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Authors

Liu, Peiying

Lin, Zixuan

Hazel, Kaisha

et al.

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RESEARCH ARTICLE

Cerebrovascular reactivity MRI as a biomarker for cerebral small vessel disease–related cognitive decline: Multi-site validation in the MarkVCID Consortium

Peiying Liu[1,2] | **Zixuan Lin**[1] | **Kaisha Hazel**[1] | **George Pottanat**[1] | **Cuimei Xu**[1] | **Dengrong Jiang**[1] | **Jay J. Pillai**[1,3] | **Emma Lucke**[1] | **Christopher E. Bauer**[4] | **Brian T. Gold**[4] | **Steven M. Greenberg**[5] | **Karl G. Helmer**[6] | **Kay Jann**[7] | **Gregory Jicha**[8] | **Joel Kramer**[9] | **Pauline Maillard**[10] | **Rachel M. Mulavelil**[11] | **Pavel Rodriguez**[11] | **Claudia L. Satizabal**[11] | **Kristin Schwab**[5] | **Sudha Seshadri**[11] | **Herpreet Singh**[6] | **Ángel G. Velarde Dediós**[11] | **Danny J. J. Wang**[7] | **Rita R. Kalyani**[12] | **Abhay Moghekar**[13] | **Paul B. Rosenberg**[14] | **Sevil Yasar**[12] | **Marilyn Albert**[13] | **Hanzhang Lu**[1,15,16]

[1]Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[2]Department of Diagnostic Radiology & Nuclear Medicine, University of Maryland School of Medicine, Baltimore, Maryland, USA

[3]Department of Division of Neuroradiology, Mayo Clinic College of Medicine and Science, Rochester, Minnesota, USA

[4]Department of Neuroscience, University of Kentucky, Lexington, Kentucky, USA

[5]Department of Neurology, Massachusetts General Hospital Stroke Research Center, Harvard Medical School, Boston, Massachusetts, USA

[6]Department of Radiology, Massachusetts General Hospital, Charlestown, Massachusetts, USA

[7]Laboratory of Functional MRI Technology, Keck School of Medicine, Stevens Neuroimaging and Informatics Institute, University of Southern California, Los Angeles, California, USA

[8]Department of Neurology, University of Kentucky, Lexington, Kentucky, USA

[9]Department of Neurology, University of California, San Francisco, San Francisco, California, USA

[10]Department of Neurology, University of California, Davis, Sacramento, California, USA

[11]Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, UT Health San Antonio, San Antonio, Texas, USA

[12]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[13]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[14]Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

[15]Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, USA

[16]F.M. Kirby Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland, USA

Correspondence
Hanzhang Lu, Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, 600 N Wolfe Street, Park 322, Baltimore, MD 21287, USA.
Email: hanzhang.lu@jhu.edu

Abstract

INTRODUCTION: Vascular contributions to cognitive impairment and dementia (VCID) represent a major factor in cognitive decline in older adults. The present study examined the relationship between cerebrovascular reactivity (CVR) measured by

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magnetic resonance imaging (MRI) and cognitive function in a multi-site study, using a predefined hypothesis.

METHODS: We conducted the study in a total of three analysis sites and 263 subjects. Each site performed an identical CVR MRI procedure using 5% carbon dioxide inhalation. A global cognitive measure of Montreal Cognitive Assessment (MoCA) and an executive function measure of item response theory (IRT) score were used as outcomes.

RESULTS: CVR and MoCA were positively associated, and this relationship was reproduced at all analysis sites. CVR was found to be positively associated with executive function.

DISCUSSION: The predefined hypothesis on the association between CVR and a global cognitive score was validated in three independent analysis sites, providing support for CVR as a biomarker in VCID.

KEYWORDS
blood oxygenation level dependent, carbon dioxide, cerebrovascular reactivity, cognitive function, magnetic resonance imaging, Montreal Cognitive Assessment, vascular cognitive impairment, vascular cognitive impairment and dementia

Highlights

- This study measured a novel functional index of small arteries referred to as cerebrovascular reactivity (CVR).
- CVR was positively associated with global cognition in older adults.
- This finding was observed in three independent cohorts at three sites.
- Our statistical analysis plan was predefined before beginning data collection.

