THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

TRANSCRIPTOME ANALYSIS OF HETEROZYGOUS RBM8A CONDITIONAL KNOCKOUT MICE

MIRANDA CHEN
SPRING 2019

A thesis submitted in partial fulfillment
of the requirements for a baccalaureate degree in Science
with honors in Biology

Reviewed and approved* by the following:

Yingwei Mao
Associate Professor of Biology
Thesis Supervisor

Bernard Luscher
Professor of Biology and Biochemistry and Molecular Biology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

RBM8A is a component of the exon junction complex (EJC), a complex of four core proteins that binds pre-mRNAs and influences their splicing, transport, and nonsense-mediated decay. Dysfunction in the core proteins has been linked to several detriments in brain development. In this study, we used next-generation RNA-sequencing to identify differentially-expressed genes (DEGs) in mice with heterozygous, conditional knockout (hcKO) of the homolog *Rbm8a* in the brain at embryonic day 12 and at postnatal day 17, in order to understand the functional role of *Rbm8a* in brain development. Additionally, we analyzed enriched gene clusters and signaling pathways within the DEGs. At the E12 time point, between the hcKO and WT mice, about 100 significant DEGs showed >2-fold change, among which we identified 20 genes related to the central nervous system (CNS). When E12 and P17 results were compared, three DEGs, *Spp1*, *Gpnmb*, and *Top2a*, appeared to peak at different developmental time points in the *Rbm8a* hcKO mice. *Spp1* and *Gpnmb* regulate bone development, while *Top2a* is specific to embryonic CNS and directly participates in DNA replication. *Fam212b*, significantly upregulated in all tested conditions, is expressed by neuronal progenitor cells (NPCs) and postnatally expressed by immature neuronal subpopulations in the cerebrum and other limited brain regions. Further observations are necessary to determine these genes' roles in CNS development. Enrichment analyses suggested altered activity in pathways affecting cellular proliferation, differentiation, and survival. The results support the hypothesis that loss of *Rbm8a* causes decreased cellular proliferation and early differentiation of neuronal subtypes, which may lead ultimately to an altered neuronal subtype composition in the brain.

TABLE OF CONTENTS

LIST OF FIGURES iii

LIST OF TABLES iv

ACKNOWLEDGEMENTS v

LIST OF ABBREVIATIONS vi

Chapter 1 Introduction 1

1.1 RNA Processing, Stabilization, and Degradation in the Mammalian Cell 1
1.2 *Rbm8a* hcKO Mouse Model 2
1.3 RNA-sequencing: Next-Generation Sequencing 4

Chapter 2 Materials and Methods 6

2.1 RNA-sequencing 6
2.2 Analysis of DEGs 6
2.3 Analysis of Enriched Gene Clusters 7

Chapter 3 Results 9

3.2 DEG Analysis in the E12 Cortex 9
3.3 DEG Analysis in the E12 Hindbrain 14
3.4 DEG Analysis of the Whole Brain at P17 17
3.5 Analysis of Enriched Pathways and Gene Clusters 21

Chapter 4 Discussion 26

4.1 DEG Analysis 26
4.2 GO Enrichment Analysis 28

BIBLIOGRAPHY 30

LIST OF FIGURES

Figure 1: *Rbm8a* targeting vector, selection, and knockout mechanism. 4

Figure 2: Volcano plot for all quantifiable expressional changes in the cortex of *Rbm8a* hcKO mice at E12. 11

Figure 3: Heat map of RNA transcript readings for all known genes with significant expressional changes in the E12 hcKO mouse cortex ($q < 0.05$). 12

Figure 4: Volcano plot for all quantifiable expressional changes in the hindbrain of *Rbm8a* hcKO mice at E12. 15

Figure 5: Heat map of RNA transcript readings for all genes with significant expressional changes in the E12 hcKO mouse hindbrain ($q < 0.05$). 16

Figure 6: Volcano plot for all quantifiable expressional changes in a whole-brain comparison between WT and *Rbm8a* hcKO mice at P17. 20

Figure 7: Overlaps between significant DEGs of the E12 cortex, E12 hindbrain, ad P17 whole brain. 21

Figure 8: Upregulated and downregulated gene clusters in *Rbm8a* hcKO mice at E12. 24

Figure 9: Upregulated and downregulated gene clusters in *Rbm8a* hcKO mice at P17. 25

LIST OF TABLES

Table 1: Gene Ontology Enrichment Analysis settings. 8

Table 2: CNS-related DEGs observed in the cortex of E12 *Rbm8a* hcKO mice. 13

Table 3: Common upregulated DEGs between the cortex and hindbrain regions at E12. 17

Table 4: CNS-related DEGs in the *Rbm8a* hcKO brain at P17. 20

ACKNOWLEDGEMENTS

First and foremost, I thank my family for their constant, enthusiastic support, and for believing in me when I lost sight of my own progress as a science student. My sister Adele has been a lifelong friend as well as the one person who won't hold back from challenging me. She has kept me sharp, and I'm glad for it. My parents have been my role models in the scientific community from the moment I set foot in undergraduate research. Dr. Chen, my dad, instilled in me an early interest in diseases and disorders of the brain, and as a volunteer in his lab, I mastered my first basic lab techniques.

