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Mecp2 deficiency decreases bone formation and reduces bone volume in a rodent model of Rett syndrome

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ABSTRACT

Rett syndrome (RTT), a neurological disorder characterized by neurological impairment and a high frequency of osteopenia which often manifests early in childhood, most often is caused by inactivating mutations in the X-linked gene encoding a regulator of epigenetic gene expression, methyl CpG binding protein, MeCP2. Clinical data show that, along with neurological defects, females with RTT frequently have marked decreases in bone mineral density (BMD) beyond that expected from disuse atrophy. To investigate the relationship between loss of Mecp2 and reduced BMD, we used a *Mecp2* null mouse model, *Mecp2* $^{-/yBIRD}$, for our histological and biochemical studies. *Mecp2* $^{-/yBIRD}$ mice have significantly shorter femurs and an overall reduced skeletal size compared to wild-type mice by post-natal day 60 (P60). Histological and histomorphometric studies identified growth plate abnormalities as well as decreased cortical and trabecular bone in P21 and especially in P60 *Mecp2* $^{-/yBIRD}$ mice. Dynamic histomorphometry revealed decreased mineral apposition rates (MAR) in Mecp2 null femoral trabecular bone as well as in calvarial bone samples. While changes in MAR of cortical bone were not significant, loss of Mecp2 significantly reduced cortical, trabecular and calvarial bone volume compared with age-matched wild-type animals. These differences indicate that Mecp2 deficiency leads to osteoblast dysfunction, which translates into reduced osteoid deposition accounting for the reduced bone volume phenotype. While individual variations were observed in OPG and Rankl concentrations, molar ratios of OPG: Rankl at P21 and P60 were comparable between wild-type and *Mecp2* $^{-/yBIRD}$ mice and showed a consistent excess of OPG. In tibial sections, TRAP staining demonstrated equivalent osteoclast number per bone surface measurements between wild-type and null animals. Our work with a *Mecp2* null mouse model suggests epigenetic regulation of bone in the *Mecp2* $^{-/yBIRD}$ mice which is associated with decreased osteoblast activity rather than increased osteoclastic bone loss.

Introduction

Over 95% of all patients with the neurodevelopmental disorder, Rett syndrome (RTT), are females who present with neurological dysfunction that becomes apparent after the first few months of life [1,2]. In addition to the prominent neurological symptoms, children with RTT frequently have reductions in skeletal growth and low BMD, which lead to pathological fractures in childhood and early adolescence [3–5]. While the neurological phenotype of the disorder has been well-characterized, only a handful of clinical reports have focused on describing the skeletal phenotype in this patient population. One of the first studies to document the risk of osteopenia in RTT compared bone mineralization in RTT girls with normal controls and individuals diagnosed with cerebral palsy (CP), who have impaired mobility and risks for disuse atrophy similar to patients with RTT [3]. In this study, the RTT patients ranged in age from 2 to 20 years with a mean age of 8.6 years, while the mean age of CP patients and normal females was 12.1 and 10.5 years, respectively. Despite adequate calcium and vitamin D intake, RTT patients in comparison to both control populations had decreases in whole body and spinal BMD as well as BMC (bone mineral content) even when corrected for age. Longitudinal and cross-sectional studies of RTT patients revealed that while bone mass does increase over time in RTT girls, it does so at a much lower rate than in normal controls [6–9]. The reduction in cortical bone thickness and BMD is further exacerbated by anticonvulsant use, immobility and scoliosis, which compound the morbidity imparted by bone fragility in RTT patients [10–13].

Most cases of RTT are caused by mutations in *MECP2* (murine ortholog *Mecp2*), encoding a methyl CpG binding protein [14]. Mecp2

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is the most notable in a family of methyl binding domain proteins that all share sequence homology with the methyl binding domain (MBD) of Mecp2 [15–17]. In addition to the MBD, a transcriptional repression domain (TRD) [18] and nuclear localization signal (NLS) have been identified. DNA methylation of cytosines in CpG dinucleotides is an epigenetic modification that is important for transcriptional regulation [19–21], X chromosome inactivation [22], and imprinting [23]. Mecp2 was identified as a protein that would preferentially bind methylated CpG dinucleotides [17,24] in the genome and repress transcription by recruiting co-repressors such as mSin3A, c-Ski, N-CoR [25] as well as histone deacetylases [26,27], although there is evidence that Mecp2 can repress transcription independent of methylation state [28]. Until recently, it was believed that Mecp2 acted solely as a global transcriptional repressor, even though microarray analyses using Mecp2 deficient systems did not yield much information regarding specific targets of Mecp2 [29–31]. Recent studies have demonstrated that Mecp2 binding is not limited to promoter regions of genes [32], nor is Mecp2 now confined to its role as a long-term transcriptional silencer. Further work has shown that Mecp2 has the ability to bind and transcriptionally regulate genes through both transcriptional repression and activation, and that CREB is a transcriptional target of Mecp2 repression [33]. These studies complement the hypothesis that the Mecp2 protein has multiple functions, which may include acting as a chromatin architectural protein (reviewed in [34,35]).

