Upstream factors of microrna identified through CC mice are involved in transcriptional regulation and neurogenesis of mNPCs

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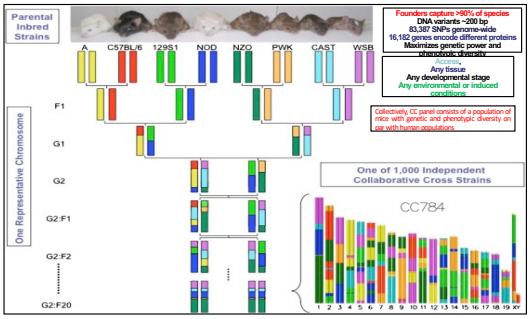
INTRODUCTION

Collaborative cross mouse model

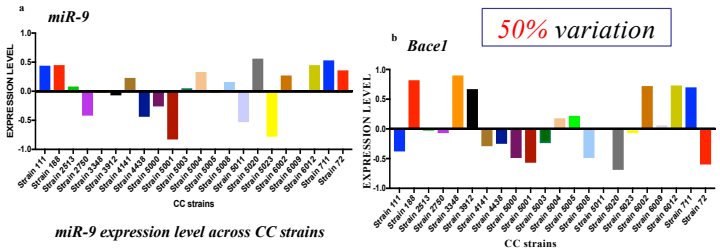


METHODS

1. Validating CC mice as a model to study genetic diversity
2. Identifying upstream multiple factors through QTL mapping, siRNA knockdown, miRNA expression change and Bioinformatics
3. Functional validation of Panx2, Mgea5 and Polr1c through miR-9 pathway in neuronal differentiation
4. Identify mechanism of upstream regulation of Panx2, Mgea5 and Polr1c in miR-9 signaling using ChIP-seq, 3C, ChIP loop and Luciferase Reporter assay

RESULTS

Using CC mice as a model to study genetic diversity

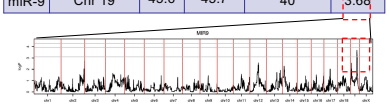


miR-9 expression level across CC strains

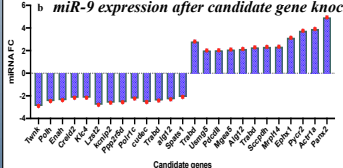
QTL mapping of the CC strains uncovers loci controlling miRNA expression

a ***QTL mapping of miR-9***

QTL	Chromosome	Start (Mb)	End (Mb)	Number of candidate genes	LOD scores
miR-9	Chr 19	45.6	45.7	40	3.68



b ***miR-9 expression after candidate gene knockdown***

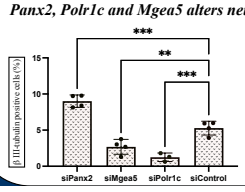


c ***Gene ontology analysis using DAVID***

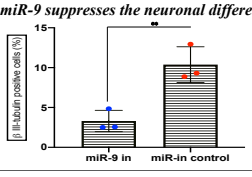
Gene name	GO Terms
Trabd	-
Ephx1	Enzyme binding (RNA Polymerase binding)
Sccpdh	Oxidoreductase activity
Nt5c2	5'-nucleotidase activity, metal ion binding, nucleotide binding (RNA binding)
Mrpl14	Ribonucleoprotein, Ribosomal protein
Ahctf1	DNA binding, DNA-binding transcription factor activity, mRNA transport
Polr1c	DNA binding, DNA-directed 5'-3' RNA polymerase activity
Cuedc2	Protein binding
Panx2	Gap junction channel activity
Creld2	calcium ion binding
Lzts2	Protein binding, Wnt signalling pathway
Mgea5	Histone acetyltransferase activity
Pdcd11	DNA binding, sequence-specific DNA binding transcription factor activity

Functional validation of Panx2, Mgea5 and Polr1c in neuronal differentiation

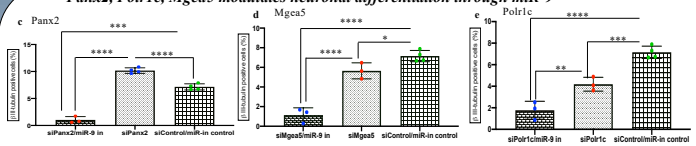
a ***Panx2, Polr1c and Mgea5 alters neuronal differentiation***



