Role of Genetic Variation in Collateral Circulation in the Evolution of Acute Stroke

A Multimodal Magnetic Resonance Imaging Study

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Background and Purpose—No studies have determined the effect of differences in pial collateral extent (number and diameter), independent of differences in environmental factors and unknown genetic factors, on severity of stroke. We examined ischemic tissue evolution during acute stroke, as measured by magnetic resonance imaging and histology, by comparing 2 congenic mouse strains with otherwise identical genetic backgrounds but with different alleles of the Determinant of collateral extent-1 (Dcel1) genetic locus. We also optimized magnetic resonance perfusion and diffusion–deficit thresholds by using histological measures of ischemic tissue.

Methods—Perfusion, diffusion, and T2-weighted magnetic resonance imaging were performed on collateral-poor (congenic-Bc) and collateral-rich (congenic-B6) mice at 1, 5, and 24 hours after permanent middle cerebral artery occlusion. Magnetic resonance imaging–derived penumbra and ischemic core volumes were confirmed by histology in a subset of mice at 5 and 24 hours after permanent middle cerebral artery occlusion.

Results—Although perfusion-deficit volumes were similar between strains 1 hour after permanent middle cerebral artery occlusion, diffusion-deficit volumes were 32% smaller in collateral-rich mice. At 5 hours, collateral-rich mice had markedly restored perfusion patterns showing reduced perfusion-deficit volumes, smaller infarct volumes, and smaller perfusion–diffusion mismatch volumes compared with the collateral-poor mice (P<0.05). At 24 hours, collateral-rich mice had 45% smaller T2-weighted lesion volumes (P<0.005) than collateral-poor mice, with no difference in perfusion–diffusion mismatch volumes because of penumbral death occurring 5 to 24 hours after permanent middle cerebral artery occlusion in collateral-poor mice.

Conclusions—Variation in collateral extent significantly alters infarct volume expansion, transiently affects perfusion and diffusion magnetic resonance imaging signatures, and impacts salvage of ischemic penumbra after stroke onset.

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Key Words: collateral circulation ■ diffusion ■ magnet resonance imaging ■ mouse ■ perfusion

Collateral vessels are anastomoses that connect adjacent arterial trees and reroute perfusion around obstructed arteries or veins. Variation among individuals in the extent of native collaterals (ie, number or diameter) may serve as a prognostic determinant of stroke severity and improve clinical risk benefit decisions for treatment options.1,2 Angiographic studies grading collateral circulation in patients with acute stroke have found that final infarct size3,4 and functional outcome deficit vary inversely with collateral flow.5-7 Patients with robust collateral circulation also have improved outcomes after tissue-type plasminogen activator (tPA) administration8 (including reduced risk of hemorrhaging9) or intra-arterial revascularization9-11 suggesting that collateral extent significantly impacts the success of therapeutic interventions for acute ischemic stroke.

Imaging of perfusion in acute stroke patients using computed tomography or magnetic resonance imaging (MRI) is routine for accurate diagnosis and volumetric assessments of ischemic tissue. Contrast-enhanced computed tomography,1 MRI,12 and arterial spin–labeled perfusion MRI13,14 are also used clinically to differentiate hemorrhagic stroke and assess collateral status. Ischemic stroke registries that centrally store these data have been used retrospectively to examine how collateral circulation alters lesion evolution.3,5,7,12,15,16 Such
analyses consider many outcome-modifying variables (eg, age, sex, comorbidities, vascular risk factors, type/time of therapeutic intervention). However, they cannot account for all variables that contribute to acute stroke heterogeneity, resulting in conclusions based on correlation rather than on causation.

Recently, a novel mouse model has been developed that permits examination of the contribution of variation in collateral extent on variation in lesion progression because genetic and environmental differences are controlled. \(^{17}\) Two congenic mouse lines were constructed that are isogenic except at a discrete locus on chromosome 7, Determinant of collateral extent-1 (Dce1), whose allelic variants determine 85% of the wide variation in collateral extent present in different inbred mouse strains: CNG-Bc are congenic wild-type BALB/cByJ (Bc) mice with sparse, small-diameter pial collaterals, while CNG-B6 are congenic B6 mice that harbor the C57BL/6J (B6) allele of Dce1, resulting in abundant, large-diameter collaterals. Because these mice differ only in collateral extent, they provide a unique opportunity to examine the role of collaterals, per se, on stroke progression.

