Breath-hold Blood-Oxygen Level Dependent MRI: a tool for the assessment of cerebrovascular reserve in children with Moyamoya

Cover Title: Breath-hold Cerebrovascular Reactivity in Moyamoya

Nomazulu Dlamini[1,2,3,4,7]; Priyanka Shah-Basak[5,8]; Jackie Leung[6]; Fenella Kirkham[7]; Manohar Shroff[8]; Andrea Kassner[6,9]; Amanda Robertson[4]; Peter Dirks[10]; Robyn Westmacott[11]; Gabrielle deVeber[1,2,4]; William Logan[1]

[1]Department of Neurology, The Hospital for Sick Children, Toronto; [2]University of Toronto, Institute of Medical Science; [3]Neurosciences and Mental Health Program, The Hospital for Sick Children, Toronto; [4]Child Health Evaluative Sciences Program, The Hospital for Sick Children, Toronto; [5] Rotman Research Institute, Baycrest, Toronto; [6]Translational Medicine, The Hospital for Sick Children, Toronto; [7] Developmental Neurosciences, UCL Great Ormond Street Institute of Child Health, London; [8]Diagnostic Imaging, The Hospital for Sick Children, Toronto; [9]University of Toronto, Department of Medical Imaging; [10] Department of Neurosurgery, The Hospital for Sick Children, Toronto; [11]Department of Neuropsychology, The Hospital for Sick Children, Toronto

Title character count: 108

Number of references: 34
Number of tables: 2
Number of figures: 4

Word count (abstract): 240
Word count (entire manuscript): 4453

Corresponding Author:
Nomazulu Dlamini
Division of Neurology
The Hospital for Sick Children
555 University Avenue, Toronto
M5G 1X8, Canada
Phone 1-416-813-7721
Fax 1-416-813-6334
Email: nomazulu.dlamini@sickkids.ca

Key words: Arteriopathy, Cerebrovascular Disease/Stroke, cerebrovascular reactivity, Ischemic Stroke, Moyamoya, Stroke in Children

Abstract

Background and Purpose: There is a critical need for a reliable and clinically-feasible imaging technique that can enable prognostication and selection for revascularization surgery in children with Moyamoya. Blood-oxygen level dependent MRI assessments of cerebrovascular reactivity, using voluntary breath-hold hypercapnic challenge is one such simple technique. However, its repeatability and reliability in children with Moyamoya is unknown. The current study sought to address this limitation.

Methods: Children with Moyamoya underwent dual breath-hold hypercapnic challenge blood-oxygen level dependent MRI cerebrovascular reactivity studies, in the same MRI session. Within-day, within-subject repeatability of cerebrovascular reactivity estimates, derived from blood-oxygen level dependent signal, was computed. Estimates were associated with demographics, and intellectual function. Inter-rater reliability of a qualitative and clinically-applicable scoring scheme was assessed.

Results: Twenty children (11 males; 12.1±3.3 years) with 30 MRI sessions (60 MRI scans) were included. Repeatability was "good" based on intra-class correlation (0.70±0.19). Agreement of qualitative scores was “substantial” (K=0.711) and intra-rater reliability of scores was “almost perfect” (K=0.83 and 1). Younger participants exhibited lower repeatability (p=0.027). Repeatability was not associated with cognitive function (p>0.05). However, abnormal cerebrovascular reactivity was associated with slower processing speed (p=0.015).

Conclusion: Breath-hold hypercapnic challenge blood-oxygen level dependent MRI is a repeatable technique for the assessment of cerebrovascular reactivity in children with Moyamoya and is reliably interpretable for use in clinical practice. Standardization of such protocols will

allow further research into its application for the assessment of ischemic risk in childhood cerebrovascular disease.

Abbreviation Key

CV = Coefficient of Variation

BOLD = Blood-oxygen level dependent

CBF = Cerebral Blood Flow

BH = Breath-hold

CVR = Cerebrovascular reactivity

ICC = Intra-class Correlation

Introduction

Arterial ischemic stroke remains a major cause of morbidity and mortality in children worldwide. The incidence ranges from 2–13 per 100,000 person-years in developed countries.[1,2] Childhood risk factors are multiple, and include cardiac disease, sickle cell disease (SCD), arteriopathy and infection.[3,4] Over two-thirds of previously healthy children presenting with their first arterial ischemic stroke have a steno-occlusive arteriopathy, the presence of which predicts recurrence and outcome.[5–7] Moyamoya is a major arteriopathy of childhood. It is a progressive steno-occlusive arteriopathy that typically affects the anterior circulation arteries of the circle of Willis. It confers a lifelong risk of recurrent stroke and neurological injury, and it is associated with early death due to chronic cerebral hypoperfusion and thrombotic vaso-occlusion.[8–10] The pathology of the primary arteriopathy is poorly understood.

