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Wild type microglia do not arrest pathology in mouse models of Rett syndrome

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Abstract

Rett syndrome (RTT) is a severe neurodevelopmental disorder caused by mutations in the X chromosomal gene *Methyl-CpG-binding Protein 2 (MECP2)* (1). RTT treatment so far is symptomatic. *Mecp2* disruption in mice phenocopies major features of the syndrome (2) that can be reversed upon re-expression of *Mecp2* (3). It has recently been reported that transplantation of wild type (WT) bone marrow (BMT) into lethally irradiated *Mecp2^{tm1.1Jae/y}* mice prevented neurologic decline and early death by restoring microglial phagocytic activity against apoptotic targets (4). Based on this report, clinical trials of BMT for patients with RTT have been initiated (5). We aimed to replicate and extend the BMT experiments in three different RTT mouse models but found that despite robust microglial engraftment, BMT from WT donors did not rescue early death or ameliorate neurologic deficits. Furthermore, early and specific genetic expression of *Mecp2* in microglia did not rescue *Mecp2*-deficient mice. In conclusion our experiments do not support BMT as therapy for RTT.

We first sought to replicate BMT-mediated rescue of male mice derived from the same *Mecp2^{tm1.1Jae/y}* colony from the original report (4), implementing established standards for conducting preclinical studies (2,6). Mice were maintained on C57Bl/6J background, which was confirmed in recipient animals by genome scanning (data available upon request). Four week-old *Mecp2^{tm1.1Jae/y}* mice and wild type littermates were subjected to the same protocol of lethal split-dose γ -irradiation and randomized to receive tail vein injection of bone marrow from *Mecp2*-deficient male littermates or bone marrow from *Mecp2*-proficient animals including C57Bl/6J male mice ubiquitously expressing GFP and *Mecp2^{+/-y}* littermates of the recipients. All animals achieved multilineage peripheral blood engraftment judged by the fraction of donor-derived GFP-expressing cells in peripheral blood 4 and 8 weeks post-transplant (Extended Data Figure 1a). PCR analysis of blood and tail tissue 4

weeks after transplant also confirmed expression of the appropriate mutant or WT variant of *Mecp2* in blood in all groups (Extended Data Figure 1b). Microglial engraftment in brain parenchyma, 30 and 90 days post-transplant was similar in mutant and WT recipients engrafted with marrow from WT mice ubiquitously expressing a GFP transgene, (Fig. 1, A and B, and Extended Data Figure 1c), and comparable to engraftment observed by Derecki *et al.* (4) and others (7).

Contrary to our expectation, *Mecp2^{tm1.1Jae/y}* mice that received *Mecp2^{+/y}* marrow had no extension of lifespan compared to *Mecp2^{tm1.1Jae/y}* marrow recipients (Fig. 1C). No difference in survival was observed in mutant animals that received *Mecp2^{+/y}* marrow from WT littermates or C57Bl/6J animals ubiquitously expressing GFP (Extended Data Figure 1d). We also observed no benefit in outcome measures at 12 weeks of age, 8 weeks after transplant, including weight, breathing, locomotion, general condition, walking gait, tremor, hindlimb claspings or neurological score (Figure 1i). Thus, the same BMT procedure with substantially greater numbers of animals, randomly assigned to treatment group, from the same *Mecp2^{tm1.1Jae/y}* mouse colony did not replicate any aspects of protection reported by Derecki *et al.* (4). Furthermore, histologic analysis blind to genotype and treatment group showed no neuropathologic evidence of differential apoptosis, microglial response, or tissue degeneration between experimental groups (Extended Data Figure 1e). No protective effect on survival was noted in two additional mouse models of Rett syndrome as well (Figure 1, e and g): *Mecp2^{LucHyg/y}* mice (Extended Data Figure 2), and *Mecp2^{R168X/y}* mice (8), despite excellent engraftment after BMT (Extended Data Figure 2). Experiments with these two models were performed in independent laboratories following the same BMT protocol (4).

