most appropriate fitting model based on Akaike information criterion and Bayesian information criterion.

Data Storage:

De-identified demographic, medical, and cognitive assessment data were entered and maintained in a database (REDCap; Vanderbilt University, Nashville TN). MR image data were organized in an Osirix database where images can be reviewed clinically, or the database can be queried for specific criteria. Approximately 17 000 DICOM image files are acquired per imaging session requiring 1.3 GB of disk space for storage without compression, 510 MB with loss-less compression. The exact number of images for each individual can vary depending on head size and the number of slices required to cover the whole brain and whether sequences needed to be repeated due to motion, for example.

Data Sharing, Ethics and Dissemination:

MR image data and demographic information are available upon request for academic purposes from qualified researchers. Investigators interested in accessing these data are required to complete a data sharing agreement available on request by emailing CalgaryNormStudy@ucalgary.ca. This brief, two-page form, requests a brief description of the study objectives and intended purpose, a list of MR sequences of interest, required medical information, and demographic information. The sharing agreement also requires that the study requesting data has approval from the appropriate REB. Once the agreement is approved by the CNS leadership and requesting principal investigators, the selected data are provided through secure electronic transfer. All data are de-identified and labelled with CNS-specific identification numbers. Information regarding MR system upgrades, protocol changes, and repeatability data may be provided upon request. Groups requesting data are asked to limit use and analysis of the data to those outlined in their data sharing agreement, not sharing with other groups or use the data for other unplanned purposes, and to acknowledge the CNS and the CNS funding agencies in presentations or publications. Further or previously unplanned analyses of requested data would require a simple amendment to the data sharing agreement

Characterization of the changes in quantitative MR biomarker measures over the adult lifespan as well as estimates of measurement variance and repeatability will be disseminated through peer-reviewed scientific publication.

Patient and Public Involvement:

This research was done without patient or public involvement in the study design and they were not consulted to develop relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

DISCUSSION:

This report demonstrates a successful recruitment strategy for a prospective, community-recruited quantitative MR study and describes the flexible design and data collection. The data collected will provide estimates of variability and repeatability of each quantitative method and provide data essential for the validation of potential biomarkers of neurodegeneration associated with aging. A distinctive aspect of this study is that it has been designed to incorporate both typical clinical MR sequences and multiple, research-based, quantitative biomarkers including MR relaxometry, diffusion imaging, quantitative susceptibility mapping and cerebral perfusion within a 60-minute scan time. While there are a number of studies that have evaluated one or two of these methods, there are very few that have incorporated this extensive multimodal approach within an imaging session.[10]

Recruitment and Population Characteristics:

The prevalence of neuroimaging findings indicative of covert cerebrovascular small vessels disease in participants in the 70+ age category was up to 20.9%. Similar prevalence of covert lacunar infarcts of presumed vascular origin, white matter hyperintensities and cerebral microbleeds in older community-dwelling populations have been reported previously.[23] We may need to consider increasing our recruitment numbers in the older age categories to maintain a balanced design of healthy individuals over the adult lifespan. The decision on whether to oversample the older participants is dependent on the definition of what is “normal” aging. Should individuals with possible evidence of cerebral small vessel disease be excluded from the analyses? This question raises some controversy and is as yet unresolved. Given the limited self-reported medical history collected in this study, it will be difficult to determine a definitive

diagnosis of cerebral small vessel disease, or other potential confounds like cardiovascular disease.

MR Protocol:

Typically, quantitative MR relaxometry, susceptibility, diffusion and perfusion methods are not all incorporated into either clinical or research studies. This may be due to the additional time required for data acquisition or the expertise and time required for data processing to generate quantitative maps. MR fingerprinting (MRF) has been proposed to overcome a number of these limitation, specifically addressing the longer acquisition times. This emerging approach to quantitative imaging simultaneously generates T1, T2, T2*, and proton density maps from a single acquisition with an approximate scan duration of as little as 16 s.[24 25] MRF uses voxel-wise pattern matching to a dictionary of MR signal evolution to estimate the MR tissue characteristics. While MRF methods overcome the time limitation for acquisition, these methods are still evolving and have limited availability.

The study design has taken into consideration the evolution of quantitative imaging techniques. Ongoing changes in the MR acquisition protocol can be implemented once an adequate number of participants in each age category have been acquired. The number of participants required can be determined for each quantitative imaging technique. An important outcome of this study will be to establish the measurement variability for each of these quantitative metrics. In practice, a limited number of changes are implemented at any given time. One limitation of this approach is the additional time required for repeatability measures prior to implementation of any intended changes to the MR acquisition protocol.

