Neurochemical alterations in the cortex and cerebellum of Mecp2 knockout mice

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Abstract

The objective of this study was to characterize time-course changes in brain metabolites, both in the cortex and the cerebellum, using localized ^{1}H-NMR spectroscopy in a mouse model of Rett syndrome. Anomalies in the metabolic profile were detected especially in the cortex at 7 to 8 weeks of age. These results agreed well with behavioral modifications measured in a parallel study.

Introduction

Rett Syndrome is a devastating neurological disorder affecting ~1 in 15,000 girls with no satisfying therapy available. Most cases of Rett Syndrome are caused by mutations in an X-linked gene encoding the transcriptional repressor methyl-CpG-binding protein 2, MeCP2. Clinical signs are characterized by mental retardation, loss of speech and use of hands, ataxia, apraxia, hand stereotypies, social withdrawal and anxiety, irregular breathing, gastro-intestinal failure and severe seizures. The only signs of neurodegeneration that have been identified are the reductions in the neuron size and dendritic arborization, likely accounting for the observed brain atrophy and decrease in synaptic formation. Disruption of the *mecp2* gene in mice causes behavioral and neurological abnormalities that closely resemble symptoms of human Rett Syndrome (1). Recent NMR data support the existence of neuronal defect and changes in glial metabolism in Mecp2 knockout (KO) mice (2). However, no biomarker has been consistently detected to predict the evolution of the disorder. Therefore, the objective of this study was to conduct a longitudinal analysis of Mecp2 KO mice to identify in two brain regions, the cortex and cerebellum, metabolic readouts that are representative of the progression of the disease. To this end, ^{1}H-NMR spectroscopy was used to measure possible changes in certain neurotransmitters (e.g. glutamate GLU) and markers of neuronal integrity (e.g. N-acetyl-aspartate NAA, phosphocholine Pcho). In parallel, Mecp2 KO mice were evaluated in different behavioral paradigms monitoring their age-related decline in motor and emotional functions. The extent to which modifications in brain metabolites correlate with behavioral defects was then discussed.

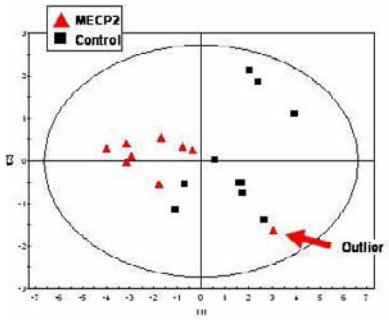
Methods

Longitudinal measurements were conducted on 5 to 9-week old mice (wild-type WT: n=4-11; KO: n=2-10/time-point). Because KO animals have gastro-intestinal difficulties threatening their survival, Mecp2 KO and WT littermates were provided with a soft transdough food in addition to their traditional nutritional diet. All NMR data were obtained using a Bruker Biospec 7T/30cm instrument equipped with a 12-cm gradient insert. MR sessions occurred at days 38, 42-45, 48-53, 56-59 and 61-66 under 1-2% isoflurane anesthesia with respiration continuously monitored. Mice were placed supine on the cradle with their head resting on top the antenna. Brain metabolites were determined using a 72-mm birdcage resonator and a 2-cm surface coil in a transmitter/receiver mode (cross-coil). Localized ^{1}H PRESS spectra (TE 13ms, TR 3s, SW 8kHz, 256 averages) were collected both in the cortex (3x1.1x5mm) and the cerebellum (2x2x2mm) in the presence of CHESS water suppression during a single session. Spectral analysis was performed by integrating peak areas after line fitting the various metabolite resonances of interest (i.e. NAA 2.0ppm, total Creatine 3.02ppm, PCho 3.2ppm, Taurine 3.4ppm, GLU 3.85ppm, Myoinositol 3.5ppm) using the Nuts-PPC software package (AcornNMR, Inc., Fremont, CA). Data are presented as mean±SE. Behavioral assessment was performed on 4 (pre-symptomatic), 6 (symptomatic), and 8 (late symptomatic)-week old Mecp2 KO and WT littermates (WT: n=10-12; KO: n=8-12). For each time-point, all animals were checked for forepaw stereotypies and hind limb clasping. In addition, their ability to climb or remain suspended on a wire mesh, grip strength, and motor coordination and balance on an accelerating rotating drum (Rotarod test) were evaluated. The mice were also assessed based on the anxiogenic Openfield (OPT), Light/Dark Transition (LDT) and Stress-Induced Hyperthermia (SIH) tests. Data are presented as mean±SEM.

Results

An age- and brain-region specific decrease in total NAA was detected by *in vivo* ^{1}H-MRS in Mecp2 KO mice (e.g. at ~7-week old, ~60% reduction in cortex vs. WT, $p<0.05$). Results also showed a transient decline in cortical levels of GLU (-40% vs WT, $p<0.05$), a slight increase in the glial marker myo-inositol in both brain regions at late symptomatic age, non-significant but steadily elevated levels of taurine in the cerebellum and no change in choline-containing metabolites. Interestingly, the most pronounced changes were observed in 48 to 53-day old mice, which corresponds to the period of fully developed symptoms. A principal component analysis (PCA) score plot of the metabolites measured in the cortex within that time-range further confirmed a clear discrimination between Mecp2 KO and WT mice (Fig. 1). In parallel, Mecp2 KO mice showed a progressive decline in motor functions such as motor coordination, grip and muscle strength, combined with forepaw stereotypies and hind limb clasping that were apparent at 4 weeks and progressively aggravated at 6 and 8 weeks of age. Mecp2 KO mice showed anxious behavior that was present at 4 weeks for the OPT test but only became significant at 8 weeks in the LDT test, most likely due to differences in the anxiogenic properties of each test. In both tests, Mecp2 KO mice displayed signs of exacerbated stress as measured by an increase in defecation vs. WT littermates. Finally, SIH data showed a tendency for an increased hyperthermia response to stress in Mecp2 KO mice that was not significant, probably because of the inherent decrease in body temperature symptomatic of these mutant animals.

Figure 1 – PCA score plot of metabolites in the cortex measured *in-vivo* by ^{1}H-MRS



Discussion

These results showed that over the time-course of their condition, Mecp2 KO mice are subject to dynamic variations in brain metabolite levels. The most dramatic changes occurred in the cortex (compared to the cerebellum) and reflected modifications in neuronal markers and transmitters, in line with reported alterations in neuronal morphology and function specific to this brain area (3). Interestingly, changes in brain metabolite closely mirrored the pervasive decline in motor and emotional capabilities exhibited by Mecp2 KO mice. Given the central role of the cortex in coordinating the activity of brain regions directly involved in the regulation of primary functions such as locomotion and anxiety, one can speculate that the anomalies in cortical metabolites are representative of a cumulative brain damage. Further work will be needed to better delineate the mechanisms leading to brain metabolite and behavioral abnormalities. However, our study showed that it is possible to identify in an animal model of the Rett syndrome valuable and specific biomarkers that can predict disease progression.

References

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