I would like to acknowledge Dr. Yingwei Mao, Dr. Colleen McSweeney, and the former members of the Mao lab for laying the groundwork that made this thesis possible. I am especially grateful for Dr. Mao's patience and reassurance while I reworked my approach to writing this thesis over and over again. Finally, I thank Fengping Dong for not only teaching me a broad skill set in the lab setting, but also making sure I understood the basis of every step of the experiments I performed. It is an honor to deliver this thesis as a member of the Mao Lab, and I hope that my work will be helpful to our future research in brain development.

LIST OF ABBREVIATIONS

Abbreviation	Full Name
Abcd2	ATP-binding cassette D2
Ano3	Anoctamin 3
Caln1	Calneruon 1
Caly	Calcyon neuron-specific vesicular protein
Casc3	Cancer susceptibility candidate 3
Ccng1	Cyclin G1
Cdkn1a	Cyclin-dependent kinase inhibitor 1A
hcKO	Heterozygous, conditional knockout
CNS	Central Nervous system
DEG	Differentially-expressed gene
Diras2	DIRAS family, GTP-binding Ras-like 2
E12	Embryonic day 12
Eda2r	Ectodysplasin A2 receptor
EIF4A3	Eukaryotic translation initiation factor 4A3
EJC	Exon junction complex
En2	Engrailed 2
Ephx1	Microsomal epoxide hydrolase 1
ESC	Embryonic stem cell
Etv4	Ets variant 4
Fam19a1	TAFA chemokine like family 1
Fam212b	Inka box actin regulator 2
Folr1	Folate receptor 1
Gda	Guanine deaminase
Gfap	Glial fibrillary acidic protein
GO	Gene Ontology
Gpnmb	Glycoprotein nmb
Hpcal4	Hippocalcin-like 4
Kcnc4	K^+ voltage-gated channel, Shaw-related subfamily 4

Lhx8	LIM homeobox protein 8
Lilrb4a	Leukocyte immunoglobulin-like receptor B4A
Lmx1a	LIM homeobox transcription factor 1a
Mal2	Mal, T cell differentiation protein 2
Neurod1	Neuronal differentiation 1
NPC	Neural progenitor cell
Nrgn	Neurogranin
Otx2	Orthodenticle homeobox 2
P17	Postnatal day 17
Pcdh11x	Protocadherin 11 X-linked
Phlda3	Pleckstrin homology like domain A3
Rbm8a	RNA-binding motif 8a
Sesn2	Sestrin 2
Sncb	Synuclein beta
Spp1	Secreted phosphoprotein 1
Sst	Somatostatin
Thy1	Thymus cell antigen 1 theta
Top2a	Topoisomerase II alpha
Vgf	VGF nerve growth factor inducible
Vsnl1	Visinin-like 1
Zfp365	Zinc finger protein 365

Chapter 1

Introduction

1.1 RNA Processing, Stabilization, and Degradation in the Mammalian Cell

RNA transcripts are modified by various processes before they are mature and functional. Fresh transcripts of pre-mRNA usually receive extensive splicing, a polyadenylation tail at the 3' end, and modified guanosine nucleotide cap at the 5' end before becoming translatable, mature mRNA.[1,2] Base modifications at certain positions serve to regulate their transport, stability, and binding by other regulatory factors.[2] Meanwhile, non-coding RNA, tRNA, and rRNA are also processed after their initial transcription, often by a dynamic suite of base modifications at specific sites in each transcript that affect their folding and stability.[2] A diverse collection of proteins carry out these different RNA modifications within the cell.