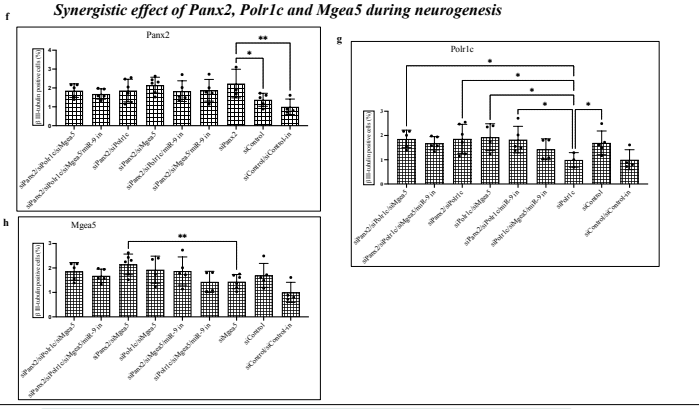
b ***miR-9 suppresses the neuronal differentiation***



Panx2, Polr1c, Mgea5 modulates neuronal differentiation through miR-9

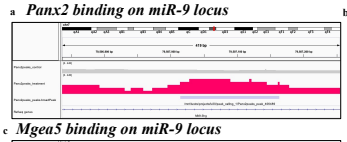


Synergistic effect of Panx2, Polr1c and Mgea5 during neurogenesis

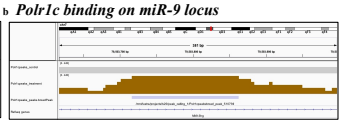


Recruitment of Panx2 Polr1c and Mgea5 on miR-9 genomic locus

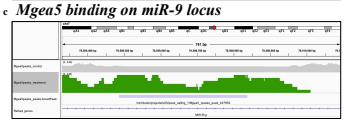
a ***Panx2 binding on miR-9 locus***



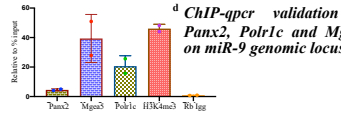
b ***Polr1c binding on miR-9 locus***



c ***Mgea5 binding on miR-9 locus***

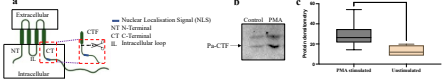


d ***ChIP-qpcr validation of Panx2, Polr1c and Mgea5 on miR-9 genomic locus***

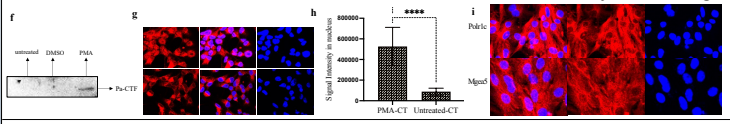


Nuclear signalling by Panx2, Polr1c and Mgea5

Translocation of Panx2-CTF to nucleus



Nuclear localization of Polr1c and Mgea5

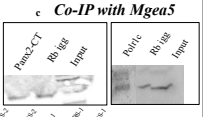


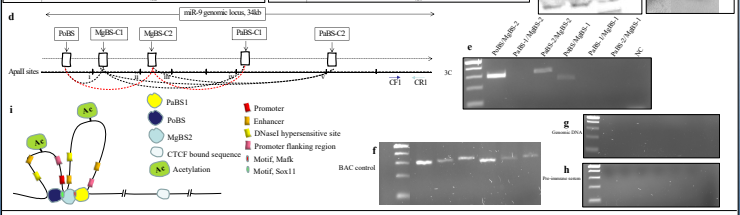
Upstream regulation of Panx2, Mgea5 and Polr1c

a ***Panx2 binding on Mgea5 locus***

b ***Polr1c binding on Mgea5 locus***

c ***Co-IP with Mgea5***





CONCLUSIONS

I. *CC mice as a useful tool to study genetic diversity*

II. *Panx2 has an additive effect whereas Polr1c and Mgea5 act synergistically in neuronal differentiation*

III. *Panx2, Polr1c and Mgea5 is recruited to the miR-9 genomic locus*

IV. *Nuclear signaling translocates Panx2-CTF to the nucleus*

V. *Panx2, Polr1c and Mgea5 forms chromatin associated loop to regulate transcription of miR-9*

Novel multigenetic factors Panx2, Polr1c and Mgea5 through miR-9 signalling using CC mice for NDD research