1 | INTRODUCTION

Cerebral small vessel disease (SVD)–related vascular contributions to cognitive impairment and dementia (VCID) represent a major factor contributing to cognitive decline in older adults.[1,2] However, there currently does not exist a validated biomarker for the diagnosis and treatment monitoring of this condition. The US National Institute on Neurological Disorders and Stroke (NINDS), a branch of the National Institutes of Health, funded the MarkVCID Consortium (https://markvcid.partners.org/) to identify and validate clinical-trial–ready biomarkers for VCID.[3,4] The study had two phases. In Phase 1 (referred to as the UH2 phase), each site in the MarkVCID Consortium collected and presented single-site data to support the proposal of their candidate biomarkers. In Phase 2 (referred to as the UH3 phase), from the set of all proposed biomarkers, the consortium selected 11 for multi-site validation, including 4 fluid-based biomarkers and 7 imaging-based biomarkers. Cerebrovascular reactivity (CVR) magnetic resonance imaging (MRI) was one of the imaging-based biomarkers evaluated and tested in the MarkVCID UH3-phase study.

CVR denotes the ability of cerebral small vessels to dilate in response to vasoactive stimuli and is thought to directly reflect the physiological function of the brain microvasculature. CVR can be measured non-invasively by administering a vasoactive challenge, such as carbon dioxide (CO_2) inhalation, while continuously collecting blood oxygen–level-dependent (BOLD) MRI.[5,6] Previous studies have suggested that CVR may be a sensitive biomarker in dementia,[7–9] mild cognitive impairment (MCI),[10–12] and SVD.[13,14] Single-site data of 72 older adult participants collected in Phase 1 of the MarkVCID Consortium revealed that whole-brain CVR was significantly lower in the cognitively impaired participants compared to the normal group, and higher whole-brain CVR was associated with better performance on the Montreal Cognitive Assessment (MoCA).[12] In addition, a comprehensive instrumental validation study demonstrated excellent inter-rater, inter-scanner, and test–retest reliability of CVR in healthy volunteers, and suggested that CVR has suitable instrumental properties for use as an imaging biomarker of cerebrovascular function in multi-site and longitudinal observational studies and clinical trials.[15] Based on these results, CVR was selected to continue into the multi-site testing in Phase 2 of the MarkVCID Consortium study from 2018 to 2021 for biological validation.

Therefore, the present work from Phase 2 of the MarkVCID Consortium aimed to report the relationship between CVR and

cognitive function at three analysis sites (data separately analyzed for each site), based on a predefined sample size and prespecified hypothesis. As part of its prespecified approach to validation,[3,4] the MarkVCID Consortium mandated that relationships between the candidate SVD biomarkers and cognition be independently tested at multiple sites to ensure generalizability to multi-center studies.

2 | METHODS

2.1 | Prespecified hypothesis

Per the MarkVCID Consortium protocol, a prespecified hypothesis was provided before the start of Phase 2, so that the primary analysis method was clearly defined. For CVR, based on our Phase 1 finding,[12] we hypothesized that CVR would be associated with the global cognitive measure of MoCA after adjusting for age, sex, and education, and that this association would be observed in data collected and analyzed at each individual site. It was also prespecified (based on phase 1 single-site data) that each site should have a minimum of 75 participants to provide sufficient power for the proposed analysis.

2.2 | Subjects

A total of 294 subjects participated in this multi-site study. The enrollment sites were: Johns Hopkins University (JHU, $N = 80$), University of Texas Health Science Center at San Antonio (UTHSCSA, $N = 102$), University of Kentucky (UKY, $N = 58$), and University of Southern California (USC, $N = 54$). The JHU data sets were collected on a 3T Philips Achieva scanner. The UTHSCSA data sets were collected on a 3T Siemens Trio scanner. Per the predefined sample-size requirement described above, the UKY and USC data sets (both of which were collected on a 3T Siemens Prisma scanner) were combined into one analysis site, with the enrollment site included as a covariate. The MarkVCID Consortium created a harmonized MRI protocol, which was optimized for each of the scanner types in use at participating sites (Siemens Trio and Prisma, Philips Achieva).[4] Each site was trained and certified for their consortium-wide MRI protocol. All sites were required to perform image quality checks every 2 months using the Alzheimer's Disease Neuroimaging Initiative phantom. The study protocol was approved by the institutional review board of each site. Informed written consent was obtained from each participant.