A network of lenticulostriate collaterals develop to by-pass the primary steno-occlusive arteriopathy, and vasodilation at the level of the capillary bed occurs so as to maintain cerebral blood flow (CBF), resulting in a reduction of cerebrovascular reserve.[11] Studies in adults with arteriopathy suggest that impairment of cerebrovascular reserve is associated with an approximate 4-fold increased risk of developing stroke or transient ischemic attacks.[12] In vivo assessment of cerebrovascular reserve can be performed by measurement of cerebrovascular reactivity (CVR), defined as a change in CBF in response to vasoactive stimuli such as carbon dioxide. In the context of steno-occlusive arteriopathy and maximal microvascular vasodilation in response to falling CBF, exhaustion of cerebrovascular reserve and vasodilatory capacity may result in paradoxical reductions in CBF and CVR following a vasodilating stimulus. [11,13–15] This negative response, termed 'steal', is an independent predictor of ischemic injury and stroke.[11,13,14]

Historically employed techniques for assessing CBF and cerebrovascular reserve such as PET and SPECT require exposure to ionizing radiation and are hence undesirable.[15] Blood-oxygen level dependent (BOLD)-MRI is a widely used technique for the non-invasive imaging of dynamic changes in CBF at the local and global level. BOLD-MRI harnesses the paramagnetic properties of deoxyhemoglobin using clinically available T2* gradient-echo MRI sequences and does not require intravenous contrast medium. Using carbon dioxide as a vasoactive stimulus, hypercapnic challenge BOLD-MRI CVR (BOLD-CVR) can be used to generate high-spatial resolution CVR maps. Thus, when cerebrovascular reserve is exhausted, negative or paradoxical reactivity on hypercapnic challenge BOLD-CVR maps, represents 'steal' and provides a visual representation of ischemic risk and impending tissue demise.[14]

Hypercapnia induced by computer-controlled carbon dioxide stimulus delivery[16] for BOLD-CVR experiments has been validated in both adults and children in research settings.[17–20] In the only published study of reliability of CVR measurements acquired in children (ten healthy children, age 16.1±1.6 years), intra-class correlations (ICCs) of within-day values were 0.857 and 0.895 in the GM and the WM, respectively.[20] However, this method relies on specialized MR-compatible equipment, limiting its applicability in clinical settings. Additionally, the use of nasal prongs or a facemask to deliver and monitor the carbon dioxide stimulus serves as barriers for CVR acquisition in young children.

An alternative approach for performing CVR assessment is breath-holding (BH), in which endogenous alveolar carbon dioxide naturally accumulates during short periods of voluntary apnea. Without the need for additional equipment and gas-delivery apparatus, BH is easier to implement and well-tolerated.[21] The repeatability and reliability of BH-BOLD-CVR (BH-CVR) in high stroke-risk pediatric populations, however, remains unknown. The purpose of

this study was to evaluate within-day, within-subject repeatability, qualitative scoring and inter-rater reliability of scoring of BH-CVR estimates. The relationship between clinical factors and intellectual function with BH performance and repeatability was also explored.

Methods

Study Population

Children diagnosed with cerebral vasculopathy consistent with a diagnosis of Moyamoya (idiopathic or syndromic), between 2010 and 2017 were recruited from the institutional Stroke Registry. All procedures were approved by our institutional Research Ethics Board, and informed written consent was obtained. Moyamoya was diagnosed if conventional or MR angiography demonstrated stenosis or occlusion of the distal internal carotid artery, the proximal middle cerebral artery and/or the anterior cerebral artery with the appearance of lenticulostriate collaterals. Children with unilateral Moyamoya with collaterals (probable Moyamoya) were included.[22] Children without a previously diagnosed condition were diagnosed as having Moyamoya disease or idiopathic Moyamoya, while those with a previously diagnosed condition such as Neurofibromatosis Type 1 or SCD were diagnosed as having Moyamoya syndrome. Children with non-Moyamoya arteriopathy and children unable to complete a repeat study were excluded.

