Investigating progranulin overexpression in Fragile X Syndrome

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Introduction

Fragile X Syndrome (FXS) is caused by loss of expression of the gene *Fmr1* but the disease mechanisms are largely unknown.

Fragile X Syndrome

~~*Fmr1*~~ → ~~FMRP~~ → ? → Intellectual disability and autism

Fig.1

Progranulin (*Grn*), a gene encoding the neurotrophic factor GRN, is upregulated in the brains of mice with FXS. Reducing progranulin expression confers therapeutic benefit. *However, progranulin's role in FXS remains poorly understood.*

Questions:
1) Why is progranulin is increased in FXS?
2) How does increased progranulin expression contribute to FXS pathology?

Hypotheses

1) Progranulin expression is upregulated in a mouse model of Fragile X Syndrome (*Fmr1*KO mice) because FMRP is a negative regulator of progranulin translation.

2) Increased progranulin expression during development is sufficient to cause FXS-associated phenotypes.

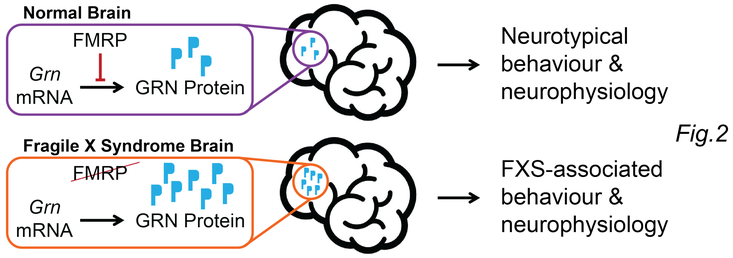

Fig.2

Approach and Methods

1. Investigating the mechanism of progranulin overexpression in FXS mice

Progranulin expression was evaluated in wild type and *Fmr1*KO mice at the RNA level by qPCR and the protein level by ELISA (**Fig.5**). To determine if FMRP interacts with progranulin mRNA, an RNA immunoprecipitation was performed (**Fig.6**) as shown below.

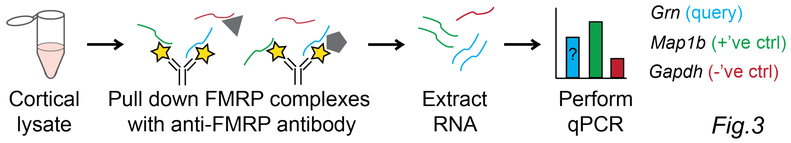

Fig.3

2. Characterization of progranulin overexpression (*Grn*^{OE}) mice

To assess the effect of increased progranulin expression on development, *Grn*^{OE} mice were characterized by ELISA (**Fig.7**) and behavioural analysis (**Fig.8**).

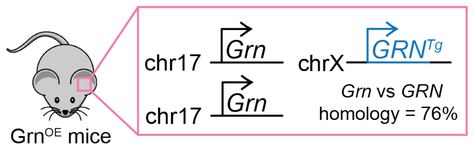

Fig.4

Results

1. *Grn* expression is upregulated post-transcriptionally in *Fmr1*KO mice

To gain insight into the mechanism of increased progranulin expression in *Fmr1*KO mice, progranulin expression was evaluated in wild type (WT) and *Fmr1*KO mice at the RNA and protein level by qPCR and ELISA, respectively.