The participants included in this study were older adults with normal cognition, MCI, or early dementia. Participants with existing neurologic conditions of etiologies different from SVD, including history of major strokes, brain trauma, multiple sclerosis, or respiratory problems such as chronic obstructive pulmonary disease or asthma, were excluded. Syndromic diagnoses at each site used the National Institute on Aging–Alzheimer's Association criteria for MCI and dementia.[16,17]

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional (e.g., PubMed) sources and meeting abstracts and presentations. While there have been several publications describing the potential utility of cerebrovascular reactivity (CVR) to carbon dioxide inhalation, as measured by magnetic resonance imaging (MRI), in the context of cognitive impairment and dementia, all previous works were single-site studies. Furthermore, none have used a predefined hypothesis approach. The relevant citations are appropriately cited.
2. **Interpretation**: Our findings of an association between CVR and cognition that were reproduced across three independent sites established a solid foundation for the field to further test CVR MRI as a candidate biomarker in vascular cognitive impairment and dementia.
3. **Future directions**: Future studies should examine the utility of CVR MRI in longitudinal settings such as testing whether CVR at baseline can predict cognitive decline over time and whether CVR changes over time precedes cognitive changes.

2.3 | Clinical and cognitive evaluation

All participants underwent clinical and cognitive evaluation, again standardized by the MarkVCID Consortium, including clinical information, physical and neurologic examination, clinical scales, and a comprehensive neuropsychological battery.[3] Specifically, the MoCA was used to evaluate global cognitive status, and an item response theory (IRT)–based score[18] was calculated to evaluate executive function. The IRT score of Uniform Data Set v3.0 executive function (UDS3-EF) is a composite score of selected tests that measures different facets of executive function, including 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).[18]

A composite vascular risk score (VRS) was calculated to quantify the vascular disease burden of the participants. Five vascular risk factors were included: hypertension, hypercholesterolemia, diabetes mellitus, smoking (smoked > 100 cigarettes in his/her life), and obesity (body mass index > 30 kg/m^2).[19] Following previous studies, each vascular risk factor was coded as a binary variable (1 if current, 0 if remote/absent).[12,19] The composite score, calculated as the sum of these five measures, therefore ranges from 0 to 5.

2.4 | CVR MRI procedure

CVR MRI requires the delivery of CO_2 gas mixture (5% CO_2, 21% O_2, 74% N_2) to the participant and monitoring of their end-tidal CO_2

($EtCO_2$). Thus, special apparatus and training are needed, above what is required for the acquisition of standard MRI scan types. The lead investigative team assembled a standardized box that contained all necessary components needed for CO_2 delivery and monitoring, and traveled to each site and conducted a 3-hour training session (1 hour of classroom training and 2 hours in the MRI suite) for site certification.

Each site performed an identical CVR procedure, based on the method of Lu et al.[20] Specifically, participants were fitted with a mouthpiece and a nose clip inside the MRI scanner, and CO_2-enriched air as described above was administered using an airbag, with a valve to switch between room air and CO_2-enriched air. After the initial 15 seconds of room-air breathing, subjects breathed 50 seconds of CO_2-enriched air and 70 seconds of room air alternatively for three repetitions, followed by an additional 45 seconds of room air, for a total duration of 420 seconds. The trace of CO_2 concentration in the exhaled air was recorded using capnography (NM3, Philips). BOLD MRI images were continuously acquired on 3T MRI scanners during the entire 420-second period.[4] The BOLD scan parameters were: voxel size = $3.4 \times 3.4 \times 3.8 mm^3$, 34 to 36 axial slices for whole-brain coverage, repetition time = 1500 ms.[4] Technical assessment of the test–retest reproducibility of this CVR mapping protocol across sites and MRI manufacturers has been reported previously.[15] Note that the CVR values obtained may depend on the hypercapnia breathing duration and processing methods,[21] but as long as all the participants received the same CVR procedure, the data heterogeneity is expected to be minimal.

