In utero MRI of cerebral vascular development in mice

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Introduction

Vascular system development involves a complex, three-dimensional branching process that is critical for normal embryogenesis. In a previous study [1], we developed a contrast-enhanced perfusion method to selectively enhance the cerebral arteries in fixed mouse embryos and demonstrated that *Gli2* mutant mice lack a basilar artery, a key arterial input to the posterior brain regions. However, imaging studies of *Gli2* and many other mutant mice with vascular defects are limited because mice do not survive postnatally. Extending vascular imaging to an *in utero* setting with potential for longitudinal vascular development studies is an exciting possibility. However, *in vivo* MRI scans routinely result in undesirable image artifact due to subject motion. In this study we utilized an *in utero* imaging method that we described previously [2], which corrects for motion using an interleaved gating acquisition and serial comparison of rapidly acquired 3D images. We demonstrate the potential of this method by examining vascular development *in utero* in E17.5 wildtype and *Gli2* mutant mice. We show that the *in vivo* methods produce high-quality images of the embryonic cerebral vasculature and are able to detect the basilar artery phenotype in *Gli2* mutants.

Methods

A modified Cartesian 3D gradient-echo sequence was used for this study [2]. In this sequence, respiratory gating data were detected from an unencoded gating signal interleaved with imaging data. Repeated, serial 3D volume images (with limited SNR) were acquired and registered together during reconstruction to eliminate shifts in embryo position during the scan. Registrations were performed based on tools produced by the Montreal Neurological Institute (MNI_AutoReg; http://www.bic.mni.mcgill.ca/software/mni_autoreg) [3,4] with a coarse, manually-drawn mask covering the embryo brain and corresponding blood vessels. All MRI data were collected on a 7.0T magnet using a Bruker Biospin Avance II console. For our initial investigation, we used a 125-µm isotropic resolution (matrix size 192x110x72, field-of-view 24x13.75x9.0, TE/TR = 3.75/35 ms, 16 degree flip angle, 28 repeats, for a total scan time of 2hrs 16mins). For imaging, pregnant mice were anesthetized with isoflurane and placed on their side with one of the uterine horns sitting above a custom surface coil. Embryos were subsequently extracted and genotyped by polymerase chain reaction (PCR) of embryo yolk sac DNA using primers for Neo and wildtype *Gli2* as previously described [5]. Following image registration, contrast was inverted in the acquired images, and semi-automatic segmentation was performed on the embryo brains using Amira software (Mercury Computer Systems, Inc.). Minimum Intensity Projections (mIPs) were acquired using Analyze software (AnalyzeDirect, Overland Park KS) after inverting the image intensity values.

Results

Figure 1 shows the application of our *in utero* imaging methods for visualization of the embryo vasculature. We clearly discerned the major vascular inputs into the developing brain including the basilar and the carotid arteries in wildtype embryos. *In vivo* 3D T2*-weighted MR brain also showed an absence of the basilar arteries and other alterations in the structure and density of blood vessels in *Gli2* mutant embryos, characteristic of the phenotype described previously in our *ex vivo* experiments.

Conclusions

With the use of gating and image registration methods, we have shown the acquisition of *in utero* 3D images of vascular development. We have also demonstrated the *in vivo* visualization of a deletion of basilar arteries in *Gli2* mutants. Taken together, our results show great potential for in vivo 3D micro-MRI to analyze the development of cerebral vasculature as well as any phenotypes associated with genomic manipulation in mutant mouse embryos. This may present important opportunities for investigating embryonic lethal mouse phenotypes, permitting longitudinal observation of vascular development or measurement of vascular perfusion *in vivo*.

Acknowledgements

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References

[1] Berrios-Otero *et al*. (2009). *Magn Reson Med* (in press); [2] Nieman *et al*. (2009). *Magn Reson Med* 61: 1148-57; [3] Collins et al. (1994). *J Comput Assist Tomogr* 18: 192-205; [4] Collins *et al.* (1995). *Hum Brain Mapp* 33: 190-208; [5] Mo *et al*. (1997). *Development* 124: 113-123.

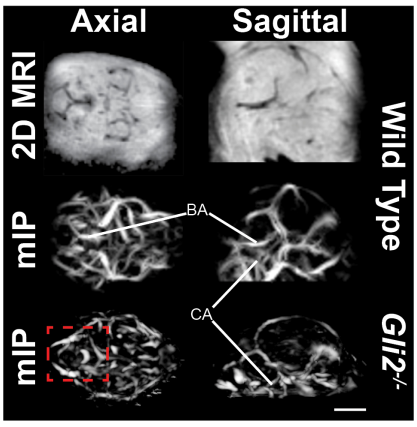

Fig 1: In vivo T2* micro-MRI slices (top two panels) and minimum Intensity Projections of E17.5 wild type and *Gli2*$^{-/-}$ mutant embryos. Note the absence of the basilar artery (red box) and the reduction in the size of the cerebral blood vessels in *Gli2*$^{-/-}$ mutants. Labels: basilar artery, BA; carotid artery, CA. Scale bar = 300µm