Repeatability and Measurement Validation:

These repeatability assessments were co-ordinated with selected scheduled changes in the MR operating system. These included major software and hardware upgrades and after the MR system main magnetic field was ramped down for renovations to the MR suite. However, we did not capture repeatability measurements after all unscheduled system changes, including repairs to or replacement of the 12-channel head, neck and spine coil. While this may be a limitation of the repeatability measurements ability to determine the impact of these changes, our MR system is regularly maintained and met the manufacturer specification and operation tolerances after each of these changes. Regular weekly quality assurance and manufacturer service to maintain the MR system within manufacturer specifications is performed, which should mitigate any systems changes.

Another limitation of the repeatability assessment is the limited number and long-term availability of selected participants for evaluation of repeatability and variability. Two participants have recently moved from the local area and new participants have been recruited for future repeatability measures. For purposes of repeatability assessment, we have generally assumed that no significantly physiological changes have occurred in the brains of these participants over either the short or long term. However, if changes were to occur within a given participant, we should be able to identify them as we have nominally three measurements at each time point. These three independent measurements are usually completed within 3 weeks for each individual.

Data Sharing:

Including conventional MR sequences in a purportedly healthy adult population along with research-based sequences, has generated greater interest in data sharing with other local and national studies. A subset of the imaging and other data has been shared with eight investigators

at the University of Calgary. Internal sharing of data was and remains the principle anticipated target group of the CNS Study; however, we also have had opportunity to share data with three investigative teams external to our institution (University of Alberta, Canada; State University of Campinas, Brazil; and University Medical Centre Utrecht, Netherlands). Data equivalent of over 1400 scans have been shared; data from some participants has been shared more than once. The research area of the investigators accessing the CNS data range from depression, inflammatory bowel disease, machine learning, migraine, epilepsy, and stroke. In most cases, only a portion of the acquired data was requested. The most frequently requested imaging data in descending order of demand were 3D-T1, rs-fMRI, FLAIR, pcASL, QSM and qT2. It also allows evaluation of these research-based sequences with the context of clinical findings that may help to better understand changes leading to traditional clinical findings. While this study does not match the size or extent of other larger MR initiatives, such as the UK Biobank[26], we have been able to successfully leverage the data by providing normal control data for other studies.

Summary:

Over 55 months from study inception in May 2013, the acquisition protocol has undergone one revision, data for four repeatability time points have been collected, 42-month follow-up data has been collected, and the data has been shared effectively. Our study sample includes ethnically diverse people represented in our local community.[27] Data collection and analyses remain ongoing. Preparation of detailed reports on repeatability of the quantitative measures, cross-sectional, and longitudinal changes over the adult lifespan are underway.

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COMPETING INTEREST STATEMENT

The authors have no competing interests to declare.

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FIGURE CAPTIONS

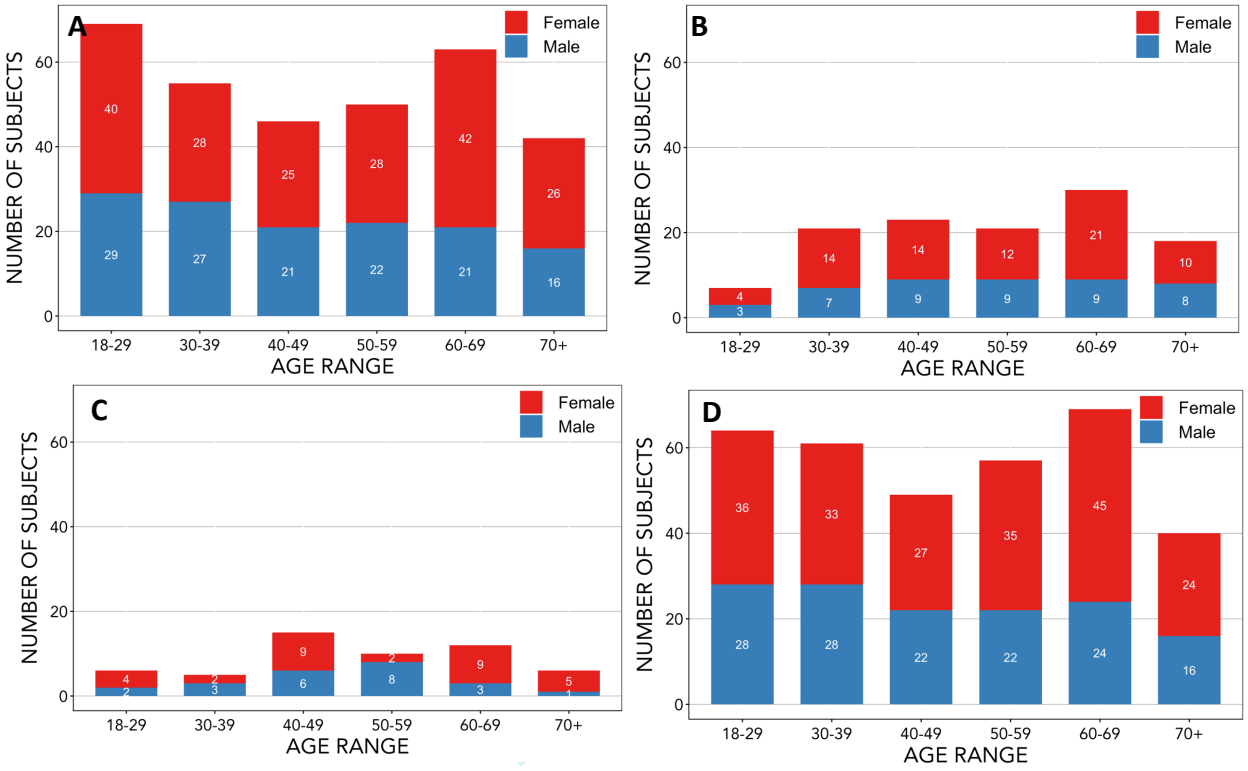
Figure 1A-D: Distribution of age at study entry and sex of participants recruited up to December 2018. The age and sex distribution of participants has been provided for A) all baseline MR scans completed B) follow-up MR scans completed C) participants whose scans were completed with Phase 1 MR acquisition protocol and D) participants whose scans were completed with Phase 2 MR acquisition protocol. Phase 1 MR acquisition protocol was used only for baseline scans; Phase 2 MR acquisition protocol was used for some baseline and all follow-up scans. The exact number of participants for each sex in a given age category are indicated in the appropriate portion of the bar representing that category.