A high-resolution 3-D T1-weighted multi-echo magnetization-prepared rapid acquisition gradient echo (MPRAGE) was performed following the harmonized imaging protocol by the MarkVCID Consortium for anatomic reference.[4] A high-resolution 3D fluid-attenuated inversion recovery (FLAIR) was also performed to evaluate white matter hyperintensities (WMH) of the brain.[4]

2.5 | Data processing and analysis

CVR data processing was performed using an online processing tool referred to as CVR-MRICloud (https://braingps.mricloud.org/cvr.v5).[22] Briefly, the BOLD data were first motion corrected and smoothed using an 8 mm Gaussian kernel. The end-tidal CO_2 ($EtCO_2$, i.e., the peak CO_2 of each breath) curve was extracted from the CO_2 trace and time shifted to align with the whole-brain BOLD signal time course. The temporal alignment between $EtCO_2$ and the BOLD signal time courses was conducted to account for the time it takes for the CO_2 gas bolus to travel from the lung to the heart, then to the brain, and induce a hemodynamic response. Specifically, we performed a series of linear regression analyses with a range of time-shifted $EtCO_2$ time courses and identified the time shift that corresponds to the best fit to the whole-brain BOLD signal time course.[22] The CVR quantification was based on the linear regression between the synchronized $EtCO_2$ and BOLD time courses.[23] The CVR map was then co-registered to the MPRAGE image space and transformed to the Montreal Neurological Institute space. Finally, the individual MPRAGE image was segmented, and the resulting gray-matter mask was applied to the BOLD data to obtain a gray-matter time course, which was used to calculate gray-matter CVR. The CVR value is written in the units of % BOLD signal change per mmHg of $EtCO_2$ (%/mmHg). We primarily focused on whole-brain gray-matter CVR in this report.

The T2 FLAIR images were reviewed by a board-certified neuroradiologist. Peri-ventricular white matter (WM) Fazekas extent grade, deep WM Fazekas extent grade, and deep WM Fazekas lesion count were evaluated to index WMH severity in periventricular WM and deep WM, with each score ranging from 0 to 3.[24] The overall Fazekas score was assigned as the maximum value of these three scores and used in the following statistical analysis.

2.6 | Statistical analyses

As the primary statistical model, multi-linear regression analysis was conducted on a site-by-site basis, with MoCA as the dependent variable; CVR as the independent variable; and age, sex, and education as covariates. The three analysis sites were JHU, UTHSCSA, and UKY/USC, as noted above.

To evaluate whether the relationship between CVR and MoCA is dependent on the diagnostic category, using data from all sites, we repeated the multi-linear regression analysis by including the diagnosis (normal vs. impaired cognition) as an additional independent variable. The impaired but not MCI, mild dementia, and MCI groups were combined to form the "impaired" group due to the small sample sizes in each diagnostic group. The relationship between CVR and MoCA was also examined separately in normal and impaired participants.

For our secondary analysis, data from all sites were pooled and the relationship between CVR and executive function was evaluated using multi-linear regression analysis, with age, sex, education and site as covariates. Furthermore, VRS and Fazekas score were additionally included as independent variables in the multi-linear regression between cognitive measures (MoCA or executive function) and CVR to examine whether CVR can explain cognitive variance beyond the traditional vascular disease scores.

3 | RESULTS

Out of the 294 participants enrolled, a total of 263 participants were included in the statistical analyses. Twenty-one participants were excluded from the analyses due to unusable CO_2 recordings, eight participants were excluded due to CO_2 delivery failure, and two participants were excluded due to excessive motion. There was not a significant difference in clinical diagnosis between the participants included in the statistical analyses and those excluded ($p = 0.56$). A summary of demographic, cognitive, and imaging characteristics of the participants included in the final analyses at each site is listed in Table 1. As can be seen from Table 1, cohorts from different sites had diverse racial and ethnic distributions.

Figure 1 shows group-averaged CVR maps from each site. Visual inspection suggested that reliable CVR maps with similar quality were obtained from all sites.

TABLE 1 Characteristics of the participants at each site.