In this study, congenic mice were subjected to permanent middle cerebral artery occlusion (pMCAo) to determine how differences in collateral extent affect ischemic tissue evolution. We used perfusion, diffusion, and T2-weighted MRI techniques that are commonly used to characterize ischemic core and estimate penumbra. \(^{18-21}\) To more specifically assess the role of collaterals and exclude confounding reperfusion factors, we used MRI at 1, 5, and 24 hours after pMCAo. To corroborate the magnetic resonance (MR) findings, we performed cleaved caspase-3 (CC3) and heme oxygenase-1 (HO-1) immunohistochemistry in a subset of animals to quantify ischemic core and penumbra, respectively. Additionally, we also used this unique data set to derive specific MR apparent diffusion coefficient (ADC)– and cerebral blood flow (CBF)–deficit thresholds that best approximate histological ground truth. Compared with collateral-poor CNG-Bc mice, the collateral-rich CNG-B6 mice showed significantly smaller ADC-deficit volume and CBF-deficit volume and more rescued penumbral tissue. Our data demonstrate that collateral extent is a key determinant of infarct evolution in mice and, if confirmed in humans, should be considered as an outcome-modifying variable in stroke clinical trials.

### Materials and Methods

#### Animal Preparation

Two congenic mouse strains on the Bc background with distinct pial collateral extent profiles (Figure 1, see Introduction) were studied (n=22/strain, male, 3–4 months of age). \(^{17}\) After an overnight fast, mice were anesthetized with 1.5% to 2% of isoflurane, and their respective rectal temperature, blood O\(_2\) saturation, and pulse/respiration rates were monitored using a MouseOx Plus (STARR Life Science Corp, Oakmont, PA) and maintained at 37±0.5°C, >97%, 380±10 bpm/100±10 bpm during surgery and scanning. A 2-mm aseptic cranietomy was performed, and pMCAo was performed by cauterization at the M2–middle cerebral artery (MCA) level. \(^{17}\) The cranietomy was closed with gelfoam (Pfizer Inc, 0009-0396-05, New York, NY) and dental cement to avoid susceptibility artifact during MR scans. After pMCAo, mice were maintained in the scanner under 0.75% to 1% isoflurane delivered in 25% O\(_2\) at a flow rate of 1 L/min. A subset of mice was scanned only once at either 1 (n=9/congenic strain), 5, or 24 hours (n=4/congenic strain) after pMCAo for histological assessment. The remaining subjects underwent longitudinal scans. All studies were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

#### MRI Acquisition

MRI data were acquired using a Bruker BioSpec 9.4 Tesla system with a BGA-9G gradient insert (Bruker Corp, Billerica, MA). A home-made surface coil (with an internal diameter of 0.8 cm) was used for brain imaging. For CBF measurement, an additional actively decoupled heart coil was used for continuous cardiac spin labeling. \(^{22}\) This was achieved by a 2-shot gradient-echo echo-planar imaging sequence with bandwidth =300 kHz, repetition time/echo time =3000/5 ms, labeling duration =2.524 s, postlabeling delay =300 ms, matrix =64×64, field of view =1.6×1.6 cm, 10 slices, and slice thickness =0.75 mm. Diffusion-weighted images were acquired with the same geometry using 2-shot spin-echo echo-planar imaging with bandwidth =300 kHz, repetition time/echo time =3000/22 ms, Δ/Δ =4/12 ms, number of B0 =5, number of directions =30, and b value =1200 s/mm\(^2\). T2-weighted anatomic images were acquired using a RARE (Rapid Acquisition With Relaxation Enhancement) sequence with spectral width =47 kHz, repetition time/echo time =2500/44 ms, matrix =144×144, RARE factor =8, number of averages =20, field of view =1.44×1.44 cm, 10 slices, and slice thickness =0.75 mm.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Genetic model of abundant vs sparse collaterals: congenic-B6 (A) and congenic-Bc mice (B). Maximally dilated precapillary vessels filled with Microfil shows larger collateral number in congenic (CNG)-B6 compared with CNG-Bc mice. Stars indicate MCA-to-ACA collaterals of 12±13 μm diameter; CNG-Bc, congenic wild-type BALB/cByJ mice (2±1 MCA-to-ACA collaterals of 12±13 μm lumen diameter); CNG-B6, congenic wild-type BALB/cByJ mice with C57BL/6J Determinant of collateral extent-1 (Dce1) genetic locus introgressed into genome (14±1 MCA-to-ACA collaterals of 20±2 μm diameter); \(^{17}\) and MCA, middle cerebral artery.
Histological Confirmation of the Ischemic Lesion

