In amnio MRI imaging for the identification of abdominal pathologies

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Introduction

Transgenic mice are integral to the study of congenital disease development and gene function. µMRI is a non-invasive technique that has been successfully used to image *ex vivo* mouse embryos [1]. However, there is currently no established approach for assessing *ex vivo* embryos within the amniotic sac using MRI. Such a method would be valuable for determining the phenotype of mutant embryos in which the abdominal wall has not closed properly and the intestine loops are either exposed to amniotic fluid (gastroschisis) or contained within a membrane (omphalocele). Determining such phenotypes is difficult using light microscopy because the structures are delicate and dissecting the amniotic sac invariably causes damage. In this study, we have developed an *in amnio* imaging methodology, in which the embryo is retained in the amniotic sac, enabling visualisation of the complex relationship between amnion, placenta and embryo. *In utero*, these phenotypes would be problematic to investigate as the fine membranous tissues involved would be very difficult to resolve due to motion. Our novel methodology enables the identification of abdominal wall and amnion pathologies.

Methods

Sample Preparation: An E17.5 mutant embryo with an indeterminate abdominal wall defect was excised from the mother, removed from the uterine muscle but retained in its amniotic sac and immediately immersed in 4% paraformaldehyde (PFA) fixative. For imaging, the embryo was placed in a 15ml Corning tube with a gauze layer above the sample to minimise movement.
Image acquisition and processing: Imaging was performed on a 9.4T VNMRS system (Agilent Technologies Inc) with a 26mm volume coil (RAPID Biomedical GmbH). The mutant embryo was imaged using a multi-slice fast spin echo sequence (Effective TE/ETL/ESP/k_0/TR/FA/NSA=60/8/15/4/5100/90/36), matrix size 256^2, FOV 16x16mm^2, slice thickness 0.25mm, slices 40. Images were visualised in Amira 5.4 (Visage Imaging, Inc. CA, USA).

Results

Figure 1a clearly shows important amniotic and embryonic anatomical structures. The border of the amniotic sac is clear and the placenta (yellow arrow), the umbilical cord (green arrow) and the protruding abdominal contents (blue arrow) characteristic of such mutants can be visualised. Figure 1b shows a different slice from the same mutant embryo, higher in the axial direction with a zoomed-in view to emphasise a thin, dark line (red arrow) extending from the abdominal region to the amniotic sac. This fine structure is likely to be a membranous tissue characteristic of a ruptured omphalocoele defect.

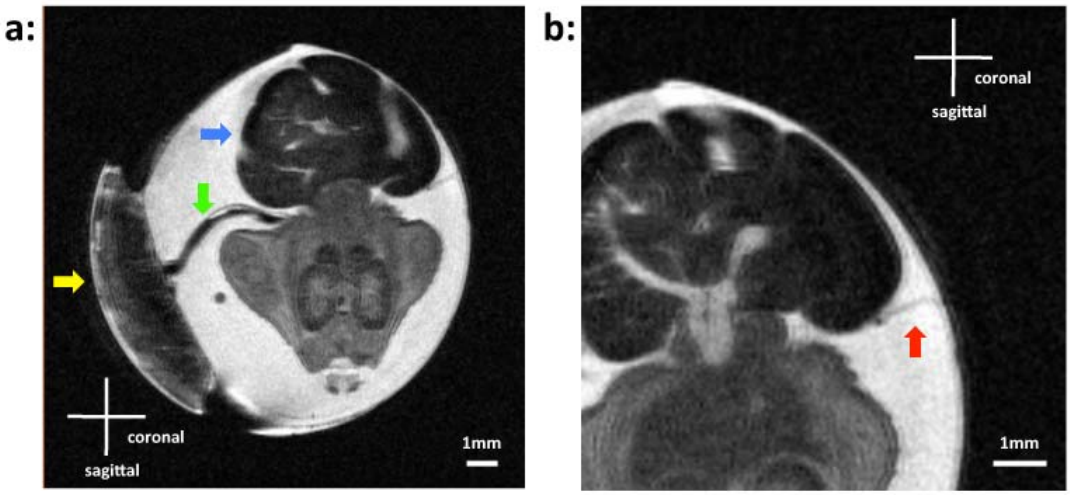

Figure 1: Axial in amnio *MRI images of a mutant mouse embryo with an abdominal defect.* ***a)*** *The amniotic fluid is hyperintense giving natural contrast to the embryo and other structures. The arrows indicate: the placenta (yellow), the herniated abdominal contents (blue) and the umbilical cord (green).* ***b)*** *A magnified image of a different slice within the same embryo illustrating the thin membranous structure (red arrow) extending from the abdomen to the amniotic sac: this is characteristic of an omphalocoele membrane which has ruptured during embryonic development.*

Discussion and Conclusion

We have developed a new MRI methodology to image mouse embryos *in amnio*. This was successfully applied to investigate a mutant with an indeterminate abdominal wall defect. The MRI images enabled visualisation of a small membrane extending from the fetal abdomen to the amniotic sac suggestive of a ruptured omphalocoele defect. The high water content of the amniotic fluid provides a bright background using our scan parameters. A fast spin echo sequence was used to reveal the ruptured omphalocoele membrane because blood in the amniotic fluid caused image artefacts when using a gradient echo sequence.

Our images represent the first attempts at *in amnio* MRI. Many structures are easily visualised without the need for contrast agents which is advantageous because direct injection of contrast agents through the amniotic sac can lead to its collapse. As the embryos are *in amnio* but *ex vivo*, fine structures are resolvable which would otherwise be extremely difficult to distinguish *in utero*. In conclusion, we present the first steps towards *in amnio* MR imaging which can be used to identify the nature of abdominal wall defects, and in future studies could easily be extended to examining a host of different developmental diseases and conditions.

References

[1] J. O. Cleary *et al*. *NeuroImage.* 54 (2011) 769–778