Site	JHU	UTHSCSA	UKY/USC
N	77	91	95
Age (years)	69.5 ± 6.8	69.4 ± 7.0	70.7 ± 9.1
Sex (M/F)	26/51	27/64	37/58
Education (years)	16.3 ± 2.6	14.8 ± 2.6	13.2 ± 5.6
Race			
White, *n* (%)	52 (68%)	89 (98%)	68 (72%)
Black, *n* (%)	24 (31%)	0 (0%)	2 (2%)
Other, *n* (%)	1 (1%)	2 (2%)	2 (2%)
Unknow, *n* (%)	0 (0%)	0 (0%)	23 (24%)
Hispanic, *n* (%)	0 (0%)	91 (100%)	46 (48%)
Cognitive diagnosis			
Normal (%)	29 (38%)	48 (53%)	64 (68%)
Impaired, not MCI (%)	0 (0%)	4 (4%)	8 (9%)
MCI (%)	42 (54%)	35 (38%)	16 (17%)
Dementia (%)	6 (8%)	4 (4%)	5 (5%)
Unknown (%)	0 (0%)	0 (0%)	2 (2%)
MoCA score	26.4 ± 2.6	24.1 ± 3.4	24.4 ± 4.0
IRT score	−0.18 ± 0.81	−0.66 ± 0.79	−0.64 ± 0.87
VRS	1.6 ± 1.2	1.9 ± 1.1	1.8 ± 1.2
Fazekas score	1.5 ± 0.8	1.4 ± 0.8	1.2 ± 0.9

Abbreviations: IRT, item response theory; JHU, Johns Hopkins University; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; UKY, University of Kentucky; USC, University of Southern California; UTHSCSA, University of Texas Health Science Center at San Antonio VRS, vascular risk scores.

Figure 2 shows the scatter plots between gray matter CVR and MoCA for each site, as well as data from all sites displayed together. Table 2 summarizes the relationship between CVR and MoCA at each site. Gray matter CVR showed a positive association with MoCA score after adjustment for age, sex, and education, in which participants with higher CVR had higher MoCA scores. This relationship was reproduced at each analysis site ($p < 0.05$ for each), confirming our pre-specified hypothesis of the associations between CVR and global cognition.

In further analysis using data from all sites, diagnosis category (normal vs. impaired cognition) also showed a significant effect ($\beta = -2.91$, 95% confidence interval [CI; −3.60, −2.21], $p < 0.0001$) on MoCA, whereas the interaction between diagnosis and CVR was not significant ($p = 0.77$). Next, we evaluated the association between CVR and MoCA in participants with normal cognition ($N = 141$) and with impaired cognition (impaired but not MCI, MCI, and dementia, $N = 120$) separately. Consistent with the results when all participants were included, after adjustment for age, sex, education, and site, the association between gray matter CVR and MoCA score was significant in both the normal participants ($\beta = 20.47$, 95% CI [12.59, 28.35], $p < 0.0001$) and in the impaired participants ($\beta = 14.97$, 95% CI [3.79, 26.14], $p = 0.009$).

As secondary analysis results, we examined the association between gray matter CVR and executive function, which is the primary cognitive domain affected by SVD and VCID. Our results showed that higher gray matter CVR was significantly associated with better executive function, indicated by higher scores ($\beta = 2.87$, 95% CI [0.96, 4.78], $p = 0.003$).

We then tested whether the associations between CVR and cognition were independent of other measures of vascular health, including VRS and Fazekas score. The results are summarized in Table 3. It was found that higher CVR ($p < 0.001$, Table 3) and lower VRS ($p = 0.038$, Table 3), but not Fazekas score ($p = 0.30$, Table 3), were independently associated with better MoCA scores after adjustment for age, sex, education, and site. Similarly, the executive function score was significantly related to CVR ($p = 0.003$, Table 3) and VRS ($p = 0.004$, Table 3), but not Fazekas score ($p = 0.92$, Table 3), after adjustment for age, sex, education, and site. These analyses were repeated using individual vascular risk conditions and the results were the same (Table S1 in supporting information). We also conducted additional analyses to examine the relationship between CVR and VRS/Fazekas score and observed that CVR was not associated with VRS ($p = 0.99$) or Fazekas score ($p = 0.70$) after controlling for age, sex, education, and site. Similarly, CVR was not associated individual vascular risk conditions ($p > 0.5$). These findings suggested that CVR provides additional predictive power to classic vascular risks in evaluating cognition in SVD/VCID patients.

