Mutation of the *Mecp2* gene causes perturbations of osmolyte and neurotransmitter metabolism in mouse brain. A ^{1}H MRS study of tissue extracts

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Introduction

Genetic disorders are characterized by abnormal expression of one or more genes, resulting in a clinical phenotype. The elucidation of mechanisms leading from genetic changes to specific symptoms is rather challenging since multiple steps are involved, all resulting in changes of the metabolic network that determines how cells perform specific tasks. Rett syndrome (RS) [1], a neurological disorder generally caused by mutations in the *MECP2* gene, is characterized by autistic features, loss of skills such as speech and purposeful hand use, and seizures, accompanied by microcephaly [2-3]. We used ^{1}H MRS of brain extracts to study effects of *Mecp2*-deletion on neurotransmitter and osmolyte metabolism in an RS mouse model.

Methods

Experiments were performed using the mouse strain B6.129P2(C)-*Mecp2*$^{tm1\text{-}1Bird}$ as described previously [4]. Freeze-clamped brains were extracted with methanol/chloroform/water. The resulting aqueous phase underwent ^{1}H MRS. Spectra were acquired on an AVANCE 400 spectrometer (Bruker, Wissembourg), using acquisition parameters described previously [5]. Spectra were evaluated with Bruker's deconvolution software (Topspin 1.3). The metabolite concentrations obtained, as well as selected metabolite ratios, were statistically analyzed employing the Mann-Whitney *U* test (n=4 for each mutants and controls), using Statview 5.0.1 (SAS, Cary, NC, USA).

Results

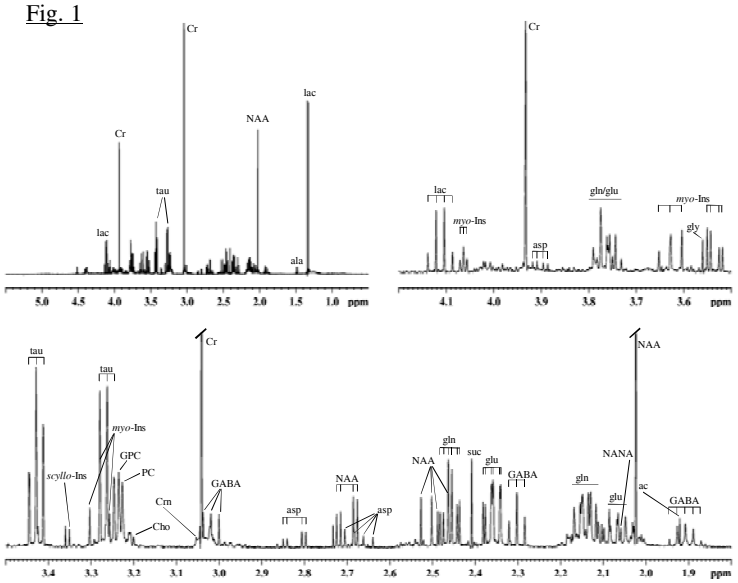
Figure 1 shows a typical ^{1}H MR spectrum representing water-soluble metabolites from the *Mecp2*-null brain. Neurotransmitter metabolites quantitated were glutamate (glu), glutamine (gln), aspartate (asp), γ-aminobutyrate (GABA), glycine (gly) and the acetylcholine metabolite, choline (Cho). The following compounds act as osmolytes : *myo*-inositol (*myo*-Ins), taurine (tau), *N*-acetylaspartate (NAA), glycerophosphocholine (GPC) and gln. Concentrations of these compounds (μmol/g tissue wet weight, means ± s.d.) are listed in Table 1 with significance levels (p values).

Table 1

	glu	gln	asp	GABA	gly
controls	5.53 ± 0.65	3.01 ± 0.26	1.46 ± 0.35	1.67 ± 0.18	0.63 ± 0.16
Mecp2-/y	5.00 ± 0.94	4.17 ± 0.71	1.31 ± 0.29	1.47 ± 0.10	0.59 ± 0.13
p	0.480	0.034*	0.480	0.157	0.724

	Cho	NAA	GPC	*myo*-Ins	tau
controls	0.13 ± 0.03	3.86 ± 1.03	0.53 ± 0.07	3.66 ± 0.63	5.99 ± 0.93
Mecp2-/y	0.08 ± 0.02	4.17 ± 1.09	0.41 ± 0.15	2.46 ± 0.47	5.85 ± 0.62
p	0.077(*)	1.000	0.289	0.034*	1.000

Fig. 1



Discussion

Between *Mecp2*-null mouse brains and controls, there was no difference for the excitatory neurotransmitter, glu; however, the increase of its presynaptic presursor, gln, indicates a global perturbation of the glu/gln cycle. This effect may be related to altered excitatory activity reported for RS patients [6], accompanied by perturbed signaling [7]. Interestingly, the neurotransmitters, asp, GABA and gly, are much less affected by *Mecp2* deletion, although variations between different brain regions cannot be excluded. Among all the water-soluble compounds quantitated, the *myo*-Ins decrease and gln increase occurred with the highest statistical significance. In addition, *myo*-inositol was decreased by about the same amount (1.21 μmol/g tissue wet weight) as gln was increased (1.16 μmol/g). Since both compounds exert an osmoregulatory function, one may assume that gln accumulation may trigger a compensatory decrease in *myo*-inositol. Such an effect has been described for cultured astrocytes [8].

Overall, the analysis of a large number of brain metabolites allowed us to obtain, for the first time, a broad characterization of the metabolic phenotype associated with *Mecp2* mutation. Modifications of neurotransmitter and osmolyte metabolisms hint at specific mechanisms of brain dysfunction due to *Mecp2* deletion. These hypotheses may open new avenues for studying mechanisms of genotype-phenotype correlations in RS.

References

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