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# Effects of postnatal dietary choline supplementation on motor regional brain volume and growth factor expression in a mouse model of Rett syndrome

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ABSTRACT

Nutritional status during pregnancy and lactation can influence behavioral and anatomical characteristics of several neurological disorders in the offspring, including Rett syndrome (RTT). RTT is associated with mutations in the X-linked gene encoding methyl-CpG binding protein 2 (MeCp2), a transcriptional repressor that binds methylated DNA. In *Mecp2*$^{1lox}$ mice, a model of RTT, enhancing maternal nutrition through choline supplementation attenuates motor coordination deficits in the mutant offspring. Here, we examine alterations in brain volume and growth factor expression in the cerebellum and striatum, motor regions that may contribute to the improved behavioral performance seen with choline supplementation. *Mecp2*$^{1lox}$ dams were given choline in drinking water, and pups nursed from birth to weaning. Brains of male offspring were collected at postnatal day 42 for volumetric and growth factor expression analyses. Compared to wild-type mice, *Mecp2*$^{1lox}$ null mice had decreased whole brain, cerebellar and striatal volume. Choline supplementation had no effect on brain volume. Nerve growth factor and insulin-like growth factor-1 expression was similar between wild-type and *Mecp2*$^{1lox}$ mice while brain derived neurotrophic factor was reduced in *Mecp2*$^{1lox}$ mice. Choline supplementation increased striatal nerve growth factor expression in wild-type and *Mecp2*$^{1lox}$ mice, suggesting that neuronal proliferation and survival may contribute to improved motor performance in this model of RTT.



## 1. Introduction

Neuronal developmental processes including neurogenesis, migration, maturation, and synapse refinement, occur predominantly in utero but also during postnatal periods (Rao and Jacobson, 2005). These processes are genetically regulated but also susceptible to environmental manipulation, such as nutritional status, which can alter the adult phenotype in several developmental disorders, including Rett syndrome (RTT; reviewed recently in Nag et al., in press-b). Using a mouse model of RTT, we have shown previously that postnatal dietary choline supplementation improves locomo-

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*Abbreviations:* ANOVA, analysis of variance; BDNF, brain derived neurotrophin factor; CB, cerebellum; ChAT, choline acetyltransferase; ELISA, enzyme-linked immunosorbent assay; IGF-1, insulin-like growth factor type 1; *Mecp2*, methyl-CpG binding protein 2; *MECP2*, methyl-CpG binding gene; MRI, magnetic resonance imaging; NGF, nerve growth factor; RTT, Rett syndrome; PD, postnatal day; STR, striatum; WB, whole brain

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tor behavior in null male *Mecp2*$^{1lox}$ mice (Nag and Berger-Sweeney, 2007) and increases total brain volume in heterozygous female but not null male *Mecp2*$^{1lox}$ mice (Ward et al., 2008). Here, we extend those findings by examining how perinatal choline supplementation influences growth factors and brain volume in two motor regions of the brain, the cerebellum and striatum, in null male *Mecp2*$^{1lox}$ mice and their wild-type littermates.

RTT is an autism spectrum disorder caused by mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional repressor that binds methylated DNA (Amir et al., 1999). RTT girls, who are generally heterozygous with one normal and one mutated *MECP2* gene, have variable MeCP2 expression (and varying phenotypes) due to random X-inactivation patterns. Whereas, males who are null for MeCP2 expression are more severely affected and often do not survive birth. The phenotype in RTT girls includes reduced head growth, regression of motor and cognitive skills (Hagberg et al., 1983; Hagberg and Witt-Engerstrom, 1986), and abnormalities in neuroanatomy and biochemistry (Armstrong et al., 1995; Armstrong, 2002; Jellinger, 2003; Wenk et al., 1991). These phenotypic features are also observed in *Mecp2* mouse models of RTT and are particularly well-replicated in the null males as compared to the heterozygous females (Chen et al., 2001; Kishi and Macklis, 2004; Stearns et al., 2007). The most consistent neurochemical changes in RTT girls include decreased cholinergic markers in the basal forebrain, basal ganglia and midbrain tegmentum (Kitt and Wilcox, 1995; Wenk et al., 1991), suggesting that cholinergic deficits may underlie some of the behavioral abnormalities in RTT.

