Evaluation of MRM-3379, a PDE4D Inhibitor, in a Mouse Model of Fragile X Syndrome

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Introduction

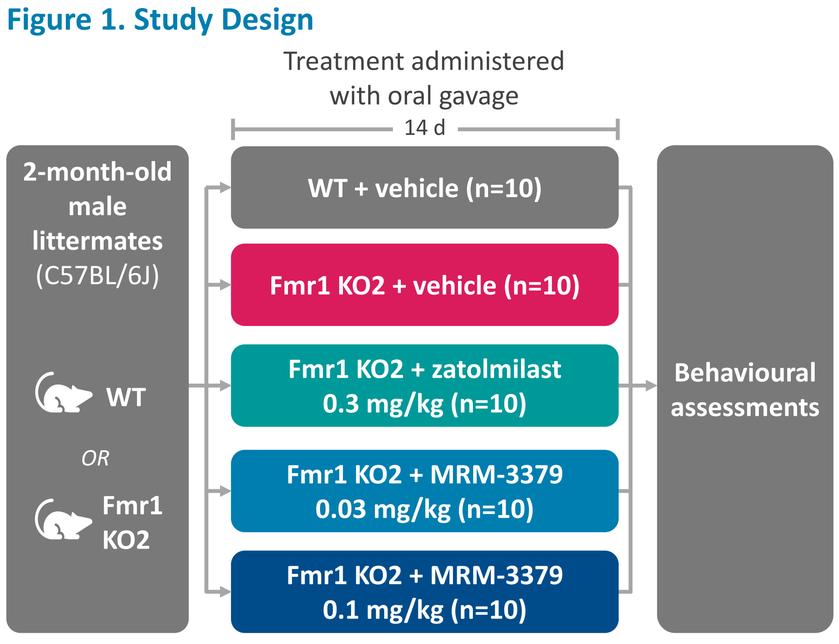
- Fragile X syndrome (FXS) is the most common form of inherited intellectual disability, and is associated with a range of developmental issues, including cognitive dysfunction, anxiety, and social and behavioural challenges.[1,2]
  - It is caused by a repeat expansion of the fragile X messenger ribonucleotide (*FMR1*) gene leading to transcriptional silencing and loss of expression of the associated protein, fragile X messenger ribonucleoprotein (FMRP).[1,3,4]
- The second-generation *Fmr1* knock-out (Fmr1 KO2) mouse model recapitulates FXS-related behavioural abnormalities, including age-dependent presentation, similar to what is observed in individuals with FXS.[5]
  - Fmr1 KO2 mice are FMRP mRNA and protein null.[6]
- Phosphodiesterase 4D (PDE4D) inhibitors are a promising therapeutic approach for the treatment of FXS, and work by enhancing cyclic adenosine monophosphate (cAMP) signalling, which is decreased in individuals with FXS.[1,7]
  - Fmr1 KO2 mice treated with the investigational PDE4D inhibitor zatolmilast showed improvements in behavioural measures compared with vehicle-treated mice.[7]
- There are currently no pharmacological agents approved to treat FXS.[2]
- MRM-3379 is an investigational allosteric inhibitor of PDE4D with high brain penetration.[8]

Objective

- To evaluate the effects of MRM-3379 on ameliorating the FXS phenotype in multiple behavioural assays in the Fmr1 KO2 mouse model.

Methods

Figure 1. Study Design



Behavioural phenotypes assessed:

Hyperactivity
Mouse placed in the corner of an open field. Distance travelled in 30 minutes was measured 30 minutes after treatment administration

Sociability
Mouse placed in a cage with one familiar (F) and one new (N) mouse. Amount of time spent with each mouse over 10 minutes was measured

Nesting
Quality of nest created overnight from pressed cotton was assessed with a 5-point scale[a]

Marble Burying
Ten marbles placed on bedding. The number buried within 30 minutes was measured

