Transgenic mouse model recapitulates brain pathophysiology of sickle cell disease

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Introduction

Sickle cell disease (SCD) is associated with an increased risk of ischemic stroke. The mechanism behind this is poorly understood, but is thought to involve increased cerebral blood flow (CBF) in response to anemia-related tissue hypoxia and altered cerebrovascular reserve (CVR)[1]. Children with SCD also have neurocognitive impairment and several studies have detected structural deficits in the brains of SCD patients, including decreased gray matter volume and cortical thinning[2-3]. The purpose of the current study was to characterize the brain physiology in a transgenic mouse model of SCD[4] in order to validate its use in further investigations into the pathophysiology and mechanism of stroke in SCD.

Methods

Female heterozygous Townes sickle cell mice and C57Bl6/J control mice were used in the study. CBF and CVR were assessed using continuous arterial spin labeling (CASL) at 6-7 weeks of age (n=5 per group). The gas mixture inhaled by the mice cycled between 30% O_2/70% N_2 and 5% CO_2/30% O_2/65% N_2 for two cycles while the pCO_2 was monitored using a transcutaneous blood gas analyzer. CASL images were acquired at 7 T using a 2D FSE pulse sequence (TR = 3000 ms, ETL = 16, TE_{eff} = 15 ms, slice thickness = 2 mm, in-plane resolution = 250 µm, scan time ~ 5 mins). Flow-induced adiabatic inversion in the common carotid artery (1 cm below the base of the brain) was applied using a 3 s labeling pulse and labeling gradient of 1.3 G/cm. CBF was quantified using a single-compartment biophysical model[5].

The diameter of the left common carotid artery and the blood flow in this vessel were measured using high-frequency ultrasound. Following this, the mice were euthanized and perfusion fixed in preparation for *ex vivo* MRI. *Ex vivo* imaging of the brains was performed using a 3D FSE pulse sequence (TR = 2000 ms, TE_{eff} = 42 ms, echo spacing = 14 ms, ETL = 6, resolution = 56 µm isotropic, scan time ~ 11.5 hrs). To assess anatomical differences between SCD and control brains, the *ex vivo* images were registered together to generate a consensus average. The Jacobian determinants from the deformation fields, which define the voxel displacements from each image to the average, were used to obtain local volume differences between each image and the average, enabling a voxel-by-voxel volume comparison between the SCD and control groups. Multiple comparisons were controlled for using the false discovery rate (FDR).

Results

Figure 1 shows the CBF and CVR in SCD and control mice at 6-7 weeks of age. The basal cerebral blood flow (A) is elevated in the SCD mice and the CVR (B) is reduced compared to controls. Ultrasound measurements showed that the carotid arteries in the SCD mice are enlarged (1.8-fold larger diameter) and blood flow is increased (3-fold).

Fig. 1

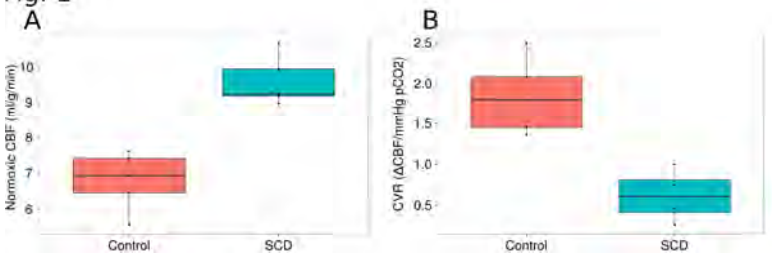
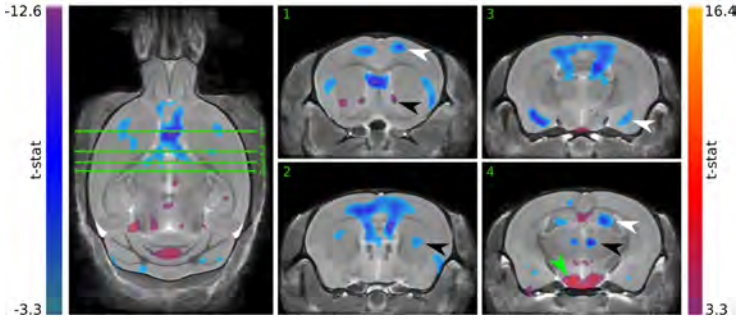


Figure 2 summarizes the results of a voxelwise comparison between SCD and control mice (10% FDR). Colour maps indicate regions that are significantly smaller (cool colours) or larger (hot colours) in the SCD group compared to controls. Significant differences were seen bilaterally throughout the brain in white matter regions including the anterior commissure (black arrow head 1) and in gray matter regions including the striatum (black arrowhead in 2), amygdala (white arrowhead in 3), thalamus (black arrowhead in 4), hippocampus (white arrowhead in 4), hypothalamus (green arrowhead in 4), and the motor cortex (white arrowhead in 1).

Fig. 2



Discussion and Conclusion

The transgenic mice used in this study show similar brain pathophysiology to SCD patients, including elevated CBF and impaired CVR, as well as changes in brain morphology. The enlarged carotid arteries and lack of response to increased [CO_2] suggest a loss of vascular tone such that vasodilation is near its maximum at baseline. This may result from a reduced bioavailability of NO due to increased free heme, as well as from chronic anemia leading to tissue hypoxia and increased cerebral blood flow demand. Localized anatomical differences were seen bilaterally in many structures throughout the brain, while the whole brain volume was unaffected. Several specific regions involved in learning and memory were smaller in the SCD mice, including the medial dorsal nucleus of the thalamus, the basolateral nucleus of the amygdala, the hippocampus and the striatum. The distribution and specificity of the anatomical differences suggest a variable sensitivity among brain structures to hypoxia and blood flow changes. Investigation into the development of these anatomical differences may help elucidate the underlying mechanism behind neurocognitive deficits in SCD. In conclusion, this transgenic mouse model displays the clinical symptoms seen in SCD and will be invaluable for investigating treatment strategies and developing an understanding of the mechanisms of stroke and neurocognitive impairment in SCD.

References: [1]Winchell AM et al. Am J Neuroradiol (2014) [2]Chen R et al. Am J Neuroradiol (2014) [3]Kirk GR et al. Cereb Cortex (2009) [4]Wu LC et al. Blood (2006) [5]Chugh et al. Magn Reson Med (2012)