Because no reliable histological biomarker can delineate the border zone between penumbra and ischemic core within the first hour of stroke, stereological morphometric analysis was performed on a subset of mice at 5 and 24 hours after pMCAo (n=5/congenic strain/end point). Mice were euthanized by Euthasol overdose (100 mg/kg, IP) and transcardially perfused with 25 mL of 1x PBS (pH 7.2) followed by 25 mL of 4% paraformaldehyde. Extracted brains were post-fixed in 4% paraformaldehyde overnight, followed by 30% sucrose until they sank and frozen to a chilled sliding microtome. Serial 30-μm-thick coronal sections were sliced between +2.2 mm anterior and +4.5 mm posterior of Bregma (as determined by atlas-derived landmarks), collecting only 1 of every 10 sections (21–23 sections/mouse/ marker) for free-floating immunohistochemistry. Sections were initially incubated in an MOM kit (PK-2200 Vector Laboratories, Burlingame, CA) according to the manufacturer’s protocol or blocking solution (1% bovine serum albumin, 0.4% Triton X-100, and 4% normal goat serum in PBS) for 20 minutes to block nonspecific binding. Sections were immunostained overnight at 4°C with either the penumbra marker mouse monoclonal antibody against HO-1 (1:50; Enzo Life Science, ADI-OSA-110, Farmingdale, NY) or the ischemic core marker rabbit polyclonal antibody against CC3 (1:100; Cell Signaling, 9661, Danvers, MA) and visualized with Alexa Fluor secondary antibodies (1:800; Molecular Probes, Carlsbad, CA). Volumes of ischemic core, penumbra, and nonischemic tissue were quantified stereologically using the Cavalieri estimator method on Stereo Investigator (v7.5; MBF Bioscience, Microbrightfield, Inc, Williston, VT) and corrected for dehydration-related shrinkage in slice thickness and edema using the indirect method.23 Systematic random samples were unbiasedly counted in 50 μm2 frames within manually defined boundaries of each hemisphere using a 10 μm optical dissector with 2 μm upper and lower guard zones. Stereological images were performed with an Olympus BX50 microscope using a 60x/1.4 NA oil-immersion objective with a calculated coefficient of error of intra-animal variation of <0.1.

Data Processing and Analysis

Image analysis was performed using Statistical Parametric Mapping in a custom-written Matlab (MathWorks Inc, Natick, MA) script.24 Images were skull-stripped, coregistered across subjects, and analyzed using established protocols that we published previously.25,26 Specifically, CBF was calculated from cardiac spin labeling data using CBF = \( \frac{\lambda T_1 S_C - S_I}{S_L + (2\alpha - 1)S_C} \), where \( S_C \) and \( S_I \) are the MR signal intensities from the control and labeled images, respectively, \( \lambda \) is the water–brain–blood partition coefficient, \( T_1 \) is that of the tissue, and \( \alpha \) is the arterial spin-labeling efficiency. For \( \lambda \), \( T_1 \), and \( \alpha \), we used 0.9, 1.9, and 0.78, respectively.27 ADC maps were calculated from the diffusion-weighted images using ADC = \( \ln \left( \frac{S_0}{S} \right) \), where \( S_0 \) is the signal intensity obtained at \( b_1 \) (1200 s/mm2) and \( S \) is the signal intensity obtained at \( b_0 \) (0 s/mm2). Group-averaged CBF, ADC, and \( T_1 \)-weighted images for each experimental group and time point were presented.