RBM8A, for RNA-binding motif 8A, is a protein that was first identified by its RNA-binding sequence.[3] RBM8A participates in an assembly of proteins known as the exon junction complex (EJC), which also contains the core protein factors eukaryotic translation initiation factor 4A3 (EIF4A3), Magoh, and cancer susceptibility candidate 3 (Casc3), and may additionally associate with other factors.[4] The EJC and its general functions are conserved across a wide range of species, with homologs being studied in *Drosophila* and murine models. Spliceosomes, which splice pre-mRNA in the nucleus, assemble the EJC on spliced pre-mRNA. The EJC can then direct further splicing, slow down transcription of the rest of the gene, or regulate mRNA transport and translation when it accompanies the mature transcript out of the nucleus.[5] In addition to binding

and modifying transcripts, the EJC has been shown to participate in nonsense-mediated decay of mRNA, which identifies mRNA with premature stop codons during translation and causes the faulty mRNA to be degraded.[3,4] The four components of the EJC also have their independent roles and binding targets when not in complex.[5] Therefore, RBM8A must have yet-unknown roles in the cell.

Our lab previously demonstrated that the mouse homolog *Rbm8a* is involved in regulating the neural progenitor cell (NPC) population and that genes downstream of *Rbm8a* expression include risk genes for intellectual disability, schizophrenia, and autism spectrum disorder.[6] We hypothesized that dysfunction of *Rbm8a* causes abnormal development of the brain at the embryonic stage, specifically by decreasing NPC renewal and increasing differentiation.[6] In this study, we analyze the changes in the transcriptome of mice with *Rbm8a* haploinsufficiency in the brain during embryonic and postnatal stages to identify functional targets of *Rbm8a* during early brain development.

1.2 *Rbm8a* hcKO Mouse Model

Recent discoveries have allowed complicated, precise manipulation of genes in model organisms. The hcKO method in this study is based on the Cre/lox and FLP/FRT systems. Cre and FLP recombinases are both site-specific, recognizing lox and FRT sites, respectively. Depending on the orientation of two lox sites, Cre recombinase can invert the direction of DNA between them, excise the region (leaving behind one lox site), or translocate the ends of two chromosomes at those sites.[7] FLP recombinase similarly acts on FRT sequences.

Previously, Colleen McSweeney and other lab members generated mice for hcKO of *Rbm8a* using FLP/FRT, Cre/loxP, and embryonic stem cell (ESC) microinjection. The mouse line produced was homozygous for our modified allele, $Rbm8a^{fl}$, on a C57BL/6 background.[8] The $Rbm8a^{fl}$ allele contains loxP sites that guide Cre recombinase to make a large excision in the gene, removing three exons and disabling it (Figure 1).

To create the *Rbm8a* hcKO mice, the $Rbm8a^{fl/fl}$ mice were crossed with nestin-Cre (Nes-Cre) transgenic mice from the Jackson Laboratory, B6.Cg-Tg(Nes-cre)1Kln/J, stock number 003771. The *Nes-Cre* mouse line has hemizygous Cre recombinase driven by a nestin promoter. Nestin has heavily biased expression in embryonic neural stem cells, allowing nervous system-specific expression of Cre at early embryonic day 10 (E10). [8]

McSweeney reported that mouse pups produced from the crossed *Nes-Cre* and $Rbm8a^{fl/fl}$ lines exhibited either typical, wild-type-like growth or severe underdevelopment. Wild-type (WT) offspring had a normal copy of *Rbm8a* and the modified but functional $Rbm8a^{fl}$. The underdeveloped, hcKO offspring had only one functional copy of the gene in their nervous system, as Cre recombinase succeeded in disrupting the $Rbm8a^{fl}$ copy. Most of these hcKO pups only survived until postnatal day 20 (P20). They had measurably lower body mass, lower brain mass, and shorter body length. A large, visible gap between the two cerebral hemispheres was typical of the hcKO brains, in contrast to the tightly aligned hemispheres in the WT brains.[8] Cre-disruption of both copies of *Rbm8a* was embryonic-lethal,[8] so we were unable to study the effects of homozygous *Rbm8a* knockout using this mouse model.

Based on our preliminary observations of the severe, physical defects in the hcKO mice, we decided to investigate the gene expressional changes that might contribute to the abnormal development of the brain downstream of *Rbm8a* hcKO.