In all models, WT mice transplanted with WT bone marrow showed no mortality, indicating the procedure was well tolerated (Figure 1, c, e, and g). Likewise, BMT was well-tolerated by mutant animals, as *Mecp2* mutant animals receiving mutant marrow exhibited either no change (*Mecp2^{LucHyg/y}* and *Mecp2^{R168X/y}* mice), or, surprisingly, slightly reduced mortality (*Mecp2^{tm1.1Jae/y}* mice) compared to naive mice not subjected to BMT (Figure 1, d, f and h). The small survival extension may be related to a salutary effect of post-irradiation antibiotic treatment of transplanted animals, to which naive animals were not exposed, or to differences in animal handling (9).

To further address the role for microglia in RTT reported by Derecki *et al.* (4), we used the Cre/lox system and a lox-stop-lox allele of *Mecp2* (*Mecp2^{LSL}*) to examine the effect of genetically-driven expression of *Mecp2* in microglia during development. First, we analyzed the suitability of the *LysM-Cre* transgene, which was used by Derecki *et al.* (4) in their genetic *Mecp2^{LSL/y}* rescue experiments (4), to drive efficient microglia-specific gene restoration. As previously reported (10), *LysM-Cre* driven dTomato reporter cells account for less than 25% of microglia, as assessed using flow cytometry of microglia derived from mice containing the *LysM-Cre* transgene and a transgene expressing Cre-dependent dTomato (Extended Data Figure 3a). Furthermore, when we generated *LysM-Cre; Mecp2^{LSL/y}* mice, we observed MeCP2 expression in neurons (large NeuN+ cells) in many brain regions (Extended Data Figure 3b). To identify a Cre transgenic line that drives efficient expression within microglia, we next evaluated *Vav1-Cre* transgene, which selectively expresses throughout the hematopoietic compartment (11). In contrast to *LysM-*

Cre, *Vav1-Cre* transgene targeted microglia with high efficiency (Figure 2a) and specificity (Figure 2b). As *Vav1-Cre*-driven expression in brain proved to be efficient and restricted to microglia, we applied this system to test whether expression of *Mecp2* in microglia rescues *Mecp2*-null mice. To quantify *Mecp2* restoration in microglia, we utilized the *fms-GFP* transgene, expression of which within brain is restricted to microglia, for flow sorting (11) (Extended Data Figure 3c). Microglia derived from *Vav1-Cre; Mecp2^{LSL/Y}* animals expressed *Mecp2* mRNA at 75% of the level of *Mecp2* mRNA in microglia derived from *Mecp2^{+/-}* animals (Figure 2c). Similar to other *Mecp2*-null mouse models, *Mecp2^{LSL/Y}* animals showed hypoactivity, poor motor coordination on parallel rod walking, increased basal and hypoxia breathing rate, increased apneas, and early death, none of which were improved by *Mecp2* expression in microglia of *Vav1-Cre; Mecp2^{LSL/Y}* animals (Figure 2, d–h). We thus conclude that, in contrast to the data reported by Derecki et al. (4), driving *Mecp2* expression developmentally in microglia does not ameliorate the phenotype of *MeCP2*-null mice.

In conclusion, we observe no benefit of BMT-mediated delivery of WT microglia into the brains of three different preclinical models of RTT, nor do we observe a causative role of microglia in the disease process. Our BMT studies included large numbers of mice derived from the same parent colony used in the original report (4), with treatment assigned randomly and analysis conducted blind to genotype and treatment group. Finally, we showed that even early and highly efficient genetically-driven *Mecp2* expression in microglia of *Mecp2*-null mice conferred no protective effect. Restoration of MeCP2 in microglia using bone marrow transplantation or genetics does not rescue the major observed phenotypes in RTT, which argues against the previously proposed therapeutic potential of BMT in patients with RTT (4).

METHODS SUMMARY

Details of procedures and reagents are described in Methods.

