In vitro and in vivo 9.4T MRI study of mutant mice for the characterization of inner ear structure defect.

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Purpose/Introduction

In the transgenic research area, the understanding of the functional consequences of genes mutations in order to investigate the origin of human disease, requires the development of non invasive investigation methods on murine models. Our aim was to implement a MRI method at 9.4T in order to study the modification of the ear in an ENU-induced mouse mutant line isolated from the PhenHoMut program in Orleans, by recording 3D MR ear images on young mutant mice, *in vitro* and *in vivo*. The ear system is a heterogeneous organ with different susceptibility effects and air-tissue interfaces, which needs an imaging strategy at high field to benefit from the potential increase of sensitivity and resolution.

Subjects and Methods

Mutant mice were selected by visual external observation showing a delay in the opening of the esternam meatus of young mice by 2 days from j11 to j13 after birth. The feasibility of MRI was determined by recording *in vitro* head mice at day 12 embedded into 1% agarose gel. Mutant and corresponding control littermate were studied.

The MRI experiments were carried out on a 9.4T horizontal imaging spectrometer (Bruker, Biospec, Wissembourg, France) equipped with a 20cm bore 950mT/m and a 3.5cm birdcage coil. The living mice were anesthetized by IP 0.1ml/10g ketamine/xylazine mixture injection and maintained at physiological temperature by warm water circulation following the animal care recommendations. For *in vitro* experiments, T1 weighted 3D FLASH (TR/TE:100ms/6ms, 15°, 118µmx118µm and 59µmx59µm in plane, 110µm in the third direction), multislice 2D SE (TR/TE:2s/14ms, 118µmx118µm thickness of 0.25mm, FOV=3cmx3cm, 117µm and 58µm in plane resolution) and proton density weighted 2D RARE (TR/TE:3,5s/56ms, 8 echoes, 118µmx118µm thickness of 0.25mm, FOV=3cmx3cm) experiments were recorded and compared in order to optimize the best sensitivity and contrast for the mean and internal ear. For *in vivo* experiments, 3D short TE FLASH gradient echo sequence was performed with T1 weighting. (TR/TE:50ms/2ms, 60°, slice thickness of 6mm, FOV=2cmx2cm, 256x256 acquisition points, 78 µm in plane resolution and 125 µm resolution in the third dimension). The total duration time was about 55 min. Image processing was achieved for 3D reconstruction to compare the ears anatomies.

Results

Concerning *in vitro* experiments, internal ear subregions composed of bones, cartilage and membrane, as well as the cochlear anatomy thanks to the lymph detection were visualized. Long duration micro imaging experiments were performed to record the details of the mice ears available at 9.4T in the heterogeneous regions of the ear. An image with an in plane resolution of about 50µm allowed to delineate the main ear components (figure 1). GE images display the best inner and medium ear details whereas SE type experiments provide a better contrast between cochlea, mean ear, and skin tissues. Image processing by 2D analysis and 3D reconstruction was performed. Both transgenic and wild type mice which were 15 days old and more showed a clearly opened vestibular system. When they are about 12 days old, mutant mice display a partially opened mean ear and wild type mice ears had a similar behavior (figure 2).. However, the size of the transgenic ears are smaller (mean 26%). When they are 15 years old, the 'ears' are opened (figure 1 and 3). Histopathological data could not be exploited as discrepancy problems between consecutive slices prevented the correct 3D reconstruction of the ear from histological slice.

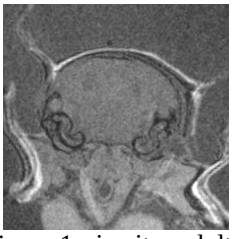

Figure 1 : *in vitro* adult transgenic mouse SE image

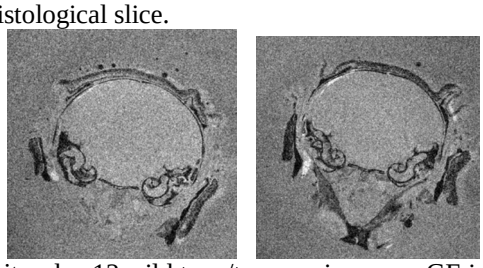

Figure 2 : *in vitro* day 12 wild type/transgenic mouse GE images

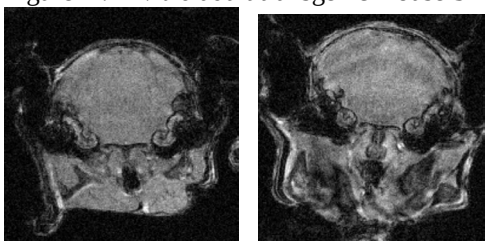

Figure 3 : In vivo day 15 wild type/transgenic mouse GE images

Discussion/Conclusion

High sensitive and resolved MRI images at 9.4T were obtained on mice ears *in vitro* and *in vivo*, enabling the 3D analysis of the complex ear system that can be applied to the study of mutant mice. Comparison of the ears between mutant and wild type young mice showed no difference of their inner ear structure at day 12, however the external mutant mice ears were smaller by about 26%. In vivo longitudinal studies in time around this age will be ran to confirm the reproducibility of the results and to further investigate the mutant and littermate mice development.