Breath-hold paradigm

Children practiced the BH paradigm seated on a chair prior to entering the MR scanner under supervision of a research technologist. Each BH paradigm began with 10 seconds of normal breathing, followed by five 60-second periods of breath-holding and normal breathing

(Figure 1a). Optimal BH duration was determined during practice in individual patients as the maximum number of seconds that they could hold their breath without discomfort. BH duration was subtracted from 60 seconds to calculate the within-cycle normal breathing duration. A 30-second rest period with normal breathing was included after the second cycle. To assess subject compliance, real-time respiration signals were monitored using respiratory bellows throughout the scan. Any patients observed to deviate from the instructions or move excessively were flagged as 'non-compliant'.

Breath-hold CVR and MRI Acquisition

Children underwent repeat BH-CVR studies (1A and 1B) on the same day during the same MRI session. Follow-up repeat studies were performed in a subset of patients at later time-points (2A-2B; 3A-3B; 4A-4B).

MRI data were acquired with a 3.0-Tesla scanner (Achieva, Philips, Best, the Netherlands) using an 8-channel head coil. The BH-CVR protocol consisted of two separate BOLD acquisition using EPI-GRE lasting 6.1 minutes each [25 slices; TR/TE=2000/30ms; voxel size=3.4×3.4×5mm^3; FOV=22cm; 180 dynamics]. The BH paradigm was employed throughout the scan duration. A high-resolution 3D T1-weighted structural image [160 slices; voxel size=0.86×0.86×1mm^3; FOV=22cm] was acquired for tissue classification and co-registering the CVR maps.

Image post-processing and CVR estimation

MRI data processing were performed using functions from AFNI (v16.1.04)[23] and FSL (v4.1.9) toolboxes. The first two dynamics of the BOLD data were truncated and subjected to slice timing correction. Dynamics were corrected for motion using a rigid body model in a

generalized linear model. BOLD dynamics exhibiting excessive motion (>0.3mm/dynamic) were automatically labelled and disregarded in subsequent analyses. Data were spatially smoothed using a Gaussian kernel of 7mm. FSL's Automated Segmentation Tool was used for defining GM and WM masks.[24] The mask of the cerebellum from the MNI152 atlas was co-registered to each patient to determine the cerebellar region.

For CVR maps, the patient's BOLD time series in each voxel of the brain was subjected to generalized linear model analysis, using the corresponding averaged cerebellar time courses as a regressor (Figure 1b). The regression coefficients (or the beta weights) were then calculated for each voxel. Negative beta weights describing an inverse relationship with the regressor are the markers of 'steal'. CVR maps consisting of voxel-wise negative and positive beta weights (describing a negative and positive relationship with the regressor respectively) were co-registered to the high-resolution T1 images in the native space for visualization.[25] A combined CVR measure was also computed as a weighted average of relative counts of positive and negative voxels.[18]

Assessment of BH-CVR Performance

The quality of BH studies was assessed using: i) BH compliance during data acquisition using respiratory waveform from the bellows, ii) evidence of excessive motion during post-processing; the determination was based on cutoff set at >15 dynamics, or 30s, affected by motion in either BH repeat study.

Qualitative Scoring of BH-CVR Maps

Whole-brain scoring of the BH-CVR maps was conducted by visual inspection for steal of the first repeat study (1A-1B) by neurologists, blinded to the patients' clinical information. Hemispheric scoring by visual inspection was conducted independently by the same neurologists for patients with multiple time-point studies (1A-1B; 2A-2B; 3A-3B; 4A-4B). (Figure 2)

Table 1. Criteria for scoring CVR maps

Visual impression	**Score**
Normal CVR	1
Reduced positive reactivity +/- minimal steal (<10%)	2
Significant steal (>10%)	3

Moyamoya Sub-Type, Stroke and Intellectual Function

Clinical records and high-resolution clinical T2-weighted images acquired at the time of each BH-CVR study were reviewed for Moyamoya sub-type, history of stroke, and the presence of vascular territory ischemic infarction.

Standardized age-appropriate measures of intelligence using Wechsler Intelligence Scale for Children (WISC IV/V),[26,27] administered as part of routine clinical care within 3 years of BH-CVR collection were reviewed and included in the analysis.