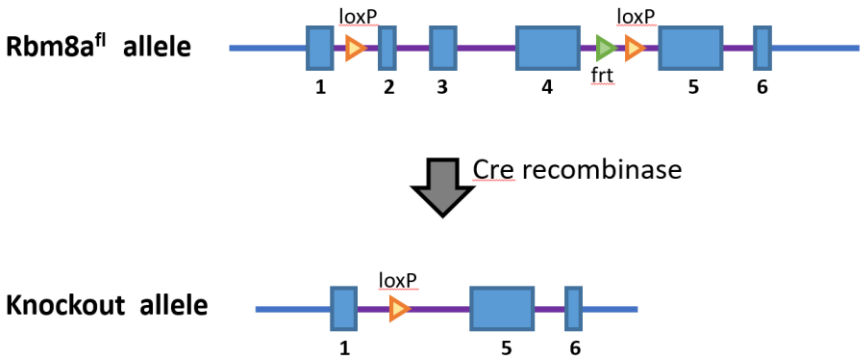

Figure 1: *Rbm8a* targeting vector, selection, and knockout mechanism.
The above diagram presents a simple overview of the disruption of the *Rbm8a*^{fl} allele. Cre recombinase excises exons 2, 3, and 4, disabling the gene. Adapted from reference.[8]

1.3 RNA-sequencing: Next-Generation Sequencing

To continue exploring the roles of *Rbm8a*, we turned to transcriptome analysis to find genes and other transcripts that are regulated downstream of *Rbm8a*. We sequenced the transcriptomes of WT and *Rbm8a* hcKO mice at embryonic and early postnatal time points using next-generation sequencing.

With the advent of next-generation RNA-sequencing technology, it became possible to analyze entire RNA transcriptomes of cells and tissues. The technology used in this study was Illumina HiSeq, designed to generate large data sets, capable of reading millions of sequences at a time.[9] Additionally, most RNA-sequencing experiments now use paired-end protocols, sequencing both the 3' end and the 5' end of the template fragment.[9,10] For RNA transcripts that can be spanned by overlapping, paired reads, this means the entire transcript can be sequenced. For longer transcripts, paired-end analysis doubles the reads and sequence coverage per fragment. In the

context of this study, this allows sequenced transcripts to be more precisely aligned to reference genomes.

The goal of this study was to find genes and pathways regulated by *Rbm8a* during early brain development, inferred from the differential expression of certain RNA transcripts when one copy of *Rbm8a* was lost. We identified over 300 transcripts that showed significant fold changes between WT and *Rbm8a* hcKO mice, including 34 genes with known functions in nervous system development. This provides a starting point for choosing a narrower subset of genes or cellular processes to observe in future studies. There is evidence that cell proliferation is decreased in the embryonic brains of *Rbm8a* hcKO mice, especially in NPCs, which likely contributes to the underdeveloped phenotype of these mice. We further observed that neural transcription factors were upregulated in the early postnatal brain, accompanied by gene expression typically associated with mature neurons in the adult brain. Considering these results, we believe that *Rbm8a* is required to delay cell differentiation and maturation, allowing the precursor cells of the nervous system to proliferate and fully populate their organs.

Chapter 2

Materials and Methods

2.1 RNA-sequencing

Sample preparation for RNA-sequencing was done by Dr. Yingwei Mao. Eight mouse embryos at E12 were collected for RNA-sequencing. Four of them were WT and four of them were *Rbm8a* hcKO. The hindbrain and cortex regions were dissected from the rest of the brain and stored separately. Six more mice, three for each condition, were euthanized at postnatal day 17 (P17); their whole brains were collected. These three sets of brain samples were sent to the Penn State Genomics Core Facility for sequencing with the Illumina HiSeq 2500 on a paired-read protocol. 20 million paired reads were run per sample, producing 40 million total reads per sample. Raw reads were processed with paired-end analysis.

2.2 Analysis of DEGs

Three sequencing data sets were obtained, corresponding to the E12 cortex, E12 hindbrain, and P17 whole brain. The raw Illumina output was processed by the Penn State Bioinformatics Consulting Center, in collaboration with Dr. István Albert. Using TopHat, reads were aligned to the NCBI *Mus musculus* genome, assembly GRCm38.p6, available to the public through the NCBI

Genome database. Subsequently, Cuffdiff was used to calculate statistical significance of expressional changes.[11]

One E12 hcKO mouse's data was not included in the analysis because it had very different read counts than the other three. The rest of the data was sorted to rank differential expression of genes by their p-values and p-adjusted values (q-values.)

After sorting DEGs by significance, DEGs were compared between the E12 cortex and hindbrain regions, as well as between the E12 and P17 time points. We identified genes that were significant at $q < 0.05$ in both conditions being compared, and noted whether these shared DEGs had changed in the same direction.

E12 cortex and P17 DEGs were further sorted to distinguish those pertinent to the CNS, to establish targets of interest for further investigation in *Rbm8a* hcKO animals. The CNS-related DEGs of the E12 cortex were categorized manually, based on literature reports of their known functions and expressional patterns. This was less feasible for the large number of DEGs at P17, so we used the Gene Ontology (GO) Enrichment Analysis tool to classify CNS-related genes DEGs in the P17 data. (Because the analysis found CNS-related genes to be enriched, it named all CNS-related genes it recognized from the submitted DEGs.)

