The Role of Collateral Circulation in Perfusion and Diffusion MRI after Stroke

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TARGET AUDIENCE Basic and Clinical Scientists in Stroke, Neuroimaging, and Vascular Biology.

INTRODUCTION Native collaterals are pre-existing anastomoses that connect adjacent arterial trees.[1] During acute ischemia, collateral extent (i.e. number and average lumen diameter) dictates retrograde perfusion from unobstructed adjacent arterials to the ischemic lesion—thus determining the severity of the injury.[2,3] Native collateral extent varies greatly in the general population.[2,3] Recently, the Determinant of collateral extent-1 (*Dce1*) locus has been identified as the major source of heritable variation in collateral extent in the mouse species (**Fig. A**).[4] This finding provides a unique opportunity to selectively examine the role of collateral abundance on stroke progression. To date, we have very limited knowledge of how collaterals affect perfusion and diffusion MRI signals during acute stroke progression. To address this question, the present study aimed to use quantitative perfusion and diffusion MRI to study stroke progression induced by permanent middle cerebral artery occlusion (MCAO) in two congenic mouse strains (CNG-B6 and CNG-BC) that differ in collateral extent based on allelic differences in the *Dce1* locus. This is the first study to test the effect of differences in collateral extent on stroke in individuals with otherwise isogenic backgrounds. It is also innovative in its use of high-field perfusion/diffusion MRI in mice, which has been highly challenging due to strong susceptibility artifact, unstable physiological conditions, and hardware constraints. We tackled these technical obstacles by using acrylic resin to reduce susceptibility at the air-tissue interface and built home-made coils for EPI-based cardiac spin-labeling (CSL) in mice.[5] Our findings show that collateral circulation ameliorates penumbral tissue loss at 5 h after MCAO by restoring the cerebral blood flow (CBF) in the peri-infarct area. These effects suppress the expansion of the ischemic core and thus reduce the final infarct size.

METHODS Permanent MCAO at the left hemisphere was performed by direct micro-cautery in adult male CNG-B6 and CNG-BC mice (3-4 mo, n=22 per strain) under 2% isoflurane anesthesia. Sections of the scalp and left temporal muscle were excised and a ~2 mm burr hole was drilled over the trunk of the left MCA where it was subsequently cauterized and transected. During MRI, mice were anesthetized with 0.75-1% isoflurane mixed with 25% O_2 and maintained on a circulating hot water bladder (respiratory rate: ~100 bpm, O_2 saturation: >97%, heat rate: ~380 bpm, body temperature: 37°C). MRI was performed on a Bruker 9.4 T Biospec scanner with a custom-made surface brain coil and a separate heart coil for CSL (**Fig. D**). CBF was measured by continuous CSL using two shot gradient-echo EPI with bandwidth=300 kHz, TR/TE=3000/5 ms, labeling duration = 2.5 s and post-labeling delay=300 ms, matrix=64x64, FOV=1.6x1.6 cm^2, and slice thickness=0.75 mm. Apparent diffusion coefficient (ADC) data were acquired with the same geometry using two shot spin-echo EPI with bandwidth=300 kHz, TR/TE=3000/22 ms, number of A0=5, number of directions=30 and b-value=1200 s/mm^2. Perfusion, diffusion and T_2-weighted images were acquired at 1, 5 and 24 h after MCAO. To avoid confounding effects of anesthesia on the lesion size, no longitudinal scans were performed during the acute phase at 1 and 5 h after MCAO (n=9 per strain at each time point). Five animals in the 1 h group were scanned again at 24 h, and another 4 animals per strain were added to bring the number of animals to 9 for the 24 h group. ADC- and CBF-derived lesion volumes were determined using the thresholds of 0.53 $\mu m^2/ms$ and 0.3 ml/g/min, respectively.[6] Infarct volumes were delineated from hyper-intense signals (≥2 SD of normal cortical tissue) in T_2-weighted images.[6] CBF, ADC, and T_2-weighted images were spatially co-registered and expressed as group averaged maps. The ratio of penumbra tissue loss was defined as the difference in ADC deficit volumes between two time points divided by the mismatch volume at earlier time point (i.e., 1 or 5 h).[7] The ratio of infarct growth was defined as the difference in ADC deficit volumes between the two time points divided by the infarct volume at the later time point (i.e., 5 or 24 h). Independent *t*-tests were used to compare strains, with a threshold of $P<0.05$.

