Brain morphology and vascular patterning analysis in Gli2-KO mutant mice using contrast enhanced micro-MRI

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Introduction.

Vascular cell proliferation and patterning is critical for normal embryonic development and also underlies many disease processes such as tumor angiogenesis. We have been using the cerebral vascular system of mouse embryos as a model for 3D studies of vascular development, using *ex vivo* micro-MRI after perfusion-fixation of Gd-contrast, similar to the methods pioneered by Smith, Johnson and co-workers [1-2]. In the current study, we have used 3D micro-MRI to analyze brain defects in *Gli2* mutant mice, which have previously been shown to have midbrain and cerebellum defects [3]. Furthermore the *Gli* gene family have been implicated as transducers in the Hedgehog signaling pathway and thought to act upstream of VEGF and Notch pathways, which are important mediators of vascular development [4]. Therefore, we also used contrast-enhanced 3D micro-MRI to analyze the cerebral vasculature of the *Gli2* mutants and wild type littermates.

Methods.

We used a retrograde Gd perfusion-fixation protocol to selectively enhance the cerebral arteries in mouse embryos at fetal day 17.5 [5]. Each embryo was surgically extracted, maintaining the vascular connections to the placenta and warmed in phosphate buffered saline (PBS). Heparin containing PBS (5,000 units/L) was perfused into the umbilical vein, followed by a fixative perfusion (2% vol/vol glutaraldehyde/1% formalin in PBS). The contrast agent, BSA-DTPA-Gd containing 1-mM Gd, dissolved in a 10% (wt/vol) gelatin solution, was then perfused through the umbilical artery. After perfusion-fixation, mouse embryos were mounted in a syringe phantom surrounded by Fomblin perfluoropolyether (Solvay Solexis). 3D T1-weighted gradient echo (TE=5ms; TR=50ms; Flip Angle=35°; FOV=$(25.6mm)^3$; Matrix=512^3; Isotropic resolution=50μm; Total imaging time=14 hr, 35 min) images were acquired on either a 7T SMIS or a 7T Bruker micro-MRI system, imaging multiple embryos simultaneously in overnight scans [6-7]. 3D image analyses were performed using Amira (Mercury systems) and Analyze software (Mayo Clinic), including segmentation and maximum intensity projection (MIP) to examine the 3D vascular structures.

Results.

3D T1-weighted MR brain images clearly demonstrated the reduction of midbrain and cerebellum in the *Gli2* mutant embryos compared to wild type littermates (Fig. 1). Interestingly, we also observed a severe reduction of the olfactory bulbs in the mutants, which has not been reported previously. After perfusion-fixation, micro-MRI showed a clear leakage of Gd contrast agent into extra-vascular spaces in the *Gli2* mutants, but not the wild type mice, suggesting that the mutants have a vascular permeability defect (Fig. 2). Micro-MRI analyses of vascular anatomy showed an absence of the vertebral and basilar arteries in *Gli2* mutant embryos, nearby the midbrain and cerebellum morphological defects noted above (Fig. 1).

Conclusions.

In this study, we used micro-MRI to demonstrate the previously described mid-hindbrain defects in *Gli2* mutant mice, as well as a previously overlooked deletion of the olfactory bulbs in these mice. Gd perfusion-fixation enabled analysis of the cerebral vasculature, which indicated a severe reduction or loss of the vertebral and basilar arteries in *Gli2* mutants. Taken together, our results show great potential for 3D micro-MRI to analyze both brain defects and associated abnormalities in the cerebral vasculature of mutant mouse embryos.

Acknowledgments.

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References.

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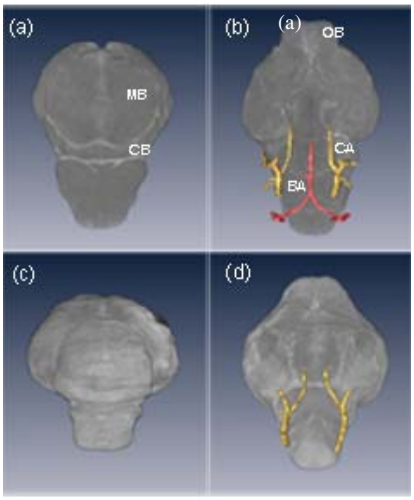

Fig 1. Volumetric rendering of wild type (a, b) and *Gli2* mutant (c,d) brains. Notice the reduction in midbrain, cerebellum and olfactory bulb size as well as the absence of the basilar artery in *Gli2* mutant brains.

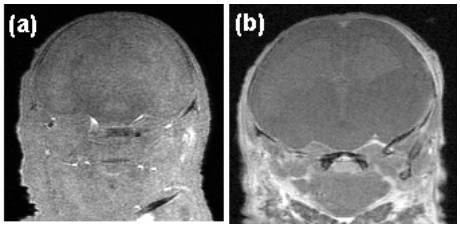

Fig 2. 50μm isotropic T1 weighted images of E17.5 wild type (a) and *Gli2* mutant embryos. Note extensive leakage of Gd contrast agent into extra vascular spaces in the *Gli2* mutant but not wild type embryo.