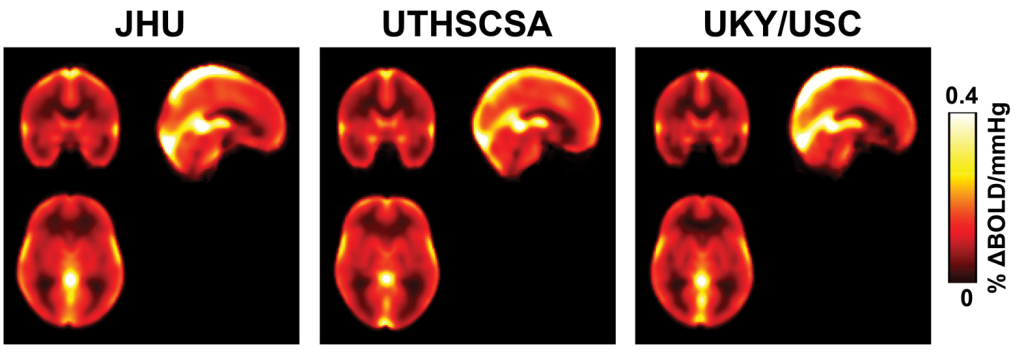

FIGURE 1 Group-averaged CVR maps from each analysis site. CVR, cerebrovascular reactivity. JHU, Johns Hopkins University; UKY, University of Kentucky; USC, University of Southern California; UTHSCSA, University of Texas Health Science Center at San Antonio

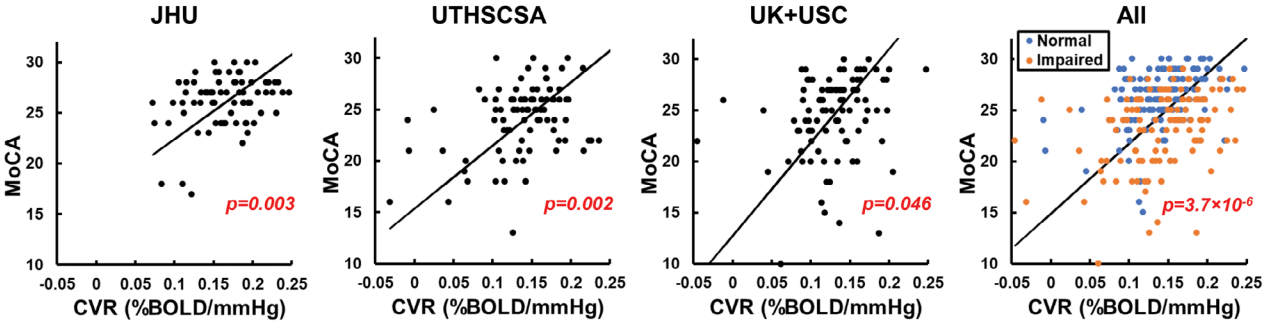

FIGURE 2 Scatter plots between gray matter CVR and MoCA. Each dot represents one participant. The lines indicate two-dimension linear fitting lines. BOLD, blood oxygen–level dependent; CVR, cerebrovascular reactivity; JHU, Johns Hopkins University; MoCA, Montreal Cognitive Assessment; UKY, University of Kentucky; USC, University of Southern California; UTHSCSA, University of Texas Health Science Center at San Antonio

TABLE 2 The associations of whole-brain gray matter CVR with MoCA score at each site.

	JHU	UTHSCSA	UKY/USC	All
β	18.72	19.51	17.75	18.60
95% CI	[6.76, 30.67]	[7.50, 31.51]	[0.32, 35.19]	[10.86, 26.35]
p value	0.003	0.002	0.046	3.7×10^{-6}

Abbreviations: CI, confidence interval; CVR, cerebrovascular reactivity; JHU, Johns Hopkins University; MoCA, Montreal Cognitive Assessment; UKY, University of Kentucky; USC, University of Southern California; UTHSCSA, University of Texas Health Science Center at San Antonio.

TABLE 3 The associations of whole-brain gray matter CVR, vascular risk factors, and Fazekas score with MoCA and executive function scores (IRT) in all subjects.

	MoCA	IRT
CVR		
β	19.09	2.97
95% CI	[11.52, 26.66]	[1.03, 4.91]
p value	1.3×10^{-6}	0.003
VRS		
β	−0.34	−0.12
95% CI	[−0.66, −0.02]	[−0.20, −0.04]
p value	0.038	0.004
Fazekas score		
β	−0.23	0.005
95% CI	[−0.68, 0.21]	[−0.10, 0.11]
p value	0.30	0.92

Abbreviations: CI, confidence interval; CVR, cerebrovascular reactivity; IRT, item response theory; JHU, Johns Hopkins University; MoCA, Montreal Cognitive Assessment; VRS, vascular risk scores.