Statistical Analysis

To determine the repeatability, ICC and coefficient of variation (CV) of within-day repeat scans were computed in the whole-brain and in the tissue (GM, WM) masks.[20] CV was computed as a ratio of the standard deviation and the mean of CVR estimates between repeat studies. Interpretation of ICC values was as follows: <0.41 "poor", >=0.41 and <0.59 "fair",

>=0.59 and <0.74 “good” and >=0.74 “excellent”.[28] Bland-Altman analysis was performed to determine the 95% limits of agreement between the repeat BH-CVR estimates. Cohen’s Kappa analysis was used to assess i) agreement between whole-brain scores of repeat scans and ii) inter-rater reliability of hemispheric scoring of repeat scans. Interpretation of the Kappa scores was as follows: 0.01 "poor"; 0.01-0.20 "slight"; 0.21-0.40 "fair"; 0.41-0.60 "moderate"; 0.61-0.80 "substantial"; 0.81-1.00 "almost perfect" agreement.[29]

Means of positive, negative and combined CVR estimates, representing the *magnitude* of CVR values, and their respective *voxel counts* were computed and compared between repeat scans using paired t-tests.

Exploratory analyses to examine relationships between ICC, CVR estimates, clinical factors (Moyamoya sub-type, history of stroke), demographics (age at CVR) and intellectual function (full scale IQ and IQ subscales) were conducted. One-way ANOVA was employed for categorical variables and linear regression for continuous independent variables. Post-hoc t-tests were done to assess significant ANOVA findings. Fisher’s exact tests were used for comparing two categorical variables.

Results

Study population

Twenty children (11 males; mean age±standard deviation: 12.1±3.3 years; median [range]: 11.7 [6.2-18.0] years) at first BH-CVR were included. Follow-up repeat scans (2A-2B) were collected in six children, three children returned for a second (3A-3B), and one child for a third (4A-4B) follow-up scan. A total of 30 pairs of within-day repeat BH-CVR studies (60 BH-CVR scans) were completed.

Eleven patients (55%) had idiopathic Moyamoya, 4 (20%) had Neurofibromatosis Type 1, 2 (10%) had SCD, while 3 others (15%) had familial, radiation induced or *ACTA 2* mutation Moyamoya (Table 2); mean age at Moyamoya diagnosis was 7.9±3.2 years; (median [range] 8.0 [1.4-16.8]) years. Seventeen patients (85%) had no prior history of stroke. Half of the children were either asymptomatic (7/20; 35%) or had headache only (3/20; 15%) at the time of Moyamoya diagnosis.

BH Performance, CVR estimates and Repeatability Measures

In six of 30 studies (20%), the children were flagged as non-compliant, all of whom were undergoing first repeat studies (1A or 1B). Four of those were noted to be non-compliant during data acquisition. Two studies had excessive motion noted during post-processing. All studies irrespective of compliance or motion were included in the assessment of repeatability (Table 2).

Mean BH durations in the first (A) and the second (B) scan were 19.6±3.3 and 20.3±3.4 seconds, respectively (p=0.1). No significant differences between repeat scans were found for the positive, negative or combined mean CVR estimates, or the counts of positive and negative voxels in the GM and the WM (all p>0.05; patient-wise estimates in Supplementary Table 1).

Out of 30 paired studies, repeatability for the whole-brain CVR estimates (ICC=0.703±0.190) was ‘excellent’ in 18 (60%), and ‘good’ in 5 (17%) studies. The remaining 7 studies (23%) fell in the ‘fair’ to ‘poor’ range (Table 2). All but one (5/6) patients flagged as either non-compliant or for excessive motion had poor ICCs. The 2 SCD patients also exhibited relatively low ICC (0.51;0.48).

Mean CV was 9.1 (±8.2)% for positive CVR and 22.5 (±18.3)% for negative CVR in the GM. CV reduced on average by 2.5% after excluding the studies flagged for non-compliance. CV and ICC were negatively correlated ($p<0.05$). A Bland-Altman plot (Figure 3) illustrated the overall variation of whole-brain CVR with a test-retest difference of -0.004±0.202.

Qualitative Scoring by Visual Inspection

Three (1A or 1B) repeat studies were excluded as not being suitable for scoring by visual inspection. In the remaining paired studies (17/20) there was 82.35% (14/17) agreement in whole-brain (1A-1B) scores. Cohen's weighted Kappa was 0.75 in keeping with 'good' strength of agreement. Inter-rater reliability for hemispheric scoring was 1.0 (left) and 0.83 (right), suggestive of 'perfect' and 'very good' strength of agreement between scorers, respectively.

