

BIOMECHANICAL PROPERTIES OF BONE IN A MURINE MODEL OF RETT SYNDROME

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Introduction

Rett Syndrome (RTT) is an X-linked genetic disease that is classically considered a neuro-developmental disorder. However, the gene mutated in RTT, methyl-CpG binding protein 2 (*Mecp2*), is expressed ubiquitously in peripheral tissues and in addition to neurological phenotypes, skeletal anomalies including reduced bone mass and fractures are a common features of the condition. RTT is generally lethal in males while girls survive but with profound disabilities. In order to explore whether *Mecp2* protein-deficiency results in altered bone properties, we conducted biomechanical testing on both cortical and cancellous bone from mice in which *Mecp2* is functionally silenced (*Mecp2*^{stop/y}) and mice in which the RTT-like phenotype is rescued by *Mecp2* gene reactivation (*Mecp2*^{stop/y/CreER}). This is the first study to explore the biomechanical integrity of bone in a (*Mecp2*^{stop/y/CreER}) mouse model of Rett Syndrome.

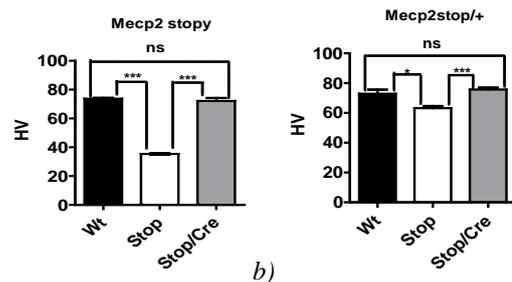
Materials and Methods

Mecp2^{stop/y} male mice (n = 5; mean age = 14wk±0.7wks) and *Mecp2*^{stop/+} female mice (n≥3; mean age = 17±1 months) along with the age-matched *Mecp2*^{stop/y/CreER} mice and wildtype controls were treated with Tamoxifen as described [Guy 2007]. The age difference between genders is necessitated by the differences in phenotype onset, severity and lethality in males. Tibial and femoral shafts were subjected to three point bending test to assess the cortical bone and the femoral necks to fracture test to measure the cancellous bone, using a Zwick/Roell Z2.0 testing machine. Microindentation testing was performed using a Wilson Wolpot Micro-Vickers 401MVA machine and at an applied load of 25gf.

Results

Both *Mecp2*^{stop/y} male mice in which *Mecp2* is silenced in all cells and female *Mecp2*^{stop/+} mice in which *Mecp2* is silenced in ~50% of cells showed significant reductions in bone

stiffness and hardness (Fig 1). Furthermore, unsilencing of *Mecp2* in adult mice by cre-mediated stop cassette deletion resulted in a restoration of tibia stiffness, load properties, bending modulus and femur microhardness values to wild-type levels.



a) b)
Fig 1: Microindentation hardness test showing significant but reversible reduction in cortical bone hardness in *MECP2*-deficient mice (*Stop*) compared to rescued (*Stop/Cre*) and wildtype controls (*WT*) for a) males and b) females.

Discussion

Previous studies [Jefferson, 2011; Shapiro, 2010; Zysman, 2006] have reported that reduced bone strength, bone mineral deficits, increase risk of fracture and bone related disorders are fairly common in RTT. We have shown the reversibility of bone defects identified in hemizygous male *Stop/y* and heterozygous female *Stop/+* mouse model following *Mecp2* gene reactivation. This significant improvement in bone properties after the *Mecp2* gene reactivation points towards the potential application of intervention gene therapy for peripheral phenotypes in RTT patients.

References

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