4 | DISCUSSION

In the present work, we performed a multi-center study to examine CVR as a potential neuroimaging biomarker of SVD/VCID. Our major finding confirmed our prespecified hypothesis that gray matter CVR is positively associated with global cognitive function measured by the MoCA at each of the participating sites. This finding supports the biological validity of CVR as a sensitive biomarker of SVD/VCID that can be performed across multiple study sites. Our results also showed that CVR is positively associated with an executive function score. Finally, our results indicated that CVR and vascular risk factors independently predict cognitive function in SVD/VCID.

4.1 | CVR as a biomarker of SVD/VCID

CVR is one of the selected imaging biomarkers being tested in the MarkVCID Consortium, the goal of which is to identify and validate clinical trial–ready biomarkers for VCID. As a dynamic property of the cerebral blood vessels, CVR provides vital information of vascular reserve that is complementary to steady-state vascular parameters, such as cerebral blood flow (CBF) and cerebral blood volume (CBV). This measure of cerebrovascular reserve may be an earlier biomarker in MCI and dementia than steady-state vascular parameters because of its specificity to microcirculatory function.[7,9,25] Several reports in the literature have examined the relationship between CVR and cognition among cognitively normal elderly, MCI, and dementia patients, with some studies reporting significant correlation,[9,26] but others reporting no correlation.[27,28] These contrasting results may be due to relatively small sample size in those studies. More recently, data collected from 72 participants from a single consortium site in Phase 1 of the MarkVCID study revealed that CVR is associated with two separate measures of global cognitive performance, the MoCA score and a composite cognitive score,[12] consistent with other reports of positive associations between CVR and global cognition measured by the Mini-Mental State Examination with similar sample size ($N = 78$).[25] The data presented in this work, collected from different cohorts at different sites, successfully reproduced and extended the findings from our Phase 1 study[12] in each analysis site.

A direct comparison between CVR and CBF in terms of their ability to predict cognitive function has also been conducted.[29] A recent single-site study measured CVR using global CBF MRI as a readout,

and thereby was able to concomitantly determine CVR and baseline CBF.[29] It was found that CVR was strongly associated with cognitive performance such as MoCA ($p = 0.001$), but baseline CBF was only weakly associated with MoCA ($p = 0.042$) and not with any other cognitive scores, physical performances, or Clinical Dementia Rating.[29] CBF measurement was not included in the multi-site Phase 2 study of the MarkVCID Consortium.

Although not the primary hypothesis of our study, our results also suggest that higher CVR is associated with better executive function, which is the primary cognitive domain affected by SVD and VCID. Importantly, the association between CVR and cognition, both global cognition and executive function, remained significant when classic vascular risks (hypertension, hypercholesterolemia, diabetes mellitus, obesity, and smoking) and WMH were taken into consideration. The lack of association between CVR and VRS could be due to the delay between VRS and its effect on the brain. Gottesman et al. reported that vascular risk factors measured in middle age are associated with increased risk of dementia 25 years later.[30] Therefore, it is plausible that there is a delay between the exposure to VRS and the changes in cerebrovascular function. The ongoing longitudinal evaluation of CVR, VRS, and cognition in the MarkVCID 2 study may provide some direct evidence on this point. Another potential reason is that CVR could be more affected by other mechanisms such as cerebral amyloid angiopathy.[31,32] Future studies are needed to better understand the relationship between CVR and vascular risk factors in SVD/VCID. The lack of association between CVR and Fazekas score is likely because overt T2 WMH abnormalities represent an almost end stage of the SVD pathological processes whereas CVR detects early-stage changes of small vessel function. These results suggested that CVR measures an independent element of vascular health above and beyond those of standard vascular disease scores.

4.2 | Readiness of CVR for multi-center clinical trials

Biomarker development is a key step toward translating scientific knowledge about disease into effective prevention and treatment strategies and is the primary goal of the MarkVCID Consortium. In addition, validation of these biomarkers for SVD/VCID is an important step in the path to clinical readiness, so that the variability of the biomarker can be incorporated into both the determination of which candidate biomarkers are promising and the analysis phase of a clinical trial. In the MarkVCID Consortium, CVR has been proposed as a susceptibility/risk biomarker. That is, CVR may be used to identify individuals at an increased risk for development of SVD/VCID, and who therefore are eligible for enrollment for SVD/VCID–related clinical trials. The present work reports a reproducible relationship between CVR and global cognition, which demonstrated the biological validity of CVR as a biomarker of VCID. CVR also has the potential to be used as a treatment monitoring biomarker. That is, CVR alterations may be tracked during treatment trials to reflect early pathological changes, before cognitive or clinical changes become apparent. Previous studies have demonstrated the sensitivity of CVR in characterizing vascular decline in longitudinal studies of brain aging.[33,34]