Age, Moyamoya Sub-Type, Stroke, Intellectual Function and Measures of BH-CVR Repeatability

Neither Moyamoya sub-type nor history of stroke were associated with ICC ranks (excellent, fair/good, poor; $p>0.05$). A significant effect of age at time of BH-CVR on the ICC ranks was found (mean age in poor, excellent and good ICC groups 8.6 (±1.7), 13.1 (±2.6) and 14.2 (±2.9) years respectively; $F(2,20) = 4.3$; $p=0.027$). ICC was not associated with FSIQ (n=9) nor subscales ($p>0.05$). However, the *count* of negative CVR voxels was a significant predictor of processing speed index (GM: $p=0.015$; WM: $p=0.047$) after controlling for BH duration and age. In addition, processing speed index was significantly reduced in patients with whole-brain qualitative scores of 3, i.e. in patients exhibiting significant steal ($p=0.009$).

Discussion

Our study demonstrates that BH-CVR implemented using standard functional MR sequences provides a feasible, repeatable and reliably interpretable tool for the assessment of CVR and cerebrovascular reserve in children with Moyamoya. On average, children older than 8.6 years, comprising 80% of our cohort, had no difficulty with our BH paradigm. In this group, within-day repeatability was 'good' to 'excellent', CV was <25% and agreement of repeat measures was acceptable. Repeatability was not affected by clinical factors such as Moyamoya etiology, history of stroke, or intellectual abilities. Negative CVR or steal, however, was associated with reduced processing speed index in a subset of our cohort. Another important goal of our study was to explore whether BH-CVR maps are reliably interpretable between different clinical users. We found 'good' to 'excellent' agreement of within-day visual inspection scores and 'almost perfect' inter-rater agreement.

A significant number of children with Moyamoya are asymptomatic at time of diagnosis and therefore require lifelong surveillance. This was reflected in our study in which 35% of our patients were asymptomatic at diagnosis. Our study also included the youngest child with Moyamoya, reported to date, to successfully complete BH-CVR studies (age 6.2 years).[19,20,30] Lowering the age limit of feasibility to under 7 years for obtaining the CVR measures will facilitate earlier instigation of longitudinal surveillance in Moyamoya than is the current practice and inform clinical management in the age group at highest risk of stroke.

There are a number of factors that affect the BOLD response and thereby measures of repeatability. This was highlighted by our two patients with SCD who had notably lower repeatability, despite completing the BH-CVR without any issues with compliance or motion. This result may be attributed to the anemia in SCD driving a global impairment in CVR[31,32] and reducing the overall signal-to-noise ratio of the BOLD response. The increased influence of

noise can adversely affect the correlation of repeat scans. In addition, negative CVR, which is frequently seen in SCD,[32] is especially prone to high variability and therefore can affect repeatability in these patients, as suggested by our data.

The BH execution and the duration of BH can also affect the BOLD response. A short BH of 3 seconds is adequate to produce a detectable BOLD response. However, a longer BH increases the magnitude and number of voxels exhibiting the response, resulting in more robust and repeatable measures of BOLD-CVR.[33] The mean BH duration in our study ranged from 19.6-20.3 seconds, suggestive of good BH duration overall. Supervised practice of the paradigm, and employment of good BH practice guidelines such as paced breathing between challenges and breath-holding after expiration both at home and outside of the MRI scanner prior to BH-CVR collection may further improve BH performance, reduce variability and in-turn improve repeatability.[21] Other strategies to minimize motion during scanning include measures to reduce distractibility between BH-CVR cycles such as visual fixation with videos and/or games.

Real-time monitoring of the carbon dioxide stimulus is the desirable method for CVR quantification.[21] Alternatively, as in our study, the dynamic cerebellar BOLD time course can be used.[34] In this method, the BOLD signal from the cerebellum represents changes in the blood flow corresponding to the accumulation of arterial carbon dioxide in each BH and provides a dynamic trace reference for quantifying BOLD in the rest of the brain tissue. While this method is reliable for within-subject assessments of CVR, the use of MR-compatible technologies for real-time acquisition of carbon dioxide in conjunction with BOLD and BH will facilitate group-based and more sophisticated quantitative analysis in future studies.

Conclusion

Measures of BH-CVR repeatability in children are ‘good’ to ‘excellent’ and maps reliably interpretable by clinical users. While clinical acquisition of MRI-CVR using computer-controlled devices has become increasingly feasible with recent iterations of this technology, the relative simplicity of BH-CVR is desirable for younger patients who can follow simple instructions. In addition, BH-CVR remains a practical substitute for many institutions when controlled-delivery carbon dioxide methods are not available or tolerable. Standardization of BH paradigms, image acquisition and processing protocols will further allow the implementation of this promising technique for clinical assessment of cerebrovascular reserve and ischemic risk in childhood cerebrovascular disease.