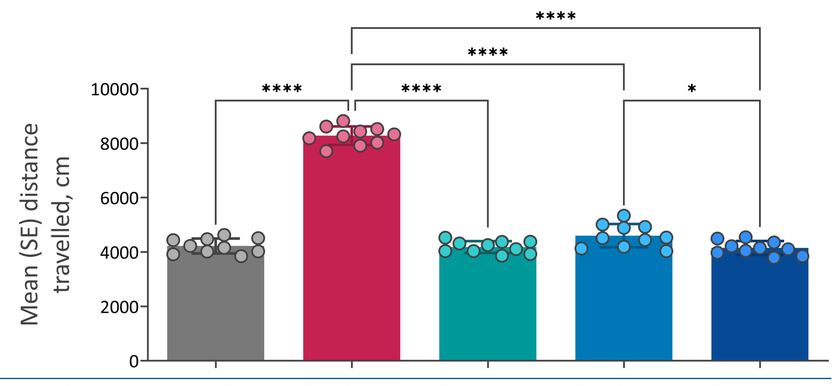
[a]The nest quality scale ranges from 1-5, with 1 indicating nestlet is largely untouched (>90% intact) and 5 indicating a (near) perfect nest (>90% of the nestlet is torn up, and the nest is a crater with walls higher than mouse body height on more than 50% of its circumference).

- Experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act, 1986.
- Data were analysed using 1-way analysis of variance (ANOVA) followed by pairwise post-hoc comparisons with Tukey's test when appropriate.

Results

Hyperactivity Phenotype Was Reversed in MRM-3379-Treated Fmr1 KO2 Mice

Figure 2. Distance Travelled in Open-Field Test



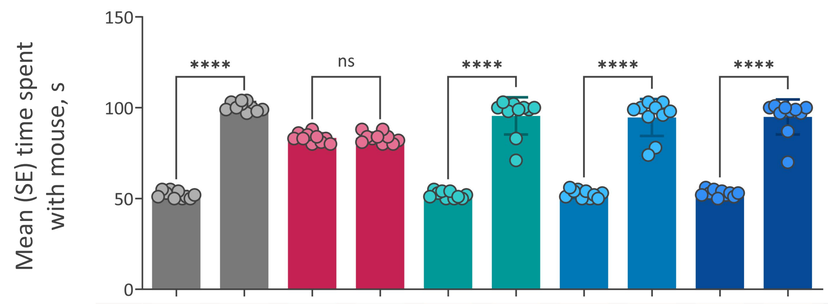
Mouse genotype	WT	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2
Vehicle	+	+	–	–	–
Zatolmilast, mg/kg	–	–	0.3	–	–
MRM-3379, mg/kg	–	–	–	0.03	0.1

* P=0.02; **** P<0.0001.

- Fmr1 KO2 mice travelled a greater distance compared with WT mice (P<0.0001).
- Fmr1 KO2 mice treated with MRM-3379 (both doses) travelled less distance compared with those treated with vehicle (both P<0.0001); similar results were observed for zatolmilast.
- After treatment with MRM-3379, the distance travelled by Fmr1 KO2 mice was not different from WT mice (ns).

Sociability Phenotype Was Reversed in MRM-3379-Treated Fmr1 KO2 Mice

Figure 3. Time Spent Interacting With Familiar vs New Mice



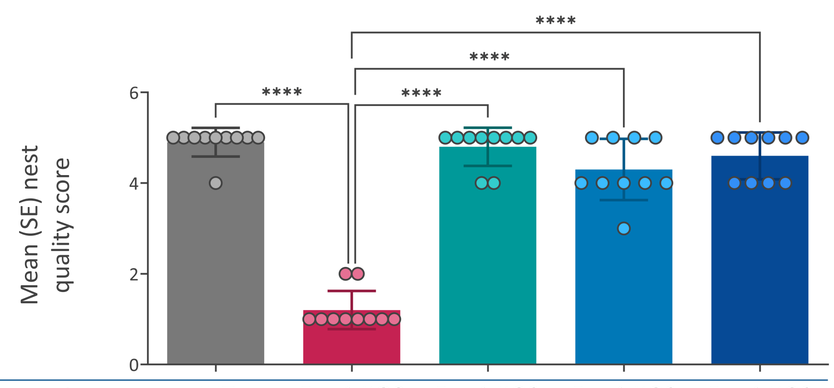
Social mouse type	F	N	F	N	F	N	F	N	F	N
Mouse genotype	WT		Fmr1 KO2		Fmr1 KO2		Fmr1 KO2		Fmr1 KO2	
Vehicle	+		+		–		–		–	
Zatolmilast, mg/kg	–		–		0.3		–		–	
MRM-3379, mg/kg	–		–		–		0.03		0.1	