To better identify a specific ADC or CBF threshold reflecting histological measure, a range of ADC (0.45–0.6 mm2/s) and CBF (0.175–0.35 mL/g/min) thresholds was used to compute their respective deficit volumes that underwent best-fit correlations with respect to histologically defined volumes using an established method.28 ADC- and CBF-deficit thresholds were calculated using Euclidean distances between true histologically defined and threshold-dependent MRI volumes. Polynomial curves up to a degree of 4 were fitted to predict distances at unmeasured threshold values, and the curves that best balanced over- and underfitting the 2 volumes were selected as the optimal threshold cutoff for ADC and CBF deficits.

The values in the ipsilesional hemisphere were calculated based on the MR perfusion and diffusion deficits that delineated with our histologically guided thresholds, while the values in the contralesional hemisphere were measured with size-matched mirror ROIs. \( T_1 \)-weighted lesion volumes were quantified by thresholding hyperintense signals (±2 SD of contralateral normal cortical tissue) as described previously.29 The ratio of MR-derived penumbral loss was defined as the difference in ADC-deficit volumes between 1 time points divided by the mismatch volume at 1 hour.15 The ratio of infarct growth was defined as the difference in ADC-deficit volumes between the 2 time points divided by the \( T_1 \)-weighted lesion volume at 24 hours.15

Statistical analysis was performed using SPSS software (IBM SPSS statistics 21, IBM Corp, Armonk, NY). To compare the difference in the deficit volumes between 2 congenic strains at multiple time points, unpaired 2-way analysis of variance followed by Tukey’s honestly significant difference and Scheffe post hoc test was performed for data-passing and not-passing homogeneity test, respectively. Significant difference in ADC or CBF value, the ratio of penumbra tissue loss, and the ratio of infarct growth between the 2 congenic strains at a particular time point was assessed by a 2-tailed independent \( t \) test. All data in text are presented as mean±SEM.

Results

Physiological parameters remained consistent throughout the MRI scans at 1, 5, and 24 hours after pMCAo. Because no significant difference in CBF and ADC intensities or volumes were observed 24 hours after pMCAo within congenic mice that either received a single scan (n=4 per strain) or longitudinal scans (n=5 per strain in the cohort for 5- and 24-hour multiple scans), data collected for each time point were compiled together for analysis. One CNG-Bc mouse was excluded from the data because of a negative mismatch volume detected 5 hours after pMCAo, likely because of artifact during imaging acquisition. Figure 2 shows group-averaged CBF, ADC, and \( T_1 \)-weighted images for each time point in CNG-B6 (n=9 per time point) and CNG-Bc mice (n=9 at 1 and 24 hours and n=8 at 5 hours) after pMCAo. By using a within-subject comparison with histological data, we identified ADC- and CBF-deficit thresholds of 0.541 mm2/s and 0.255 mL/g/min after pMCAo best approximated the tissue-deficit volume defined by CC3 (necrotic/apoptotic cells) and CC3+HO-1 (necrotic/apoptotic cells+penumbra) expression, respectively (Figure 3A and 3B). These thresholds were used for subsequent analyses. With these optimized thresholds, we also found significant correlations between ADC-deficit and CC3 lesion volumes (Figure 3C) and also CBF-deficit and CC3+HO-1 lesion volumes (Figure 3D).

At 1 hour after pMCAo, we observed marked reductions in CBF values in the lesion side compared with the contralesional (normal) hemisphere as expected (Figure 4A). We observed no significant differences in the MR-derived CBF-deficit volume at this time point (Figure 5A). The mean ADC values were not different between the 2 groups, but ADC-deficit volumes were significantly smaller in CNG-B6 mice (15.957±1.305 mm3) than in CNG-Bc mice (23.325±4.592 mm3; \( P<0.005 \); Figure 5B). No significant difference in perfusion–diffusion mismatch volumes was found at 1 hour (16.562±6.817 mm3 for CNG-B6 and 14.370±7.372 mm3 for CNG-Bc; \( P>0.05 \); Figure 5C).