Acknowledgements

We would like to acknowledge the support of The Auxillium Foundation, the Stroke Imaging Laboratory for Children and the Ontasian Imaging Laboratory.

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Lynch JK, Hirtz DG, DeVeber G, Nelson KB. Report of the National Institute of Neurological Disorders and Stroke workshop on perinatal and childhood stroke. *Pediatrics*. 2002;109(1):116-23.

2. Fullerton HJ, Wu YW, Zhao S, Johnston SC. Risk of stroke in children: ethnic and gender disparities. *Neurology*. 2003;61(2):189-94.

3. Fullerton HJ, Wu YW, Sidney S, Johnston SC. Risk of recurrent childhood arterial ischemic stroke in a population-based cohort: the importance of cerebrovascular imaging. *Pediatrics*. 2007;119(3):495-501.

4. Mackay MT, Wiznitzer M, Benedict SL, et al. Arterial ischemic stroke risk factors: the International Pediatric Stroke Study. *Ann Neurol*. 2011;69(1):130-40.

5. Ganesan V, Prengler M, Wade A, Kirkham FJ. Clinical and radiological recurrence after childhood arterial ischemic stroke. *Circulation*. 2006;114(20):2170-7.

6. Braun KPJ, Bulder MMM, Chabrier S, et al. The course and outcome of unilateral intracranial arteriopathy in 79 children with ischaemic stroke. *Brain*. 2009;132(Pt 2):544-57.

7. Amlie-Lefond C, Bernard TJ, Sébire G, et al. Predictors of cerebral arteriopathy in children with arterial ischemic stroke: results of the International Pediatric Stroke Study. *Circulation*. 2009;119(10):1417-23.

8. Dobson SR, Holden KR, Nietert PJ, et al. Moyamoya syndrome in childhood sickle cell disease: a predictive factor for recurrent cerebrovascular events. *Blood*. 2002;99(9):3144-50.

9. Rafay MF, Armstrong D, Dirks P, MacGregor DL, DeVeber G. Patterns of cerebral ischemia in children with moyamoya. *Pediatr Neurol*. 2015;52(1):65-72.

10. Guey S, Tournier-Lasserve E, Hervé D, Kossorotoff M. Moyamoya disease and syndromes: from genetics to clinical management. *Appl Clin Genet*. 2015;8:49-68.

11. Sobczyk O, Battisti-Charbonney A, Fierstra J, et al. A conceptual model for CO2-induced redistribution of cerebral blood flow with experimental confirmation using BOLD MRI. *Neuroimage*. 2014;92:56-68.

12. Gupta A, Chazen JL, Hartman M, et al. Cerebrovascular reserve and stroke risk in patients with carotid stenosis or occlusion: a systematic review and meta-analysis. *Stroke*. 2012;43(11):2884-91.

13. Conklin J, Fierstra J, Crawley AP, et al. Impaired Cerebrovascular Reactivity With Steal Phenomenon Is Associated With Increased Diffusion in White Matter of Patients With Moyamoya Disease. *Stroke*. 2010;41:1610-1616.

14. Reinhard M, Schwarzer G, Briel M, et al. Cerebrovascular reactivity predicts stroke in high-grade carotid artery disease. *Neurology*. 2014;83(16):1424-31.

15. Lee M, Zaharchuk G, Guzman R, Achrol A, Bell-Stephens T, Steinberg GK. Quantitative

hemodynamic studies in moyamoya disease: a review. *Neurosurg Focus*. 2009;26(4):E5.

16. Slessarev M, Han J, Mardimae A, et al. Prospective targeting and control of end-tidal CO2 and O2 concentrations. *J Physiol*. 2007;581(Pt 3):1207-1219.

17. Kassner A, Winter JD, Poublanc J, Mikulis DJ, Crawley AP. Blood-oxygen level dependent MRI measures of cerebrovascular reactivity using a controlled respiratory challenge: reproducibility and gender differences. *J Magn Reson Imaging*. 2010;31(2):298-304.

18. Poublanc J, Crawley A, Mandell D, et al. Quantification of cerebrovascular reactivity by blood oxygen level-dependent MR imaging and correlation with conventional angiography in patients with Moyamoya disease. *AJNR Am J Neuroradiol*. 2010;31(5):862-7.

19. Han JS, Mikulis DJ, Mardimae A, et al. Measurement of cerebrovascular reactivity in pediatric patients with cerebral vasculopathy using blood oxygen level-dependent MRI. *Stroke*. 2011;42:1261-1269.

