Anatomical Phenotyping of Rett Syndrome in the Mouse

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Introduction – Rett Syndrome (RTT) is an X-linked disorder, which primarily affects females. RTT is located under the Autism Spectrum of Disorders (ASD), and is caused by mutations to the Mecp2 gene. A commonly used mouse model of RTT involves a truncation of the Mecp2 gene at codon 308. The behavioural phenotype of the Mecp2^{308} mouse has includes abnormalities in social interaction and home-cage behaviours (1), as well as learning and memory impairments (2). A previous MRI study of the RTT knockout mouse model has detected decreases in size of gross anatomical structures such as the cerebellum and motor cortex (3). Recently, anatomical phenotyping in the fixed mouse brain using MRI has been shown in a number of mutants to be quite useful in determining specific volumetric changes (4). Therefore, the purpose of this study was to examine the volume changes in the Mecp2^{308} RTT mouse model with high resolution MRI.

Methods – *Specimen Preparation* – Thirty-four C57/B6 fixed mice brains were examined, 17 wild-type and 17 knockdown Mecp2^{308} mice. Of the Mecp2^{308} mice, 6 were heterozygous females, 5 were homozygous females, and 6 were hemizygous males. The mice were anesthetized and intracardially perfused. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures containing the brain were placed in 4% PFA and 2mM Prohance (a Gadolinium contrast agent) overnight and then transferred to PBS, 0.02% sodium azide, and 2mM Prohance for at least 7 days prior to the MRI acquisition. *MRI Acquisition* - A multi-channel 7.0 Tesla MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set (max gradient strength 100 G/cm) was used to acquire anatomical images of brains within skulls. Three custom-built solenoid coils were used to image three brains in parallel. Parameters used in the anatomical conventional MRI scans were optimized for gray/white matter contrast: a T2- weighted, 3-D fast spin-echo sequence, with a TR of 325 ms, and TEs of 10 ms per echo for 6 echos, four averages, field-of-view of 14 × 14 × 25 mm^3 and matrix size = 432 × 432 × 780 giving an image with 0.032 mm isotropic voxels. Total imaging time was ~11 h. *Data Analysis* - To visualize and compare volumetric changes and white matter structural changes, the brains were registered together. For the volume measurements the registration resulted in deformation fields for each individual brain, which were used to calculate individual volumes from the segmented population average. From this data the volume of 62 different structures (5) can be assessed for all 34 brains.

Results and Discussion – Of the 62 different regions assessed 15 were found to be significantly different when comparing the hemizygous male (Mecp2^{308}(-/y)) with the corresponding male wild type, 17 and 24 were found to be significantly different when comparing heterozygous (Mecp2^{308}(-/x)) or homozygous (Mecp2^{308}(-/-)) females with the corresponding female wild type, respectively. Figure 1 shows the significant changes between the homozygous female mouse and wild type, for either increases (red) such as in the ventricles (Fig.1-right), or decreases (blue), as seen the in areas of the cerebral cortex (Fig.1-middle). Table 1 lists specific changes in 7 notable regions. Interestingly, the hemizygous males had significant decreases in white matter structures such as the corpus callosum and anterior commisure that were not found in either female models (Table 1). The most significant differences were found in the homozygous females, with increases in the ventricles and cerebellar cortex as well as decreases in 2 regions in the cerebral cortex (Table 1). Decreases in the cerebral cortex may coincide with previously reported decreases in the motor cortex of the Mecp2 knockout mouse (3). However; increases were also found in the cerebellum, in both female Mecp2^{308} mutants which is not consistent with that study.

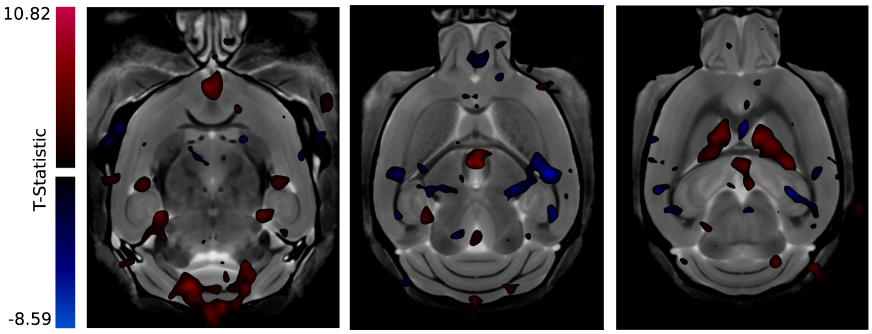

Figure 1 - Significant volume changes in the Mecp2^{308}(-/-) female mice when compared with the female wild type mice. Changes highlighted in blue indicate decreases, and changes indicated in red indicate increases.

Conclusions – Most changes found show decreases in volume, but the ventricles and cerebellar cortex are larger in all Mecp2^{308} mutants. Furthermore, the changes are more pronounced in the female homozygous model than the male hemizygous model.

References - 1) Moretti et al. *Human Mol. Genetics* 14: 205-220 (2005), 2) Moretti et al. *J of Neurosci.* 26:319-327 (2006), 3) Saywell et al. *BBRC* 340: 776-783 (2006), 4) Nieman et al. *Physiol Genomics* 24: 154-162 (2006), 5) Dorr et al. *Neuroimage* 42:60-69 (2008)

Table 1 – Regional Changes in Volume (mean ± standard deviation)

Region	Volume (mm^3)				
	WT (x/y)	Mecp2^{308} (-/y)	WT (x/x)	Mecp2^{308} (-/x)	Mecp2^{308} (-/-)
Anterior Commisure	1.89 ± 0.08	1.77 ± 0.09 *	1.79 ± 0.05	1.77 ± 0.09	1.76 ± 0.07
Cerebellar Cortex	43.87 ± 1.19	45.25 ± 2.03	44.99 ± 0.52	47.86 ± 1.26 *	48.01 ± 0.42 *
Cerebral Cortex – Frontal	44.67 ± 1.51	42.85 ± 1.12 *	44.26 ± 1.21	43.25 ± 1.46	42.92 ± 0.89 *
Cerebral Cortex – Parieto-Temporal	73.41 ± 2.41	70.45 ± 2.48	71.99 ± 2.46	70.88 ± 2.21	69.06 ± 0.23 *
Corpus Callosum	21.46 ± 0.71	19.97 ± 1.10 *	20.68 ± 0.98	20.10 ± 0.60	19.74 ± 0.56
Fourth Ventricle	0.37 ± 0.02	0.45 ± 0.06 *	0.43 ± 0.04	0.49 ± 0.06 *	0.53 ± 0.04 *
Lateral Venticle	3.93 ± 0.34	3.97 ± 0.30	3.61 ± 0.15	4.34 ± 0.45 *	4.68 ± 0.54 *

* indicates significance when compared with same sex wild type ($p < 0.05$, unpaired t-test)