Extended Data

Extended Data Figure 1.

a, Multilineage peripheral blood engraftment with donor cells in *Mecp2^{tm1.1Jae/y}* and WT mice. WT and *Mecp2^{tm1.1Jae/y}* animals received transplant from WT animals ubiquitously expressing green fluorescent protein (GFP) (Jackson Labs, C57BL/6-Tg(UBC-GFP)20Scha/J, stock 004353). Peripheral blood engraftment in indicated blood lineages was measure by flow cytometry (GFP) 4 weeks and 8 weeks posttransplant. **b, PCR analysis of**

blood and tail tissue 4 weeks after transplant. PCR analysis shows expression of only the appropriate mutant or WT variant of *Mecp2* from the donor in blood in all four groups, with retention of the original genotype in tail tissue as expected. Specifically, *Mecp2*^{+/-} → *Mecp2*^{+/-} mice show only the WT allele at 190 bp, while *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{tm1.1Jae/y} mice show only the mutant allele at 250 bp, as previously described in the original report of generation of these mice (Chen *et al. Nature* **27**, 327–331, 2001). *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{+/-} mice, however, show only the mutant allele in blood tissue and retention of host WT allele in tail tissue. Accordingly, *Mecp2*^{+/-} → *Mecp2*^{tm1.1Jae/y} mice show only the WT allele in blood tissue, with retention of the host mutant allele in tail tissue. Tail tissue in these latter two groups shows some of the allele from the donor as well, presumably due to blood contained within the tail clips used for analysis. Importantly, the *Mecp2* allele expressed in blood is always restricted to the donor genotype, indicating successful transplantation with complete replacement of the hematopoietic system in the host. Samples are labelled with a 'T' for tail and 'B' for blood, followed by the number of the animal, indicating that 6 different animals were analyzed for each condition. HMGD1 = high mobility group protein-like domain 1; HMGD2 = high mobility group protein-like domain 2; MBD= methyl binding domain; TRD= transcription repression domain; NLS= nuclear localization signal; CTD = C-terminus domain alpha and beta. **c, Robust and early microglial engraftment of donor cells following bone marrow transplantation in *Mecp2*^{tm1.1Jae/y} and WT mice.** Microglial engraftment was visualized using double immunofluorescence staining in sections quenched for autofluorescence by incubation in Sudan black solution. All sections were stained with an anti-Iba-1 primary with CY-3 secondary and an anti-GFP primary with CY-5 secondary. All microglia are Iba-1-positive, and thus successfully engrafted GFP-expressing donor derived microglia were observed as GFP and Iba-1-positive, while native microglia were only Iba-1-positive. Engraftment of microglia into WT and *Mecp2*^{LucHyg/y} mice was determined by dividing the GFP⁺/Iba-1⁺ cells by the number of total Iba-1⁺ cells. Cell counts were performed in cerebellum, cortex and brainstem from mice. Percent engraftment in WT and *Mecp2*^{tm1.1Jae/y} mice yielded similar results to previously published engraftment results at 30 days post-transplant (9). **d, BMT was well-tolerated in animals.** No difference in survival was observed in mutant animals that received *Mecp2*^{+/-} marrow from their WT littermates (WT, N=13) and C57Bl/6J animals ubiquitously expressing GFP (WT-GFP, N=13). **e, Representative H&E-stained sections of cerebellum, brainstem and hippocampus (400×) from age-matched WT and *Mecp2*^{tm1.1Jae/y} mice sacrificed at 7 weeks of age.** Sections demonstrate comparable histologic features between WT and *Mecp2*^{tm1.1Jae/y} brains and a lack of gliosis, cell loss, cellular debris, microglia or macrophages in *Mecp2*^{tm1.1Jae/y} brains.

