MRI FOR *IN VIVO* STRUCTURAL BRAIN PHENOTYPING OF A MIGRAINE MOUSE MODEL

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Introduction

Migraine is a neurological paroxysmal disorder affecting up to 16% in the general population. Patients suffer from throbbing, often unilateral headaches lasting 4 to 72 hours that are accompanied by nausea, vomiting and/or photo- and phonophobia. Familial hemiplegic migraine type-1 (FHM1) is an autosomal dominant subtype of migraine with aura caused by mutations in the *CACNA1A* gene. This gene encodes the pore forming α1-subunit of $Ca_v2.1$ (P/Q-type) calcium channels. Recently, a transgenic knockin (KI) mouse model of migraine has been generated by our group that contains a human pathogenic R192Q FHM1 mutation in the orthologous mouse *Cacna1a* gene [1]. In patients, the R192Q mutation is associated with FHM1 without additional clinical features [2].

The purpose of this work was to use magnetic resonance imaging (MRI) for *in vivo* characterization of brain structures in the R192Q KI migraine mouse model. The *in vivo* brain morphology was analyzed by two different approaches:

1. using semi-automatic segmentation for volume measurements of different brain regions.
2. by generating deformation fields using a free-form non linear image registration for a pixel-by-pixel shape analysis.

Methods

In vivo imaging was performed on 11 homozygous R192Q KI mice and 11 wild-type mice aged 19.9 ± 3.3 months. Mice were anesthetized with isoflurane in a mixture of air and O_2 during the MRI procedure. The respiratory rate was monitored and kept stable by regulating depth of the anesthesia. After imaging, brains were removed from the skull and fixed by bath-fixation in 4% phosphate buffered paraformaldehyde, followed by dehydration and embedding into paraffin. Paraffin sections (5 µm) were prepared and stained with hematoxylin and eosin and Klüver-Barrera staining using standard protocols.

Imaging was performed on a Bruker 9.4T vertical 89-mm-bore magnet with a Bruker Micro2.5 gradient system of 1T/m and a transmit/receive birdcage radio frequency coil with an inner diameter of 30 mm. Bruker ParaVision 3.0 software was used for image acquisition. Anatomical images were acquired using a T_2-weighted multi-slice spin echo sequence. Imaging parameters were: TE = 35 ms, TR = 6 s, FOV = 25.6 mm, MTX = 256×256, 40 slices of 0.2 mm thickness, AVG = 4.

Whole brain, total ventricular system, forth ventricle and cerebellum were first automatically segmented using an atlas-based approach (SHIVA, Laboratory of Neuroimaging, UCLA, Los Angeles, CA) that was manually corrected when required. Volumes of segmented brain regions were calculated by multiplying the number of voxels-by-voxel size. For comparison, all volumes were corrected for head size. For the morphological group comparison, we normalized all the images to a standard space and generated an average image for each group. A free-form deformation image registration method [3] was used to co-register the average wild-type image (moving image) on the average R192Q KI image (fixed image). The output of the registration is a non-linear deformation field that gives for each voxel of the moving image a displacement vector toward the matched voxel in the fixed image. From each displacement vector, the orientation and the amplitude can be derived.

Results

Table 1 shows volumetric measurements of whole brain. Cerebellar volume was slightly increased with respect to whole brain volume in the R192Q KI mice (p=0.030), while ventricle volumes remained unchanged. In addition, we found a difference in shape of the cerebellum between the two groups. **Figure 1** shows the outcome of the deformation field analysis, showing the extent and direction of changes between control and R192Q KI mice. Vectors indicate cerebellar displacement in caudal direction. No pathology was found on histological evaluation.

Table 1 Volumetric results and p-values

	Wild-type	R192Q KI	p-value
Absolute Volumes			
Total brain volume	469.4 ± 12.2	466.7 ± 18.3	0.696
Normalized			
Cerebellum	10.84 ± 0.28	11.12 ± 0.29	0.030†
Ventricles	1.73 ± 0.24	1.76 ± 0.21	0.694
4th Ventricle	0.38 ± 0.07	0.34 ± 0.05	0.130

Absolute volumes in µl ± SD. Normalized values in % of total brain volume ± SD. Two-tailed Student's t-test. †$p < 0.05$

Discussion

We analyzed *in vivo* brain morphology of R192Q KI mice using MRI combined with segmentation-based volume measurements and morphological analysis using deformation fields. A slight increase in cerebellar volume and a caudal displacement was found. We hypothesize that edema might be the underlying cause.

Particularly in migraine with aura patients with high attack frequency, sub-clinical white matter abnormalities were found mainly in regions of the cerebellum and brainstem [4]. This indicates that the migraine aura may be an important factor in the pathogenesis of these lesions. Neuroimaging findings indicate that the migraine aura is due to cortical spreading depression (CSD), a wave of transient intense spike activity that progresses slowly along the cortex and is followed by a long-lasting neuronal suppression [5]. Previous results showed that the transgenic R192Q FHM1 mice exhibited an increased susceptibility to CSD [1]. CSD is known to induce blood-brain barrier disruption and leads to edema in a rat model [6]. Therefore, cerebellar volume increase and caudal displacement may be caused by edema, which is not visible on histological sections due to tissue fixation and dehydration that preceded paraffin embedding.

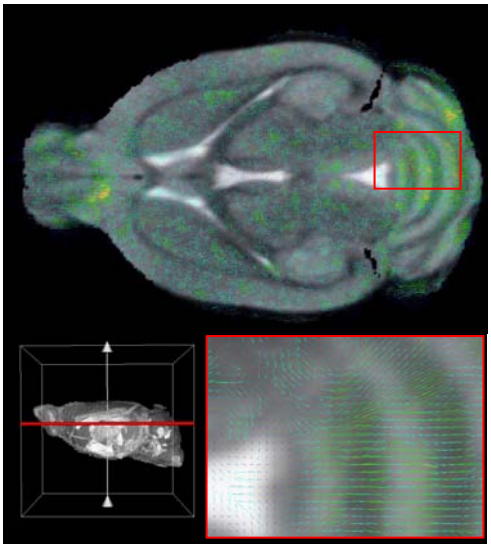

Figure 1 Deformation field analysis showing deformation mainly at the olfactory bulb and cerebellum.

Acknowledgements

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