Additionally, no differences were observed between congenic strains in \( T_1 \)-weighted infarct volume at 1 hour (Figure 5C and 5D), likely because of the low sensitivity of this method to detect ischemic tissue during the hyperacute phase of pMCAo.

At 5 hours after pMCAo, CBF-deficit volumes were significantly reduced in collateral-rich CNG-B6 mice (19.692±5.208
mm³) compared with collateral-poor CNG-Bc mice (32.425±8.213 mm³; \( P < 0.005 \); Figure 5A), suggesting significant restoration of tissue perfusion by collaterals. The mean ADC values were not different between the 2 groups, but ADC-deficit volumes were significantly smaller in CNG-B6 mice (16.165±3.212 mm³) than in CNG-Bc mice (24.481±4.777; \( P < 0.005 \); Figure 5B). A significantly smaller perfusion–diffusion mismatch in CNG-B6 was observed (3.528±2.596 mm³ compared with 10.758±7.661 mm³ in CNG-Bc; \( P < 0.05 \); Figure 5C).

The infarct volume defined by \( T_{2} \)-weighted images in CNG-B6 mice (9.876±1.883 mm³) was also significantly smaller than in CNG-Bc mice (16.327±3.516 mm³; \( P < 0.005 \); Figure 5D). No significant difference in the loss of perfusion–diffusion mismatch tissue (1.257±6.854 mm³ for CNG-B6 and 8.047±11.754 mm³ for CNG-Bc; \( P > 0.05 \); Figure 6A), as well as the growth of \( T_{2} \)-weighted lesion volume (1.149±2.939 mm³ for CNG-B6 and 3.484±5.089 mm³ for CNG-Bc; \( P > 0.05 \); Figure 6B), were found from 1 to 5 hours.
At 24 hours after pMCAo, the CBF-deficit volumes remained significantly reduced in collateral-rich CNG-B6 mice (19.370±5.179 mm³) compared with those in collateral-poor CNG-Bc mice (32.021±8.957 mm³; P<0.005; Figure 5A). The mean ADC values were not different between the 2 groups, but ADC-deficit volumes were significantly smaller in CNG-B6 mice (17.379±5.945 mm³) than in CNG-Bc mice (28.713±3.6826 mm³; P<0.005; Figure 5B). No significant difference in perfusion–diffusion mismatch volumes was found (1.473±1.245 mm³ for CNG-B6 and 3.331±2.648 mm³ for CNG-Bc; P>0.05; Figure 5C). The infarct volume defined by T2-weighted images in CNG-B6 mice (18.133±4.231 mm³) was significantly smaller than in CNG-Bc mice (33.527±3.829 mm³; P<0.005; Figure 5D). In addition, loss of perfusion–diffusion mismatch tissue (approximate penumbra) from 5 to 24 hours became significantly different between CNG-B6 (6.532±7.697%) and CNG-Bc (36.376±8.289%) mice. The growth of T₂-weighted lesion volume also differed between CNG-B6 (6.137±3.225%) and CNG-Bc (15.076±2.229%) mice (both P<0.05; Figure 6A and 6B).

**Discussion**

The present study sought to differentiate the contribution of variation in pial collateral circulation to the evolving perfusion and diffusion MRI signals within the first 24 hours after acute stroke. Using congenic mouse strains that differ solely in their collateral extent, we report that (1) genetic specification of poor versus good collaterals alters the initial ADC-deficit volumes and the ability to salvage at-risk tissue within 5 hours after stroke. (2) Although CBF-deficit volume does not differ between the 2 congenic strains at 1 hour after pMCAo, collateral perfusion in collateral-rich mice is higher and sufficient to restrict expansion of initial ADC-deficit volume. (3) Within 5 hours after pMCAo, collateral-rich mice display small CBF-deficit volume, resulting in significant rescue of penumbra, while collateral-poor mice experience a larger area that remains at risk, demonstrating that collateral extent plays a major role in altering the outcome of the second wave of cell death during permanent focal ischemia. (4) The imaging protocols and ADC/CBF thresholds established herein in mice demonstrate sufficient sensitivity and specificity to detect ischemic tissue evolution and, thus, offer a technical foundation for future studies investigating the efficacy of collateral therapeutics.