**** P<0.0001.

- WT mice spent more time with a new mouse than a familiar mouse (P<0.0001); Fmr1 KO2 mice showed no preference.
- Fmr1 KO2 mice treated with MRM-3379 (both doses) spent more time with a new mouse than a familiar mouse (both P<0.0001); similar results were observed with zatolmilast.

Nesting Phenotype Was Reversed in MRM-3379-Treated Fmr1 KO2 Mice

Figure 4. Nest Quality Scores



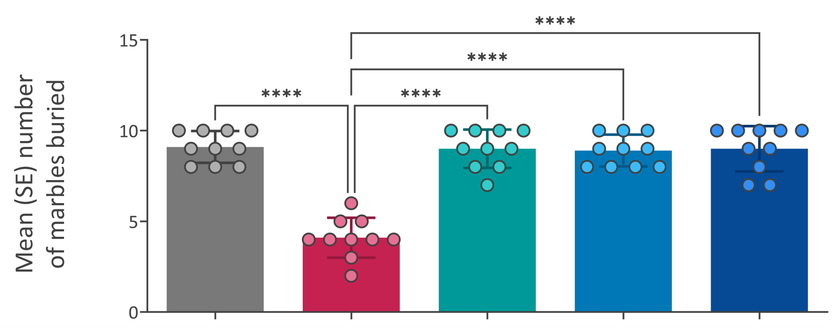
Mouse genotype	WT	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2
Vehicle	+	+	–	–	–
Zatolmilast, mg/kg	–	–	0.3	–	–
MRM-3379, mg/kg	–	–	–	0.03	0.1

**** P<0.0001.

- Fmr1 KO2 mice had lower nest quality scores compared with WT mice (P<0.0001).
- Fmr1 KO2 mice treated with MRM-3379 (both doses) had higher nest quality scores compared with those treated with vehicle (both P<0.0001); similar results were observed for zatolmilast.
- After treatment with MRM-3379, nest quality scores for Fmr1 KO2 mice were not different from WT mice (ns).

Marble Burying Phenotype Was Reversed in MRM-3379-Treated Fmr1 KO2 Mice

Figure 5. Number of Marbles Buried



Mouse genotype	WT	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2	Fmr1 KO2
Vehicle	+	+	–	–	–
Zatolmilast, mg/kg	–	–	0.3	–	–
MRM-3379, mg/kg	–	–	–	0.03	0.1

**** P<0.0001.

- Fmr1 KO2 mice buried fewer marbles compared with WT mice (P<0.0001).
- Fmr1 KO2 mice treated with MRM-3379 (both doses) buried more marbles compared to those treated with vehicle (both P<0.0001); similar results were observed for zatolmilast.
- After treatment with MRM-3379, the number of marbles buried by Fmr1 KO2 mice was not different from WT mice (ns).

Conclusions

- These findings demonstrate that MRM-3379 can improve multiple behavioural domains relevant to the FXS phenotype in a mouse model.
- MRM-3379 fully recapitulated WT activity in a mouse model of FXS at a 10-fold lower dose than zatolmilast.
- The consistent effect of MRM-3379 across doses and behavioural assays supports its potential as a therapeutic candidate for the treatment of FXS.

Abbreviations
ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; F, familiar; *FMR1*, fragile X messenger ribonucleotide; Fmr1 KO2, second-generation *Fmr1* knock-out; FMRP, fragile X messenger ribonucleoprotein; FXS, fragile X syndrome; N, new; ns, not significant; PDE4, phosphodiesterase 4; PDE4D, phosphodiesterase 4D; WT, wild-type.

Disclosures
LC, SH, and CK are employees of and shareholders in Mirum Pharmaceuticals, Inc. RJMD and PC have nothing to disclose.

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