Extended Data Figure 2. Early transplantation of wild-type microglia into the brain does not rescue additional models of *Mecp2*-null mice: *Mecp2^{LucHyg}* mice and *C57B6/J-Mecp2^{R168X}* mice
a, Generation of *Mecp2^{LucHyg}* mice. Luciferase/Hygromycin (LucHyg) fusion gene vector correctly targeted to the *Mecp2* locus in ES cells. Positions of the probes and enzyme restriction sites are indicated. The targeting vector's homology arms are depicted in black, and its backbone in grey. **b, Confirmation of genetic targeting for *Mecp2^{LucHyg}* mice.** Southern blotting of NdeI or KpnI-digested DNA extracted from clone C4 cells, used for blastocyst injections, hybridized with either the Hygromycin probe (Hygro) or External

probe confirms correctly targeted event. **c**, Luciferase activity in clone C4 cells before (Day 0) or after subjecting cells to retinoic acid (RA)-induced differentiation (Day 5). Following adsorption to eliminate feeder mouse embryonic fibroblasts, clone C4 ES cells were treated with RA (100 nM) in differentiation medium for 5 days and Luciferase activity was measured before and after RA treatment. Mean values are plotted relative to that of the wild-type cells (n=3, error bars represent standard deviation). RA-induced differentiation leads to an increase in luciferase activity consistent with an increase in *Mecp2* expression level as measured in **d**. **d**, mRNA level of *Mecp2* increased and mRNA level of ES marker *Nanog* decreased in clone C4 cells subjected to RA-induced differentiation. mRNA levels were measured before and after treatment by real-time quantitative PCR. Mean values are plotted relative to day 0 for each mRNA (n=3, error bars represent standard deviation). **e**, Western blot analysis of Mecp2 expression in brains of wild-type and *Mecp2^{LucHyg}* male mice. Mecp2 protein is not detected in *Mecp2* luciferase males. The ponceau S staining serves as a loading control. **f, g, Robust peripheral blood and microglial engraftment of donor cells following bone marrow transplantation (BMT) in *Mecp2^{LucHyg/y}* mice.** WT and *Mecp2^{LucHyg/y}* mice received WT bone marrow marked with GFP or CD41.1. Peripheral blood engraftment was measured by flow cytometry (GFP or CD45.1) in the indicated lineage 4–8 weeks posttransplant. For CNS engraftment, flow cytometry was performed on isolated mononuclear cells from the cortex, brain stem, cerebellum, hippocampus and striatum. Engraftment of BMT-derived cells was determined by dividing the CD11b+CD45+GFP+ cell population by total CD11b+CD45+ monocytes/microglia. **h, Robust peripheral blood engraftment of donor cells 7 weeks following bone marrow transplantation in *Mecp2^{R168X}* mice.** Reconstitution of bone marrow from *B6.SJL-Ptprc^a Pepc^b/BoyJ* mice into wild-type mice and *C57B6/J-Mecp2^{R168X}* mice showed robust engraftment in peripheral blood. Reconstitution of bone marrow was determined by FACS analysis of peripheral blood using anti GR-1, anti CD4, anti CD8 antibodies and CD45.1 for the donor cells (*B6.SJL-Ptprc^a Pepc^b/BoyJ* mice, white bars) and CD45.2 for host cells (wild-type and *C57B6/J-Mecp2^{R168X}* mice, grey bars).

Extended Data Figure 3. Flow sorting and histological characterization of *LysM-Cre* or *Vav1-Cre* transgenic mice

a, Stepwise process to characterize the amount of microglia (CD45^{lo} expressing) cells that also express tdTomato in a *LysM-Cre* dependent fashion. **b**, High power images of cortex from *LysM-Cre*^{Tg/+}; *Mecp2*^{LSL/Y} animals. Each panel shows single channel images and merged image. The first panel shows microglia (Iba1 expression) in green, the second panel shows MeCP2 protein in red, the third panel shows NeuN in white, and the fourth panel shows the merged image. Scale bar in all is 50 μ m. **c**, Merged high power images from