Wide variation in collateral extent among 21 inbred mouse strains is found to be tightly correlated with similarly wide variation in infarct volume after pMCAo and links to variants of the **Dce1** locus in these strains, suggesting that genetic polymorphisms underlying differences in collateral extent may be important risk factors for poor stroke outcome. However, other gene variants not associated with differences in collateral extent can also alter stroke severity, including the expression and activity of proteins that could affect infarct progression poststroke. In addition, uncontrollable

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**Figure 4.** Cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) values in CNG-B6 and CNG-Bc mice. CBF (A) and ADC (B) values in corresponding lesion volume at 1, 5, and 24 h after permanent middle cerebral artery occlusion (pMCAo). No significant change in CBF or ADC in contralesional hemisphere in both strains. Significantly higher CBF present in lesion area in CNG-B6 1 h after pMCAo. Means±SEM; *P<0.05 vs CNG-Bc. CNG-Bc indicates congenic-line5 wild-type BALB/cByJ mice; and CNG-B6, congenic-line5 wild-type BALB/cByJ mice with C57BL/6J **Determinant of collateral extent-1 (Dce1)** genetic locus introgressed into genome.

**Figure 5.** CNG-B6 mice shows smaller cerebral blood flow deficit, apparent diffusion coefficient deficit (A and B), mismatch (C and D), and T₂-weighted lesion volumes after permanent middle cerebral artery occlusion. Means±SEM; *,**,##P<0.05, <0.005 vs CNG-Bc; ###P<0.05, <0.005 vs time. CNG-Bc indicates congenic-line5 wild-type BALB/cByJ mice; and CNG-B6, congenic-line5 wild-type BALB/cByJ mice with C57BL/6J **Determinant of collateral extent-1 (Dce1)** genetic locus introgressed into genome.
physiological differences between wild-type mouse trains, for instance, mean arterial pressure and immune response, might contribute to lesion evolution. The use of congenic mice with genetic variability restricted to a single locus shown to be the major determinant of anatomic variation in collaterals in mice allowed us to test for causation between collateral extent and the severity of the ischemic lesion and its evolution. To our knowledge, this is the first study to dissect the causal role of collateral extent, per se, in ischemic lesion evolution during the hyperacute, acute, and early-chronic phases after pMCAo. pMCAo in mice immediately reduces averaged CBF in the middle cerebral artery territory by 50% and irreversibly results in necrotic death within minutes in regions where perfusion drops by $\geq 80%$.32 pMCAo in both congenic lines resulted in significant reductions in CBF, yet mean CBF within perfusion-deficit volumes were significantly greater in collateral-rich compared with collateral-poor mice 1 hour after stroke onset (40.69±4.31% and 38.23±2.05%, respectively) and reduced the initial ischemic core volume by 31.50% as defined by ADC-deficit thresholds. This immediate retrograde collateral perfusion of the MCA territory is established within seconds after occlusion because of the large increase in cerebral perfusion pressure across the collateral network.33,34 Although mathematical models have predicted that pial collaterals can continue to maintain 15% of overall MCA baseline CBF immediately after pMCAo,35 retrograde collateral perfusion to the proximal MCA territory is insufficient to preserve cellular function during this hyperacute phase, resulting in death for most cells in this region if CBF is not sufficiently restored.

