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BRAIN RESEARCH

Research Report

Ube3a mRNA and protein expression are not decreased in *Mecp2*^{R168X} mutant mice

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ARTICLE INFO

Article history:
Accepted 10 August 2007
Available online 24 August 2007

Keywords:
Rett syndrome
Angelman syndrome
MeCP2
UBE3A
Mouse model

ABSTRACT

Mutations in the transcriptional repressor *methyl CpG binding protein 2* (*MeCP2*) are responsible for most cases of Rett Syndrome (RS), a severe neurodevelopmental disorder characterized by developmental regression, minimal speech, seizures, postnatal microcephaly and hand sterotypies. Absence of the maternal copy of ubiquitin protein ligase 3A (*UBE3A*) results in Angelman syndrome, also a severe developmental disorder that shares some clinical features with RS. As *MeCP2* regulates gene expression, this has led to the hypothesis that *MeCP2* may regulate *UBE3A* expression; however, there are conflicting reports regarding the expression of Ube3a in *MeCP2* null mutant mice. We have generated a novel *MeCP2* mutant knock-in mouse with the mutation R168X, one of the most common mutations in patients with RS. These mice show features similar to RS, including hypoactivity, forelimb stereotypies, breathing irregularities, weight changes, hind limb atrophy, and scoliosis. The male mice experience early death. Analysis of Ube3a mRNA and protein levels in the *Mecp2*^{R168X} male mice showed no significant difference in expression compared to their wild type littermates.

1. Introduction

Rett syndrome (RS) is a severe neurodevelopmental disorder characterized by apparently normal initial development followed by slowing of development and head growth (Hagberg et al., 1983, 1985, 2002; Rett, 1966). Purposeful hand skills are lost and replaced by characteristic stereotypies. Spoken language is lost. Patients may also develop seizures, breathing irregularities, sleep disturbance, autistic symptoms and scoliosis. Mutations or deletions in *methyl-CpG-binding protein 2* (*MECP2*) located at Xq28 are detectable in 96% of patients with RS (Amir et al., 1999; Moretti and Zoghbi, 2006). A small percentage of RS patients with early onset seizures have mutations in *cyclin dependent kinase-like 5* (*CDKL5*) (Evans et al., 2005; Scala et al., 2005; Tao et al., 2004). CDKL5 may play a role in phosphorylation and regulation of *MeCP2* (Mari et al., 2005).

Because *MeCP2* binds to methyl CpG dinucleotides, an early leading hypothesis was that it serves as a global repressor of transcription. However, transcriptional profiling studies have failed to reveal a global de-repression of transcription in the

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Abbreviations: AS, Angelman syndrome; *MeCP2*, *methyl CpG binding protein 2*; qRT–PCR, quantitative real-time reverse transcriptase polymerase chain reaction; RS, Rett syndrome; *UBE3A*, *ubiquitin protein ligase 3A*; *UBE3A*-ATS, *UBE3A* antisense transcript

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doi:10.1016/j.brainres.2007.08.039

setting of *MeCP2* deficiency (Tudor et al., 2002). An alternate hypothesis that *MeCP2* instead regulates transcription in a highly specific and selective manner has thus been raised. Strong evidence for this hypothesis is the finding that *MeCP2* regulates *BDNF* in a calcium- and phosphorylation-dependent manner (Chen et al., 2003; Zhou et al., 2006).

Angelman syndrome (AS) is an imprinting disorder caused by a decrease in or loss of function of the maternal copy of *ubiquitin protein ligase E3A*(*UBE3A*) located at 15q11q13 (Kishino et al., 1997; Magenis et al., 1987). This protein, unlike *MeCP2*, is not a regulator of gene transcription but is involved in the ubiquitination pathway, which targets specific proteins for degradation. AS patients exhibit profound speech deficits, gait ataxia, seizures, characteristic EEG, postnatal acquired microcephaly, sleep disturbance, and an unusually happy demeanor with propensity to paroxysms of laughter (Angelman, 1965; Clayton-Smith and Laan, 2003; Williams et al., 1995). Several of these features are in common with RS, including the speech deficits, acquired microcephaly, sleep disturbance, and seizures. These similarities suggest that *UBE3A* could be a target for regulation by *MeCP2*. However, recent studies designed to test this hypothesis yielded conflicting results (Jordan and Francke, 2006; Makedonski et al., 2005; Samaco et al., 2005).