Cortex, Pons, Medulla from *LysM-Cre; Mecp2^{LSL/Y}* animal. Colors are as in **b**. Upward facing carrots identify large NeuN staining cells that express MeCP2 (NeuN+; MeCP2+). Downward-facing triangles mark microglia not expressing MeCP2 (Iba+; MeCP2-). Scale bar is equal to 20µm. **d**, Gating strategy for microglia sorting for *Mecp2* expression quantification in *Vav1-Cre; Mecp2^{LSL/Y}* and control animals is presented: **i**. Size/Complexity (size/cytoplasmic granularity for cells but not debris); **ii**. Forward scatter pulse height/area (eliminates doublet cells); **iii**. Side scatter pulse height/width (eliminates doublet cells); **iv**. SYTOX® Red staining; dead cells are SYTOX® Red-positive and removed from the following analysis; **v**. fms-GFP expression analysis enables the purification of microglia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Neul JL. The relationship of Rett syndrome and MECP2 disorders to autism. *Dialogues Clin Neurosci.* 2012; 14:253–62. [PubMed: 23226951]
2. Katz DM, et al. Preclinical research in Rett syndrome: setting the foundation for translational success. *Dis Mod and Mech.* 2012; 5:733–745.
3. Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nature Genet.* 2001; 27:322–326. [PubMed: 11242117]
4. Derecki NC, et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature.* 2012; 18:105–9. [PubMed: 22425995]
5. MT2013-31:Allogeneic Hematopoietic Cell Transplantation for Inherited Metabolic Disorders, Sever Osteoporosis and Males with Rett Syndrome Following Conditioning with Busulfan (Therapeutic Drug Monitoring), Fludarabine +/- ATG. University of Minnesota; Minneapolis, MN USA:
6. Landis SC, et al. A call for transparent reporting to optimize the predictive value of preclinical research. *Nature.* 2012; 490:187–91. [PubMed: 23060188]
7. Yang Y, et al. Perivascular, but not parenchymal, cerebral engraftment of donor cells after non-meloablative bone marrow transplantation. *Exp Mol Pathol.* 2013; 95:7–17. [PubMed: 23567123]

8. Brendel C, et al. Readthrough of nonsense mutations in Rett syndrome: evaluation of novel aminoglycosides and generation of a new mouse model. *J Mol Med (Berl)*. 2011; 89:389–398. [PubMed: 21120444]
9. Pitcher MR, et al. Insulinotropic treatments exacerbate metabolic syndrome in mice lacking MeCP2 function. *Hum Mol Genet*. 2013; 22:2626–33. [PubMed: 23462290]
10. Goldmann T, et al. A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. *Nat Neurosci*. 2013; 11:1618–26. [PubMed: 24077561]
11. Sasmono RT, et al. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood*. 2003; 101:1155–63. [PubMed: 12393599]

Figure 1. Early transplantation of wild-type microglia into the brain does not rescue *Mecp2*-null mice

a, Transplantation of bone marrow from C57BL/6 mice with ubiquitous GFP transgene expression into *Mecp2*^{tm1.1Jae/y} mice confers robust donor engraftment at the indicated time posttransplant shown via immunohistochemical detection of GFP-positive cells of microglial morphology in entorhinal cortex and hippocampus, in both *Mecp2*^{+/-}-GFP donors → *Mecp2*^{tm1.1Jae/y}, and *Mecp2*^{+/-}-GFP donors → *Mecp2*^{+/-} groups. **b**, Double immunofluorescent labeling with GFP and Iba1 in cerebellar tissue from *Mecp2*^{+/-}-GFP