20. Leung J, Kim JA, Kassner A. Reproducibility of cerebrovascular reactivity measures in children using BOLD MRI. *J Magn Reson Imaging*. 2016;43(5):1191-1195.

21. Bright MG, Murphy K. Reliable quantification of BOLD fMRI cerebrovascular reactivity despite poor breath-hold performance. *Neuroimage*. 2013;83:559-68.

22. Fukui M. Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Japan. *Clin Neurol Neurosurg*. 1997;99 Suppl 2:S238-40.

23. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res*. 1996;29(3):162-73.

24. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *IEEE Trans Med Imaging*. 2001;20(1):45-57.

25. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. *Med Image Anal*. 2001;5(2):143-156.

26. Wechsler D. *Wechsler Intelligence Scale for Children-Fifth Edition. Technical and Interpretive Manual*. Bloomington, MN: Pearson; 2014.

27. Wechsler D. *Wechsler Intelligence Scale for Children - Fourth Edition Technical and Interpretive Manual*. San Antonio, TX: The Psychological Corporation; 2003.

28. Lipp I, Murphy K, Caseras X, Wise RG. Agreement and repeatability of vascular reactivity estimates based on a breath-hold task and a resting state scan. *Neuroimage*. 2015;113:387-96.

29. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977;33(1):159-74.

30. Mandell DM, Han JS, Poublanc J, et al. Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in Patients with arterial steno-occlusive disease: comparison with arterial spin labeling MRI. *Stroke*. 2008;39(7):2021-2028.

31. Kosinski PD, Croal PL, Leung J, et al. The severity of anaemia depletes cerebrovascular dilatory reserve in children with sickle cell disease: a quantitative magnetic resonance imaging study. *Br J Haematol*. 2017;176(2):280-287.

32. Prohovnik I, Hurlet-Jensen A, Adams R, De Vivo D, Pavlakis SG. Hemodynamic etiology of elevated flow velocity and stroke in sickle-cell disease. *J Cereb blood flow Metab*. 2009;29(4):803-810.

33. Abbott DF, Opdam HI, Briellmann RS, Jackson GD. Brief breath holding may confound functional magnetic resonance imaging studies. *Hum Brain Mapp*. 2005;24(4):284-90.

34. Raut R V, Nair VA, Sattin JA, Prabhakaran V. Hypercapnic evaluation of vascular reactivity in healthy aging and acute stroke via functional MRI. *NeuroImage Clin*. 2016;12:173-9.

Table 2. Patient demographics and corresponding repeatability metrics of BH CVR studies.

Patient ID	Age at diagnosis (years)	Sex	Moyamoya Classification	Clinical Presentation	Study #	Age at CVR (years)	BH Issues	GM		WM		*Study quality using ICC
								ICC [95% CI]	CV	ICC [95% CI]	CV	
P01	6.0	M	Familial	Stroke	1	12.5		0.93 [0.92,0.93]	2,4	0.83 [0.81,0.85]	9,4	Excellent
P02	8.3	M	Neurofibromatosis Type 1	Asymptomatic¶	1	15.3	NC	0.74 [0.72,0.76]	1,27	0.67 [0.64,0.69]	4,41	Excellent
P03	7.0	M	SCD	Asymptomatic¶	1	9.9		0.51 [0.47,0.55]	15,49	0.55 [0.51,0.59]	23,44	Fair
P04	5.5	F	Idiopathic	TIA and Headache	1	8.6		0.80 [0.78,0.82]	0,10	0.74 [0.71,0.76]	9,8	Excellent
					2	12.6		0.82 [0.80,0.83]	2,3	0.84 [0.82,0.85]	8,10	Excellent
P05	8.3	F	Idiopathic	Headache	1	11.8		0.92 [0.91,0.92]	1,0	0.91 [0.91,0.92]	2,0	Excellent
					2	16.0		0.89 [0.88,0.90]	3,16	0.88 [0.86,0.89]	1,11	Excellent
P06	6.0	M	Idiopathic	TIA	1	10.1	NC	0.32 [0.27,0.36]	11,62	0.32 [0.27,0.36]	18,64	Poor
P07	10.0	M	Idiopathic	TIA	1	16.6		0.90 [0.89,0.91]	16,20	0.88 [0.87,0.90]	17,31	Excellent
P08	9.0	M	Idiopathic	Stroke	1	11.5		0.71 [0.69,0.73]	4,26	0.69 [0.66,0.72]	2,20	Good
P09	8.0	F	Neurofibromatosis Type 1	Asymptomatic¶	1	14.0		0.74 [0.72,0.76]	13,14	0.66 [0.63,0.69]	6,5	Good
					2	16.0		0.83 [0.81,0.84]	9,17	0.79 [0.78,0.81]	0,9	Excellent
P10	6.0	F	Idiopathic	TIA	1	13.2		0.61 [0.58,0.64]	7,18	0.59 [0.55,0.63]	4,17	Good
					2	14.3		0.81 [0.79,0.82]	0,14	0.83 [0.81,0.85]	3,11	Excellent
					3	16.4		0.73 [0.70,0.75]	9,14	0.77 [0.75,0.79]	3,9	Excellent
P11	8.0	M	Idiopathic	Headache	1	8.9	NC	0.33 [0.29,0.37]	5,10	0.43 [0.39,0.47]	9,15	Poor
					2	11.1		0.79 [0.78,0.81]	10,2	0.82 [0.81,0.84]	10,6	Excellent
					3	12.1		0.82 [0.80,0.83]	7,8	0.85 [0.84,0.86]	11,18	Excellent