The scalability of the CVR MRI measurement to multi-vendor, multi-center studies has also been shown recently.[15] As part of the MarkVCID study, a comprehensive multi-vendor, multi-center study was conducted to evaluate the instrumental validity of CVR, which demonstrated good inter-rater, inter-scanner, and test–retest reliabilities in healthy volunteers.[15] Together, these findings suggested that CVR is a promising biomarker of SVD/VCID that is ready for larger scale clinical studies. Recently, the MarkVCID Consortium has expanded the number of sites to a total of 16 medical centers around the United States, which are participating in longitudinal observational studies of 1800 high vascular risk older individuals. CVR has been selected as one of the four biomarkers to continue to the next phase of clinical validation, defined as readiness for incorporation into clinical trials at the level of individual participants, in the MarkVCID 2 study.

4.3 | Limitations

One limitation of the present study is that, in our CVR measurement, we did not control the participants' ingestion of vasoactive drugs or beverages prior to the MRI scan. These factors may change the participant's vascular tone, thereby affecting CVR.[35] For example, caffeine can reduce CBF by up to 25%,[36] and thus consumption of coffee may alter the measured CVR value. Our recent study revealed a 32.7% reduction in CBF-based CVR after taking a 200 mg caffeine tablet (equivalent to 2 cups of coffee).[37] Such CVR reductions were observed in both caffeine-naïve and caffeine-habituated subjects. These normal physiological variations may be part of the reason that the scatter plots shown in Figure 2 still have substantial spread. Future work minimizing these physiological variations in CVR measurements is expected to further improve the sensitivity of CVR in clinical studies of SVD and VCID.

We would also like to point out that the present study used BOLD MRI signal as a readout for the vasodilation effects. A physiologically more meaningful measure would be CBF, which can provide an assessment of both CVR (in terms of percent CBF increase with hypercapnia) and baseline CBF. However, it is known that CBF images are noisier than BOLD MRI. Thus, BOLD was used in this study as opposed to CBF MRI (similar to the reason that most functional MRI used BOLD instead of CBF MRI). It should also be pointed out that, for global CBF-based CVR, it has been shown that CBF-CVR is also strongly associated with MoCA.[29]

Another limitation of the present work is that we only demonstrated the validity of CVR in a cross-sectional study. The ongoing MarkVCID 2 study will provide large-scale, multi-center CVR data in a longitudinal setting. Moreover, only syndromic diagnosis was performed in this study. Because we did not perform cerebrospinal fluid sampling or positron emission tomography scanning in these participants, biomarker-based classification was not available. Previous studies reported that > 80% of patients with a diagnosis of dementia have certain levels of small vessel pathology,[38] it is therefore expected

that the majority of the impaired participants in this study have SVD and VCID pathology. It is recognized that Alzheimer's disease (AD) pathology may also contribute to the cognitive performance of these participants. In a previous single-site report as part of the Phase 1 study of the MarkVCID, CVR and AD pathology were found to simultaneously and independently predict part of the variance in cognitive performance.[12] In the ongoing MarkVCID 2 study, additional recruitment criteria have been implemented to ensure that the participants have at least one significant vascular risk, including clinical factors (diabetes or current hypertension) or MRI factors (Fazekas score ≥ 2 or microbleed count ≥ 1 or lacunar infarct ≥ 1).

4.4 | Conclusions

The present study evaluated the relationship between CVR and cognition in a multi-center setting. CVR was found to be positively associated with global cognitive function measured by the MoCA, independent and complementary to standard vascular risk factors. These observations replicated the findings in a previous single-site study and were shown to be reproducible across different sites with diverse cohorts. These findings support the utility of CVR as a biomarker in future clinical trials of SVD and VCID.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest or financial disclosures to report. Author disclosures are available in the supporting information.

CONSENT STATEMENT

All human subjects provided informed consent.

ORCID

Hanzhang Lu https://orcid.org/0000-0003-3871-1564

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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