donors → *Mecp2*^{tm1.1Jae/y} mice examined 30 days post BMT confirms early microglial engraftment in brain parenchyma. Quantification of microglial engraftment 30 days posttransplant using double immunofluorescence labeling for GFP and Iba1 is presented in Extended Data Figure 1c. **c–h**, No differences in survival were noted between *Mecp2*^{+/y} → *Mecp2*-null mice and *Mecp2*-null → *Mecp2*-null mice, indicating that engraftment of WT microglia into the brains of *Mecp2*-null mice did not protect *Mecp2*-null mice from premature death. In addition, no differences in survival were noted between *Mecp2*-null → *Mecp2*^{+/y} mice and *Mecp2*^{+/y} → *Mecp2*^{+/y} mice, indicating that engraftment of *Mecp2*-null microglia into the brains of WT mice does not shorten survival as seen in *Mecp2*-null mice. **i**, No differences were seen in other outcome measures at 12 weeks of age, 8 weeks after BMT, between *Mecp2*^{+/y} → *Mecp2*^{tm1.1Jae/y} mice (n=31) and *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{tm1.1Jae/y} mice (n=25), including weight, frequency of breathing apneas, locomotor activity (beam breaks), general condition, walking gait, tremor, hindlimb clasping or neurological score. Here, data is presented as relative outcome measure. Values for each measure were determined for *Mecp2*^{+/y} → *Mecp2*^{tm1.1Jae/y} mice and *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{tm1.1Jae/y} mice, and means and standard deviation were calculated. All values (both means and standard deviations) were then divided by the mean value for *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{tm1.1Jae/y} mice, for relative comparison in the composite graph. Specific values obtained for *Mecp2*^{tm1.1Jae/y} → *Mecp2*^{tm1.1Jae/y} mice and *Mecp2*^{+/y} → *Mecp2*^{tm1.1Jae/y} mice are as follows: weight in grams (18.22 ± 0.93 vs 18.96 ± 0.8); # apneas/15 min (35.4 ± 5.96 vs 35.2 ± 5.24); # beam breaks/12 hours (3729.2 ± 253.3 vs 4330.2 ± 305.2); general condition score (0.52 ± 0.1 vs 0.35 ± 0.1); walking gait score (0.24 ± 0.087 vs 0.23 ± 0.076); tremor score (0.24 ± 0.087 vs 0.13 ± 0.061); hindlimb clasping score (0.48 ± 0.12 vs 0.29 ± 0.083); neurological score (1.48 ± 0.29 vs 1 ± 0.2). None of the differences were statistically significant.

Figure 2. Genetic reconstitution of *Mecp2* in microglia does not rescue *Mecp2*-null mice
a, b, Evaluation of efficiency and specificity of Vav1-Cre in microglia. **a**, Representative flow sorting of microglia derived from brains from Vav1-Cre; Rosa26:LSL:tdTomato mice shown on left, with quantification (n=3) of cells that express both fluorescent reporter (tdTomato) and microglia marker (CD45) shown on right. **b**, Histological characterization of tdTomato expression in brain from Vav1-Cre; Rosa26:LSL:tdTomato. tdTomato expression is seen in red, Iba1 (microglia) in green, and neurons (NeuN) in white. Upper left shows low-power image of a mid-sagittal section. Lower left shows higher power image of cortex.

Images on right are individual color channels contributing to merged image in lower left. Right facing arrows indicate microglia expressing tdTomato (Iba1+; tdTomato+). Scale bar in low power image is equal to 5mm, in high power images is equal to 50 μ m. **c**, qPCR results of *Mecp2* expression from flow sorted microglia derived from wild-type (n=2), *Mecp2*^{lox-stop/Y} (n=2), and *Vav1-Cre; Mecp2*^{lox-stop/Y} (n=3) animals. **d**, Distance traveled in open field assay. **e**, Number of footslips per distance traveled on parallel rods. **f**, Breathing rate at baseline and during hypoxia challenge. **g**, Number of apneas per 10,000 breaths. **h**, Survival curve. c-h, Genotypes and labels: *Mecp2*^{+Y}=WT (n=8), black; *Vav1-Cre*^{Tg/+}=CRE (n=10), gray; *Mecp2*^{LSL/Y}=NR (n=12), orange; *Mecp2*^{LSL/Y}; *Vav1-Cre*^{Tg/+}=RESC (n=13), green Mean values are displayed, Error bars +/-SEM, *p<0.05.