

# Morphological assessment in a transgenic *Cacna1a* knockin migraine mouse model using automatic segmentation of high-resolution whole brain MR

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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 (FHM-1) is an autosomal dominant subtype of migraine with aura caused by mutations in the *CACNA1A* gene. This gene encodes the pore forming subunit of the Ca<sub>v</sub>2.1 (P/Q-type) calcium channels. Recently, a transgenic knockin mouse model of migraine has been generated by us containing a human pathogenic R192Q FHM1 mutation in the mouse *Cacna1a* gene [1]. In patients, the R192Q mutation is associated with FHM without additional clinical features, such as ataxia [2]. Here, MRI was used for phenotypical characterization of brain structures in the migraine mouse models, assessing cerebellar white matter and ventricular volume in particular.

**Material and Methods** *In vivo* imaging was performed on 10 homozygous R192Q mice (4 males, 6 females) and 14 wild type (WT) controls (4 males, 10 females). All mice were aged 18 ± 4.5 months. Mice were anaesthetized with 4% isoflurane in air (50%) and O<sub>2</sub> (50%) and maintained with ~1.5% isoflurane during all procedures. The respiratory rate was monitored via an air-pressure cushion connected to a laptop using Biotrig software (Bruker, Rheinstetten, Germany).

**MRI** The experiments were performed on a 9.4T vertical 89-mm-bore magnets (Bruker BioSpin, Rheinstetten, Germany) with a Bruker Micro2.5 gradient system of 1T/m and a transmit/receive birdcage radiofrequency coil with an inner diameter of 30 mm. Bruker ParaVision 3.0 software was used for image acquisition. Anatomical images were acquired using a T2-weighted multi-slice spin echo sequence. Imaging parameters were: TE = 35 ms, TR = 6 s, FOV = 25.6 mm, matrix = 256x256, 40 slices of 0.2 mm thickness, 4 averages.

An average *in-vivo* T2 brain image has first been generated from 6 randomly selected images that were manually aligned to the shiva ex-vivo brain template [3] using 12-parameter affine transformation (see figure 1.a). This average image has then been automatically registered to every single mouse image using again 12-parameters affine transformation. Using the transformation matrix resulting from the automatic registration, we mapped the masks of the intra-cranial, cerebellum and a manually defined brain ventricle area (see figure 1.b) on every image. Using automatic fuzzy clustering guided by the mapped masks, we segmented automatically the brain ventricles and the white matter in the cerebellum (see figure 1.c). For group comparison, all the images and the corresponding segmentations have been normalized to the shiva standard space using automatic affine (12 -parameters) registration. This normalization step corrects for differences in head size and orientation.

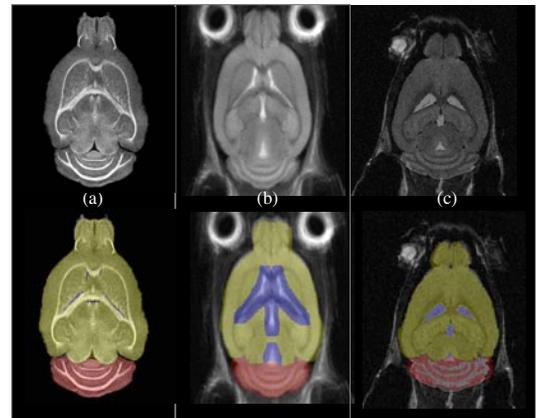
**Results** - Table 1 shows volumetric measurements of the white matter and the ventricles for the different groups. No significant differences were found between the groups for any of the values. However, we did find a difference in shape of the cerebellum between the two groups. Figure 2 a shows a difference image (absolute values) of the average migraine mouse and the average control mouse. The larger the image intensity, the larger the shape difference. One can note that most of the differences were found in the cerebellum and the ventricle areas. Figures 2.b-d show an elongation of the white matter in the cerebellum.

**Discussion** - Earlier results showed that the transgenic R192Q FHM1 mice exhibited a lowered threshold for cortical spreading depression compared to the WT mice, indicating that the gene mutation affects the neuronal excitability [1]. Here we show that in the transgenic mice, the mutation is not associated with gross differences in ventricular volume or in the white matter/grey matter ratio of the cerebellum. The observed elongation, however, was not predicted and was never reported in humans. The reason for this might be related to the fact that white matter segmentation in the human cerebellum is not as straight forward as in mice. Our results encourage further research on the shape of the cerebellum and its white matter distribution in humans.

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## References

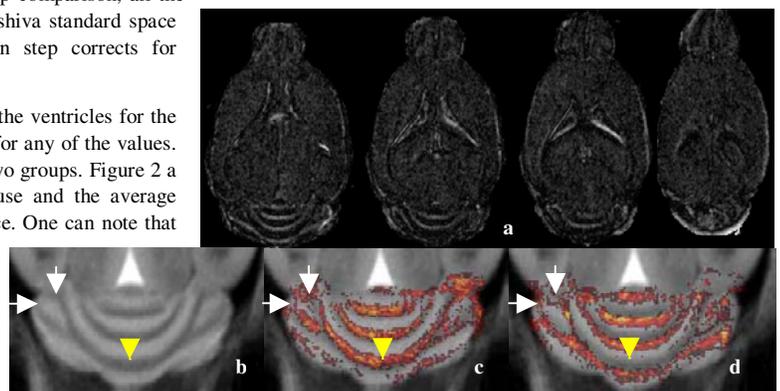
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**Fig. 1** (a) The Shiva template (b) The *in vivo* template (c) Automatic segmentation outcome. The above row shows the MR images while the bottom one shows the same image overlaid by the corresponding masks.

	N	Vol. WM (μl)	Vol. Ventr. (μl)
WT	14	13.64 (±1.04)	5.26 (±1.04)
R192Q	10	13.46 (±1.7)	5.66 (±0.87)
P-values		0.75	0.33

**Table 1** Volumetric results (mean (±SD)) and p-



**Fig 2** (a) Difference image at different levels of the brain. (b) Average image of the control mice with some landmarks. (c) Average control image overlaid with the white matter distribution in control mice. (d) Average control image overlaid with the white matter distribution of the migraine mice. Note the elongation of the white matter. No shift is present at the white arrows, but only at the yellow arrow.