					4	13.2		0.77 [0.75,0.79]	19,40	0.79 [0.77,0.81]	20,14	Excellent
P12	11.3	F	ACTA 2	Asymptomatic	1	17.4		0.62 [0.59,0.65]	8,34	0.64 [0.61,0.67]	5,17	Good
P13	4.0	F	Idiopathic	TIA	1	12.0	NC	0.44 [0.40,0.48]	12,24	0.33 [0.28,0.38]	3,18	Fair
					2	14.4		0.77 [0.75,0.79]	6,12	0.76 [0.73,0.78]	3,12	Excellent
					3	15.9		0.67 [0.65,0.70]	7,14	0.70 [0.67,0.73]	7,9	Good
P14	10.3	F	SCD	Asymptomatic¶	1	17.0		0.48 [0.44,0.52]	17,44	0.46 [0.42,0.51]	13,40	Fair
P15	6.0	F	Idiopathic	TIA	1	9.3		0.79 [0.77,0.81]	5,27	0.86 [0.85,0.88]	0,13	Excellent
P16	9.0	M	Idiopathic	TIA	1	9.7		0.80 [0.78,0.82]	20,42	0.80 [0.78,0.81]	17,35	Excellent
P17	7.5	M	Neurofibromatosis Type 1	Asymptomatic¶	1	9.1	Motion	0.31 [0.27,0.36]	24,75	0.30 [0.25,0.34]	34,68	Poor
P18	10.5	F	Neurofibromatosis Type 1	Headache	1	11.1		0.86 [0.85,0.87]	1,0	0.84 [0.82,0.85]	3,11	Excellent
P19	1.4	M	Idiopathic	Stroke	1	6.2	Motion	0.33 [0.28,0.38]	35,37	0.23 [0.18,0.29]	14,32	Poor
P20	16.8	M	Radiation vasculopathy	Asymptomatic¶	1	18.0		0.88 [0.86,0.89]	5,14	0.87 [0.86,0.89]	1,5	Excellent
				Mean±SD				**0.70±0.19**	**9.1±8.2, -22.5±18.3**	**0.69±0.20**	**8.6±8.0, -20.±17.1**	
				Mean±SD (excluding NC and Motion)				**0.77±0.12**	**7.6±6.1, -18.9±13.8**	**0.76±0.12**	**7.3±6.3, -16.1±12.1**	

M=male; F=female; SCD=sickle cell disease; TIA=transient ischaemic attacks; NC=non-compliant; Motion=excessive motion; ICC=intraclass correlation; CI=confidence interval; CV=% coefficient of variation for positive and negative CVR estimates; ¶Diagnosis made following screening imaging study; *Study quality based on whole-brain ICC

Figure legends

Figure 1. (a) Schematic of breath-hold paradigm (b) Cerebellar BOLD signal time course obtained from a representative participant (P11).

Figure 2: (a) Normal appearing BH-CVR maps demonstrating positive BH-CVR reactivity. (P07: Whole-brain score: 1. Hemispheric score: 1:1). (b) Abnormal BH-CVR maps demonstrating bilateral abnormal (left > right) negative reactivity (P15: Whole brain score: 3. Hemispheric score: 3:3); L=left; a.u.= arbitrary units.

Figure 3: Bland-Altman plot showing a low mean between-scan difference of 0.004 (dotted line) with a critical difference of 0.202 (broken lines).