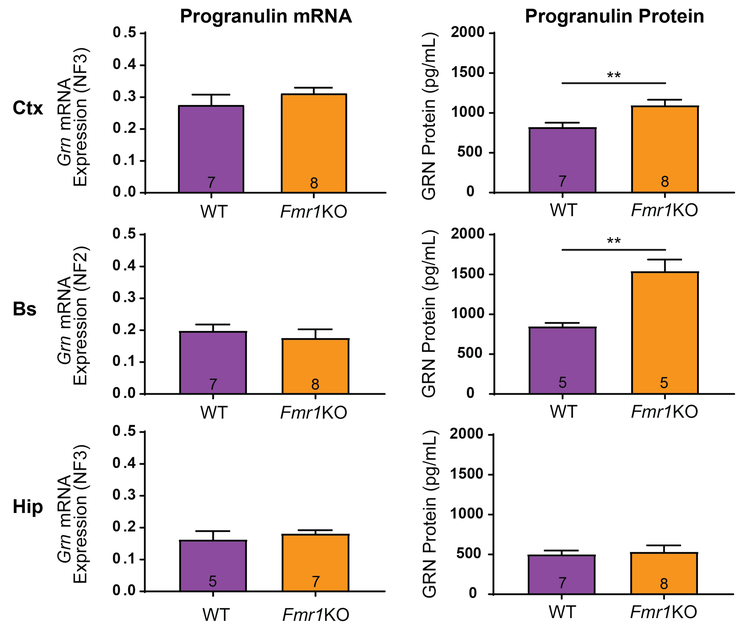

Figure 5: Progranulin expression in the cortex (Ctx), brainstem (Bs), and hippocampus (Hip) of 2-month-old male wild type and *Fmr1*KO mice. ** denotes p<0.01 by unpaired Mann-Whitney U Test.

2. FMRP interacts directly with *Grn* mRNA

Given that progranulin expression is increased post-transcriptionally in the absence of FMRP, we sought to determine if progranulin mRNA is a target of FMRP by determining if these two species interact using an RNA immunoprecipitation.

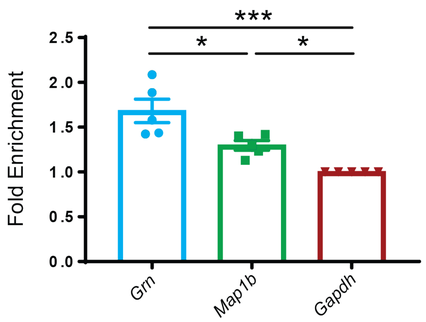

Figure 6: FMRP RNA immuno-precipitation of n=5 post-natal day 13 male mouse cortices.

mRNA levels of *Grn*, *Map1b*, and *Gapdh* are reported as enrichment relative to *Gapdh*.

* denotes P<0.05, *** denotes P<0.001 by one-way ANOVA.

3. *Grn*^{OE} mice express increased progranulin relative to wild type mice

To determine the extent of increased progranulin expression in the *Grn*^{OE} mice, we compared mouse and human progranulin expression in wild type and *Grn*^{OE} mice.

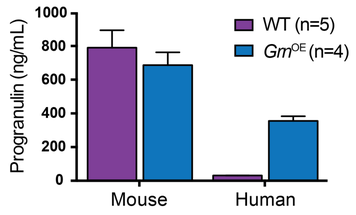

Figure 7: Mouse and human progranulin expression in the plasma of 2-month male wild type and *Grn*^{OE} mice by ELISA.

4. Increased progranulin expression causes hyperactivity in mice without other FXS-associated phenotypes

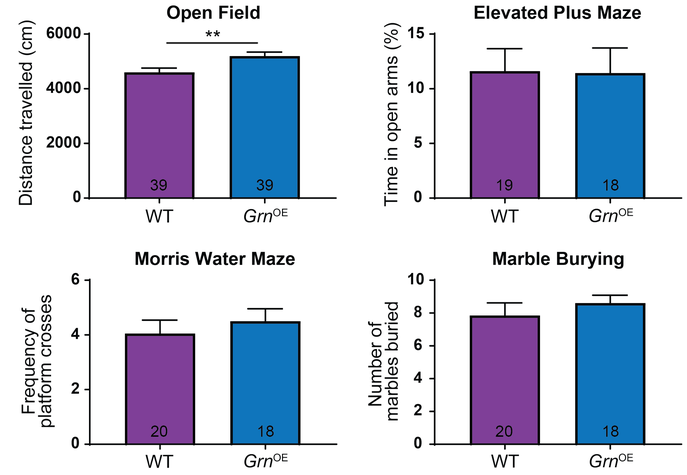

Figure 8: FXS-associated behavioural phenotypes were evaluated in 2-6 month wild type and *Grn*^{OE} mice. ** denotes p<0.01 by unpaired Mann-Whitney U Test.

Conclusions

1) Progranulin overexpression in *Fmr1*KO mice is post-transcriptional & tissue specific.
2) Progranulin mRNA interacts with FMRP, suggesting that progranulin mRNA is a target of FMRP.
3) While increased progranulin expression may be sufficient to cause hyperactivity, it is not sufficient to fully recapitulate FXS-associated behavioural phenotypes.
4) Characterization of *Grn*^{OE} mouse electrophysiology and synaptic morphology is needed to understand the neurodevelopmental consequences of increased progranulin expression

Acknowledgements

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