In rodents, choline supplementation during critical periods of brain development enhances transmission at cholinergic synapses (Blusztajn et al., 1998; Blusztajn and Wurtman, 1983), protects against neurodegeneration (Guo-Ross et al., 2002), improves performance on several behavioral tasks (Meck et al., 1988; Ricceri and Berger-Sweeney, 1998), and up-regulates growth factor expression (Sandstrom et al., 2002; Wong-Goodrich et al., 2008). In *Mecp2*$^{1lox}$ null mice, dietary choline supplementation from postnatal day (PD) 1–21 improves motor deficits as measured by rotor-rod performance, while cognitive deficits are unaltered (Nag and Berger-Sweeney, 2007). These results suggest that postnatal choline supplementation acts on brain motor regions of *Mecp2*$^{1lox}$ mice, therefore, in the current study we focus on two motor regions of the brain, the cerebellum and striatum. In addition, we focus these studies on *Mecp2*$^{1lox}$ null male mice because their phenotype more closely resembles that of RTT girls than the heterozygous female *Mecp2*$^{1lox}$ mice (Stearns et al., 2007), and we can detect behavioral improvements in the males in response to choline supplementation but not in the females, who have only very subtle motor and cognitive deficits in early adulthood (Nag and Berger-Sweeney, 2007). Furthermore, the *Mecp2*$^{1lox}$ null male mice provide an interesting opportunity to examine how choline supplementation can improve behavioral responses in the absence of Mecp2 protein, in other words, in the absence of this transcriptional repressor that binds methylated DNA.

It is not clear how perinatal choline supplementation causes long-term alterations in brain and behavior. Choline is essential for the structural integrity of cell membranes, methyl metabolism, transmembrane signal transduction, and cholinergic neurotransmission (Blusztajn, 1998; Zeisel, 1981, 2006; Zeisel and Blusztajn, 1994). Given the essential functions of choline in the organism, one possible mechanism by which supplementation may improve behavior in a mouse model of RTT is by increasing neuronal integrity, which may be reflected globally in volumetric changes in the brain. Indeed, longitudinal magnetic resonance imaging (MRI) reveals increases in whole brain volume in heterozygous female *Mecp2*$^{1lox}$ mice between PD 21 and 42 in response to postnatal choline supplementation, but not in null male *Mecp2*$^{1lox}$ mice who lack the *Mecp2* gene (Ward et al., in press). Using MRI, it is difficult to resolve specific regions within the brain. Therefore, in the current study, we measured specific brain regions associated with motor activity, namely the cerebellum and striatum, in histological sections using the computer program AMIRA™ to look for more subtle regional volume changes that may not be apparent by examining whole brain volume.

We hypothesized that choline supplementation improves motor behaviors in null male *Mecp2*$^{1lox}$ mice by increasing brain volume and stimulating neuronal growth factors. Growth factors promote neurite outgrowth, neuronal differentiation, and maintenance of cell function and survival (Ebendal, 1992). *Mecp2* null mice have reduced cortical dendritic arborizations (Kishi and Macklis, 2004) that may be partially restored with choline supplementation. Three extensively studied growth factors are insulin-like growth factor 1 (IGF-1), nerve growth factor (NGF), and brain derived growth factor (BDNF). The latter two are neurotrophins, a class of growth factors, and play a significant role in postnatal survival and neurite outgrowth. While all three growth factors have different expression profiles and act on different classes of neurons, they all promote neuronal integrity.

In the mouse brain, IGF-1 is associated with overall brain size. Over-expression of IGF-1 results in increased brain volume (Han, 1995), whereas IGF-1 null mice have reduced brain volume (Beck et al., 1995). IGF-1 mRNA is widely expressed in fetal brain tissue, with more discrete postnatal expression in olfactory bulb, hippocampus, and cerebellum (Bondy et al., 1992), being particularly important in cerebellar development (Torres-Aleman et al., 1994). RTT girls have unchanged levels of cerebrospinal and serum IGF-1 levels compared to controls (Riikonen, 2003, Vanhala et al., 2000), however no study has reported IGF-1 expression in the brains of RTT girls.

NGF and BDNF expression are evident in the hippocampus, cerebellum, cortex, striatum and basal forebrain (Large et al., 1986; Mobley et al., 1989; Senut et al., 1990; Shelton and Reichardt, 1986). NGF up-regulates the expression of choline acetyltransferase (ChAT), the synthetic enzyme for acetylcholine. NGF regulates expression of cholinergic cells of the striatum (Van Vulpen and Van Der Kooy, 1999), as well as the basal forebrain (Berse et al., 1999; Li et al., 1995; Tian et al., 1996). There are conflicting reports about NGF levels in RTT. It is either reduced (Lappalainen et al., 1996; Lipani et al., 2000; Riikonen, 2003; Riikonen and Vanhala, 1999) or unaltered compared to control subjects (Vanhala et al., 1998; Wenk and Hauss-Wegrzyniak, 1999), with the results being seemingly dependent on age, pathology, and sample collection site. We have found no reports of NGF levels in mouse models of RTT.