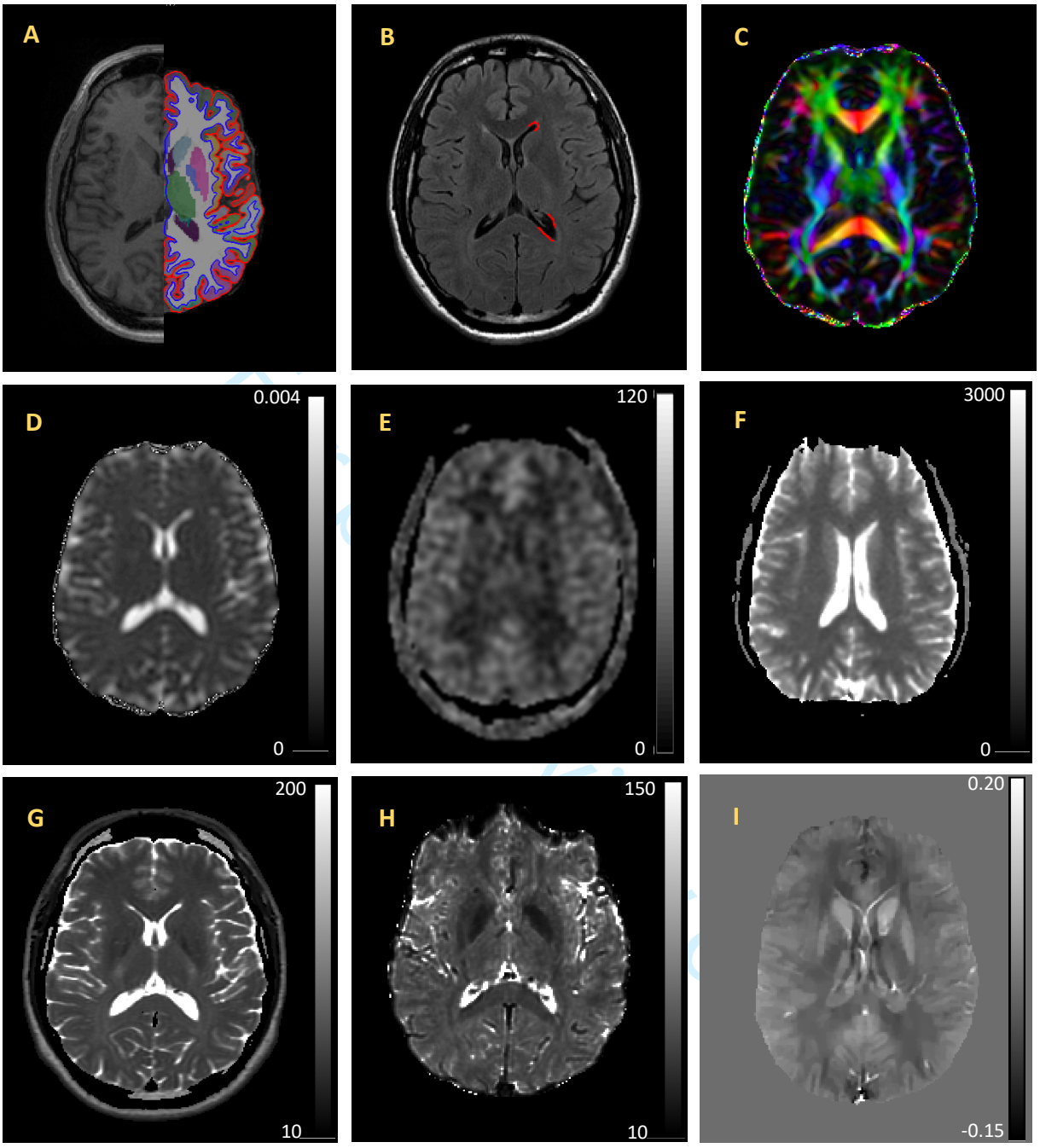
Figure 2: Example quantitative imaging maps and associated processing overlays. A) 3D-T1 image shown with FreeSurfer segmentation and parcellation results overlay on the left hemisphere. B) FLAIR with WMH mask (red) on the left hemisphere C) FA color map indicating the primary diffusion direction of white matter tracts (red indicates left/right; green indicates anterior/posterior; and blue indicates inferior/superior). D) mean diffusivity (MD) map with voxel intensity values are in mm^2/s. E) Cerebral blood flow map. Voxel intensity values are in mm/min/100g tissue. F) Quantitative T1 map. Voxel intensity values are in ms. G) T2 map Voxel intensity values are in ms. H) T2* map. Voxel intensity values are in ms. I) Quantitative susceptibility map. Voxel intensity values are in parts per million (ppm). All images are from the same individual and are shown in radiological orientation.