The study by Samaco et al. looked at two lines of mutant *Mecp2* adult mice, as well as *postmortem* brain tissue from RS patients (Samaco et al., 2005). One mouse line studied, $Mecp2^{tm1.1Jae}$, was constructed with an exon 3 deletion and characterized as functionally *Mecp2* null (Chen et al., 2001). The other mouse line studied was $Mecp2^{tm1.1Bird}$, which is *Mecp2* null (Guy et al., 2001). They reported a significant reduction in expression of both *UBE3A* RNA and protein products. Next, Makedonski et al. investigated newborns of one of the same mouse lines studied by Samaco et al. ($Mecp2^{tm1.1Bird}$) as well as *postmortem* brain tissue from RS patients and a lymphoblast cell line from an RS patient (Makedonski et al., 2005). They also reported reductions in *UBE3A* RNA and protein expression. Jordan and Francke then reported studies on both mouse lines, $Mecp2^{tm1.1Jae}$ and $Mecp2^{tm1.1Bird}$, at 3 and 21 days of age (Jordan and Francke, 2006). However, in direct contrast to the previous two reports, no significant changes in Ube3a RNA or protein expression were detected. This lack of consensus prompted us to investigate Ube3a expression in a novel *Mecp2* mutant mouse line designed in our laboratory.

2. Results

2.1. $Mecp2^{R168X}$ mice show RS features

We designed a line of *Mecp2* mutant mice, which have the most common mutation associated with RS, R168X, knocked-in the mouse gene ($Mecp2^{R168X}$) (Bienvenu and Chelly, 2006). This sequence change replaces a codon for arginine with a stop codon. Using site directed mutagenesis, we altered the AGA sequence coding for arginine at codon 168 to a TGA coding for stop (Fig. 1a). The mutant transcript is transcribed and is easily detectable by RT–PCR (Fig. 1b). The amplicon was confirmed to be *Mecp2* by sequencing, which also confirmed the presence of the mutation in the mouse line. The mutant transcript is relatively stable compared to the WT transcript (Fig. 1c). The

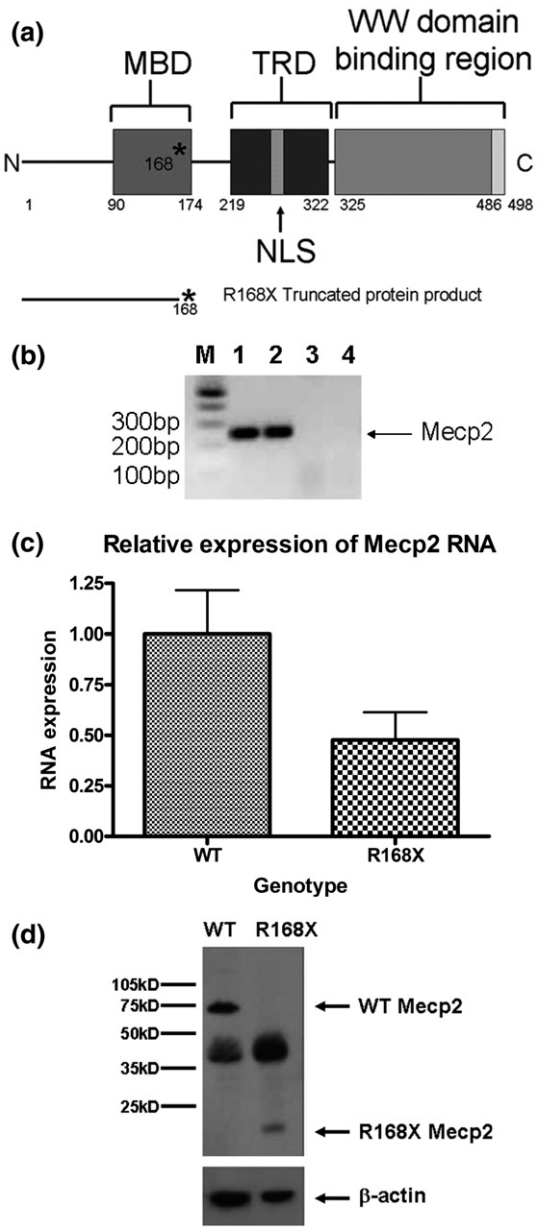

Fig. 1 – $Mecp2^{R168X}$ mutant mice. (a) An A to T point mutation was engineered to create a premature stop codon in place of an arginine at codon 168. (b) Confirmation of the mutant transcript by RT–PCR. RT–PCR was performed with primers flanking the mutation using cDNA reverse transcribed from WT mouse RNA (lane 1), cDNA reverse-transcribed from mutant mouse RNA (lane 2), WT mouse RNA (lane 3), and mutant mouse RNA (lane 4). The expected 238 bp product was detected in lanes 1 and 2 and confirmed as *MeCP2* by sequencing. (c) The $Mecp2^{R168X}$ mutant RNA is relatively stabled compared to the WT transcript. Error bars show standard error of the mean. (d) $Mecp2^{R168X}$ mutant mice (right lane) express a small protein which may be a prematurely truncated mutant *MeCP2* protein, but do not express the full length WT protein. The WT mice (left lane) express the full length protein.