At 5 hours after pMCAo, a significantly smaller CBF-deficit volume was found in collateral-rich CNG-B6 mice compared with their collateral-poor counterparts. Studies in mice have shown that during the first few hours of collateral perfusion, presumed fluid shear stress–mediated dilation results in a 26% increase in collateral diameter after major vessel occlusion. Collaterals subsequently undergo outward remodeling of lumen diameter that requires 3 to 6 days, depending on genetic background, to achieve maximal remodeling (≈2- to 4-fold increase in anatomic lumen diameter).22 Changes in smooth muscle tone, plus the initial driving force of cerebral perfusion pressure across the collateral network induced by occlusion, are the primary determinants of collateral blood flow during the acute phase of pMCAo. However, further changes in collateral flow induced by alterations in cerebral perfusion pressure, such as those produced by the downstream collapse of the venous network from insufficient inflow, will also impact delivery of oxygen to the territory at risk.33,36 The lack of change in ADC threshold volumes between 1 and 5 hours after pMCAo in collateral-rich mice, coupled with a significant reduction in perfusion–diffusion mismatch volume, indicates that collateral circulation can rescue penumbral tissue from death. On the other hand, in the collateral-poor mice, collateral flow is insufficient to prevent the second wave of cellular death resulting from an inability to restore adequate circulation to the entire MCA penumbral territory. Using the oxidative stress marker HO-1 expressed in penumbral tissue after pMCAo,37 we found that mismatch of perfusion-deficit volumes <0.255 mL/g/min and diffusion-deficit volumes <0.541 mm$^2$/s accurately estimate penumbral volume in both congenic strains.

Infarct volume at 24 hours after pMCAo, as confirmed by ADC-deficit volumes and T$_2$-weighted imaging, was significantly smaller in collateral-rich mice compared with collateral-poor mice 1 hour after pMCAo in mice.38 Our detection of a significant divergence of ADC- and CBF-deficit volumes between congenic strains demonstrates that collateral circulation dictates initial infarct size, infarct expansion, and whether penumbral tissue will be rescued. Multimodal MRI techniques are improving the efficiency of clinical stroke diagnosis and allow rapid estimation of penumbral tissue volume as early as the acute phase of ischemic.
stroke, which is important in balancing the risk versus benefit of reperfusion therapy.39,40 The controversy surrounding the use of perfusion–diffusion mismatch to indicate penumbra and the course of stroke treatment is in part attributed to the lack of consistency in defining deficit thresholds, overestimation of the tissue at risk, assumption of the irreversible diffusion lesions, and differences in the parameters used during acquisition.40,42 Furthermore, the dynamic nature and heterogeneous distribution of the penumbra can cause significant fluctuations in perfusion- and diffusion-deficit volumes during acute stroke43,44—variation that is only accentuated when collateral extent varies among subjects.6,7,45 Our findings suggest caution to be taken when referring to the penumbra or perfusion–diffusion mismatch volume at a fixed time point as a prognostic or treatment criteria, without carefully considering the role of collateral circulation and the consequent temporal evolution of ischemic penumbra.

Mouse perfusion and diffusion MRI have many challenges, including strong susceptibility artifacts and lack of commercially available hardware for high-quality 2-coil arterial spin labeling.23,46,47 More importantly, no gold standard ADC and CBF thresholds exist for probing ischemic tissue in rodents. We addressed these problems herein by optimizing our thresholds against histologically defined volumes, leading to a strategy for mapping the evolution of ischemic tissue in mice. One limitation of our study is that $T_1$ relaxation times increase after stroke and may result in decreased CBF signals.48,49 Thus, even though mismatch volumes in our study may better approximate penumbra volumes in mice with different collateral extents, they may not represent the absolute size of ischemic penumbra.

Conclusions

In summary, we performed quantitative perfusion and diffusion MRI in congenic mice differing in collateral extent to investigate the relationship between the ischemic penumbra and the vigor of the collateral circulation. Our findings using mice with identical genetic background but with congenically specified good versus poor collaterals demonstrate that the presence of good collaterals results in penumbral salvage within the first 5 hours of pMCAo followed by reduced perfusion–diffusion mismatch tissue loss during the 5- to 24-hour period. The results demonstrate the utility of this mouse model to explore the development of therapeutic strategies to enhance collateral perfusion poststroke onset.50 Additional studies combining this genetic mouse model with transient middle cerebral artery occlusion followed by postlabeling delays will allow investigation of how reperfusion interacts with inherent differences in collateral circulation among individuals to determine the dynamic behavior of the ischemic penumbra.

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Disclosures

None.

References


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