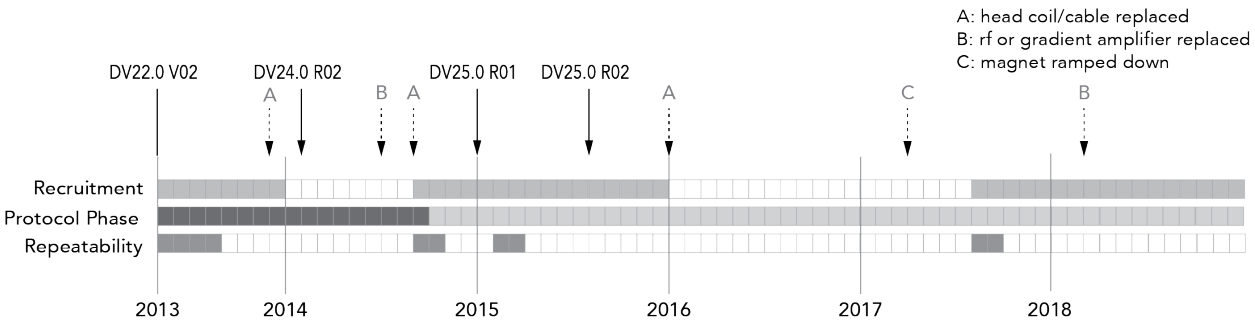
Figure 3: Timeline of MR protocols, recruitment phases, and scanner hardware and software upgrades. The filled squares, each representing one month, indicate when participants were actively recruited and scanned, when the MR acquisition protocol was revised and when repeatability measurements were completed. The solid arrows indicate version changes to the scanner software with the version number indicated above each arrow. The update between DV24.0 and DV25.0 included installation of some additional hardware. The dashed arrows indicate hardware repair or changes.

AUTHORS' CONTRIBUTIONS:

Cheryl R McCreary	conceptualization, investigation, data collection and curation, formal analyses, writing original manuscript draft, review and editing
Marina Salluzzi,	conceptualization, investigation, data collection and curation, analyses, review and editing manuscript
Linda B Andersen	conceptualization, data collection and curation, review and editing manuscript
David Gobbi	methodology, review and editing manuscript
M Louis Lauzon	methodology, review and editing manuscript
Feryal Saad	investigation, review and editing manuscript
Eric E Smith	methodology, investigation, review and editing manuscript
Richard Frayne	conceptualization, funding acquisition, review and editing manuscript







STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5-14
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-14
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-14
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-13
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	15[A]
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	NA
		(*e*) Describe any sensitivity analyses	NA

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Study design paper-NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	15
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	*NA*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	17-20
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17-20
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	21

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

[A] As a study protocol paper the results section is not included. The outcomes and participant information will be the subject of future publications.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.