RESULTS & DISCUSSION CNG-B6 had more pial collateral vessels than CNG-BC (**Fig. B and C**).[4] Both strains had marked CBF reduction in the ipsilateral cortex 1 h after stroke (**Fig. E**), while the area with ADC deficit in CNG-B6 was significantly smaller than in CNG-BC ($P<0.005$; **Fig. F**). This resulted in a significantly larger perfusion-diffusion mismatch area in CNG-B6 1 h after stroke ($P<0.05$; **Fig. H**). By 5 h after stroke, CBF was restored in CNG-B6 (**Fig. E**), which significantly reduced the tissue loss in the penumbral area and slowed expansion of infarct core ($P<0.005$; **Fig. I and J**). Smaller CBF and ADC deficit volumes in CNG-B6 also resulted in a significantly smaller perfusion-diffusion mismatch area in CNG-B6 compared to CNG-BC at 5 h ($P<0.005$; **Fig. H**). These data suggested that greater collateral extent provides an alternate source of perfusion in the ischemic region and suppresses the expansion of ischemic core. Significant differences in CBF, ADC deficit, final infarct volumes, penumbra tissue loss and infarct growth were also observed between the 2 strains at 24 h after stroke ($p<0.005$; **Fig. E-G, I and J**).[8] In CNG-B6, we observed a significantly larger perfusion-diffusion mismatch area at 1 h after stroke that was reduced by 5 h ($P<0.05$), indicating that collateral circulation is a significant determinant of tissue fate in the ischemic penumbra (**Fig. H**). Our data suggested that with same initial occlusion, extent of perfusion-diffusion mismatch depends on the time of measurement after stroke as well as collateral extent. This is the first demonstration of the effect of differences in collateral circulation on variation in CBF, ADC deficit, and thus ischemic penumbra and infarct volume, where non-collateral genetic-dependent differences (eg, neuronal sensitivity to hypoxia) are controlled.

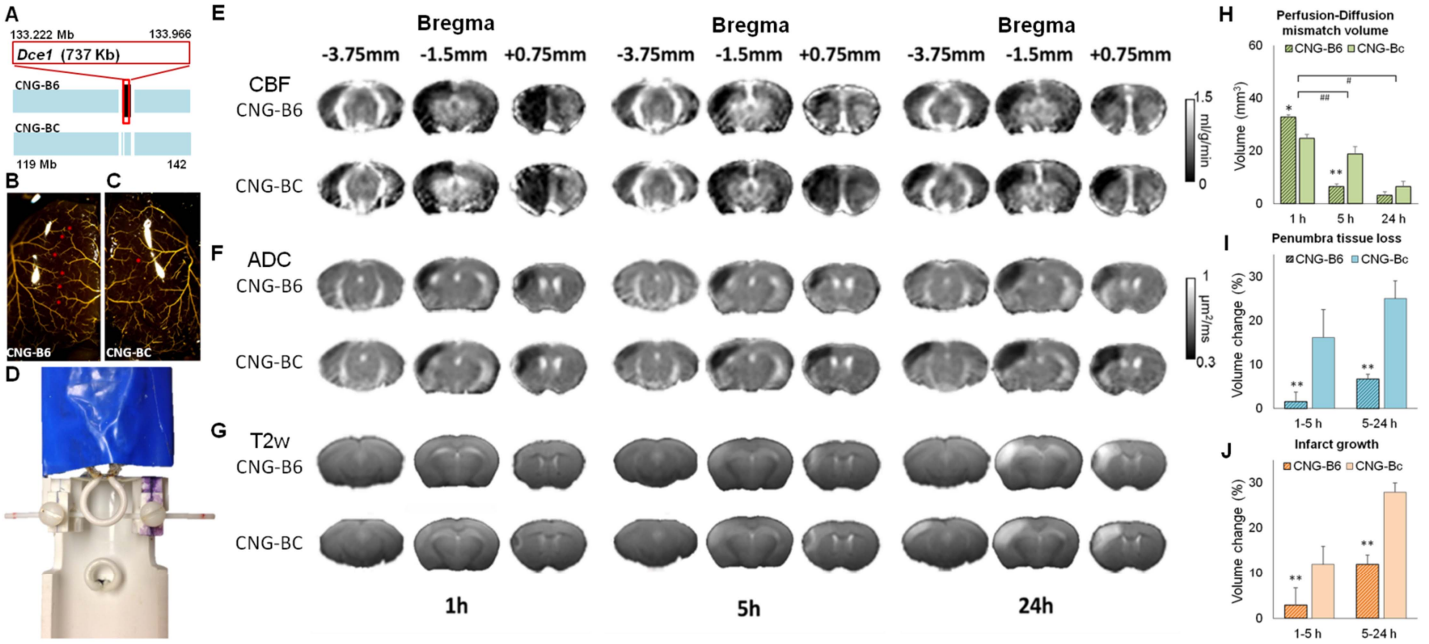

Figure Perfusion and diffusion MRI in mice with experimental stroke and different collateral extents. **(A)** Congenic strains showing the B6 allele of *Dce1* (the red open rectangular) introgressed onto the BC background. Black genotype: B6; blue: BC; white: regions of uncertainty. **(B & C)** Filled pial pre-capillary vessels in CNG-B6 and CNG-BC mice. Red stars indicate MCA-to-ACA collaterals. **(D)** Custom-made hardware for two-coil cardiac spin-labeling for mice. **(E-G)** Group-averaged CBF, ADC, and T_2-weighted images (n=9 per strain at each time point). **(H)** Volume showing perfusion-diffusion mismatch at 1 h, 5 h and 24 h after stroke. **(I & J)** Normalized volume change in penumbra tissue loss and infarct growth from 1 h to 5 h and 5 h to 24 h. * and ** indicates significant difference between two strains ($P<0.05$ and $P<0.005$, respectively). Error bars represent SEM.

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