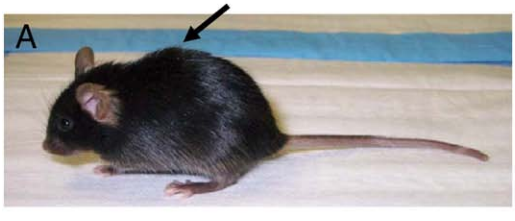
Several Mecp2 deficient rodent models have been developed that harbor various inactivating mutations in the *Mecp2* locus. The strain developed by the Bird laboratory, B6.129P2(C)-Mecp2tm1.1Bird/J, carries a deletion of exons 3 and 4 generated by a constitutive Cre-mediated recombination event [36]. The line is carried through heterozygous females and all studies were performed using the hemizygous males (*Mecp2* $^{-/yBIRD}$) because individual differences in X-inactivation patterns in females lead to marked variability in phenotype. While extensive work has established many neurological similarities between this mouse model and RTT patients, we sought to determine if this mouse model would recapitulate the bone phenotype and provide insight into the mechanism by which Mecp2 regulates BMD. To eliminate any potential artifacts resulting from secondary effects owed to neurological deficits in feeding or activity, only the mildly to moderately symptomatic animals were included in this study.

Materials and methods

Animal care and use

Female B6.129P2(C)-Mecp2tm1.1Bird/J mice developed by the Bird laboratory, which are heterozygous for the *Mecp2* gene, were obtained from Jackson Laboratory in addition to wild-type C57Bl/6J male mice [36]. Although the strain was originally developed on a mixed background, it has undergone extensive numbers of backcrosses and now is congenic to the C57Bl/6J strain. Subsequent breeding and genotyping were performed at the A.I. duPont Hospital for Children/Nemours Foundation Life Science Center. Mice were supplied with water and standard mouse chow ad libitum. Heterozygous breeding female diets were supplemented with sunflower seeds [37], dried banana chips, and Supreme MiniTreats (Bio-Serv) to enhance maternal care of new litters. All subsequent studies were performed using the hemizygous males (*Mecp2* $^{-/yBIRD}$, $n \geq 4$ for each timepoint) following procedures approved by the IACUC at the A.I. duPont Hospital for Children/Nemours Foundation or University of Delaware, as appropriate. When possible, littermates were used in the following studies.

In addition to the progressive growth deficiency exhibited by *Mecp2* $^{-/yBIRD}$ mice between P21 and P60, they also developed behaviors that included rapid breathing and short periods of "freezing" becoming more frequent as the mice aged. To minimize potentially confounding variables introduced by the neurological symptoms, all P21 mice (weight range 4.4–11.1 g) used in the following studies were healthy and active and the P60 *Mecp2* null mice (weight range 11.7–29.4 g), although symptomatic, were also lively and active. The behavioral abnormalities exhibited by symptomatic mice at P60 did not typically lead to extended periods of hypoactivity. The animals selected for the microcomputed tomography (μCT) and histological analyses were all nourished, mobile and active and although they were generally smaller (nose to tail) than their wild-type counterparts, their weights were not significantly different than wild-type (Fig. 1).



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	Average Weight (g)
P21 Wild-type	9.00 +/- 1.75
P21 *Mecp2* $^{-/yBIRD}$	8.28 +/- 0.95
P60 Wild-type	24.30 +/- 2.33
P60 *Mecp2* $^{-/yBIRD}$	22.02 +/- 3.66

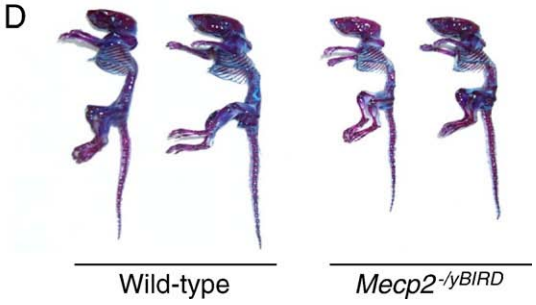

Fig. 1. *Mecp2* $^{-/yBIRD}$ male mice have reduced skeletal size compared with their wild-type littermates by P60. (A) Kyphotic posturing in a P60 Mecp2 null male mouse compared with (B) an age-matched wild-type control. (C) The average body weight of P21 and P60 wild-type and *Mecp2* null male mice used for the histological and μCT evaluations in this study ($n \geq 4$). (D) Skeletal preparations from P60 wild-type and *Mecp2* null male mice stained with Alcian blue depicting cartilage content (blue) and Alizarin red staining mineralized tissue (red-purple).