2.3 Analysis of Enriched Gene Clusters

Overrepresented gene clusters and pathways were identified among significantly upregulated and downregulated DEGs using the Gene Ontology Consortium's free online resource, GO Enrichment Analysis.[12,13,14] GO Enrichment Analysis groups genes by function and pathway, then estimates how many genes from each group are expected in a list of a given number

of genes. If the actual number of genes from the same group significantly exceeds the expected number, then that group of genes is determined to be enriched. The software requires an input list with a sufficient number of genes to accurately identify gene cluster enrichments. We began by inputting the DEGs significant at $q < 0.05$, and if this was not sufficient, expanded the input to include DEGs significant at $p < 0.01$. The E12 cortex and hindbrain and the P17 whole brain were analyzed individually, with inputted DEGs further separated by direction of change (upregulation or downregulation.) GO Analysis uses the PANTHER Overrepresentation Test to recognize gene cluster enrichments. The specific settings used in this study are shown in Table 1.

Table 1: Gene Ontology Enrichment Analysis settings.

Analysis Type	PANTHER Overrepresentation Test
Annotation Version and Release Date	GO Ontology Database, released 01 January 2019
Reference List	Mus musculus (all genes in database)
Annotation Data Set	GO biological process, complete
Test Type	Fisher's Exact
Correction	Bonferroni correction for multiple testing

Chapter 3

Results

Using RNA-seq, we were able to identify over 300 genes that were significantly up- or down-regulated ($q < 0.05$) at some point during development of *Rbm8a* hcKO mice in comparison to WT mice. In the E12 cortex and P17 whole brain, we also distinguished DEGs that were CNS-related in function or localization, which may be more closely observed in further experiments with *Rbm8a* knockout models. Finally, we used GO Enrichment Analysis to identify gene clusters that were relatively enriched or depleted. Enrichment analysis revealed which overarching pathways and processes were likely to be affected by *Rbm8a* haploinsufficiency.

3.2 DEG Analysis in the E12 Cortex

In the E12 RNA-seq samples, reads from the Illumina HiSeq 2500 were mappable to about 50,000 known transcripts in the mouse genome. A volcano plot was generated to display all genes that had quantifiable transcript readings in both the WT and hcKO cortices (Figure 2). This accounts for about 28,000 genes, microRNAs, and long non-coding RNAs (lncRNAs), and covers more than half of the referenced transcripts. In the E12 cortex, 430 genes showed altered expression with significant p-values ($p < 0.01$) and 87 DEGs genes showed altered expression of at least two-fold with significant q-values ($q < 0.05$). Of these 87 DEGs, 73 had previously-

identified functions. The original RNA transcript readings for these 73 DEGs are compared between the WT and KO mice (Figure 3).

20 of the DEGs were identified to be relevant to the central nervous system (CNS), in function and/or expression (Table 2). The two downregulated genes among these were *Abcd2* and *Top2a*. *Abcd2* is a membrane transporter that is involved in production and/or maintenance of the myelin sheath,[15] and *Top2a* is a topoisomerase, a protein that directly participates in DNA replication, specifically expressed in the CNS.[16] Meanwhile, another gene involved in myelin maintenance, *Mal2*,[17] is upregulated more than two-fold.

Two CNS-related DEGs were upregulated more than three-fold. These were *Kcnc4*, a voltage-gated potassium ion channel,[18] and *Gda*, a guanine deaminase concentrated in the brain that regulates microtubule assembly.[19] *Kcnc4* conducts potassium outward following an action potential, restoring the membrane to resting state.[20] An increased expression of this channel could indicate that the *Rbm8a* hcKO embryos had abnormal action potentials. Likewise, the increase in *Gda* expression could indicate a misregulation of the cytoskeleton or an increase in cellular outgrowths.

Among the other upregulated, CNS-related DEGs, *Hpcal4*, *Caln1*, and *Nrgn* are involved in calcium-dependent signaling pathways in the neuronal cytoplasm. *Hpcal4* and *Caln1* directly bind Ca^{2+}, potentially acting as calcium sensors in the presynaptic terminal.[22,23] *Nrgn* binds calmodulin at synapses and may participate in long-term potentiation (LTP).[24] This suggests that *Rbm8a* might influence both immediate synaptic transmission and long-term synaptic excitability by regulating genes involved in calcium signaling.

In summary, in the E12 mouse cortex, we observed changes in gene expression relevant to myelination, DNA replication, and Ca^{2+} signal transduction.

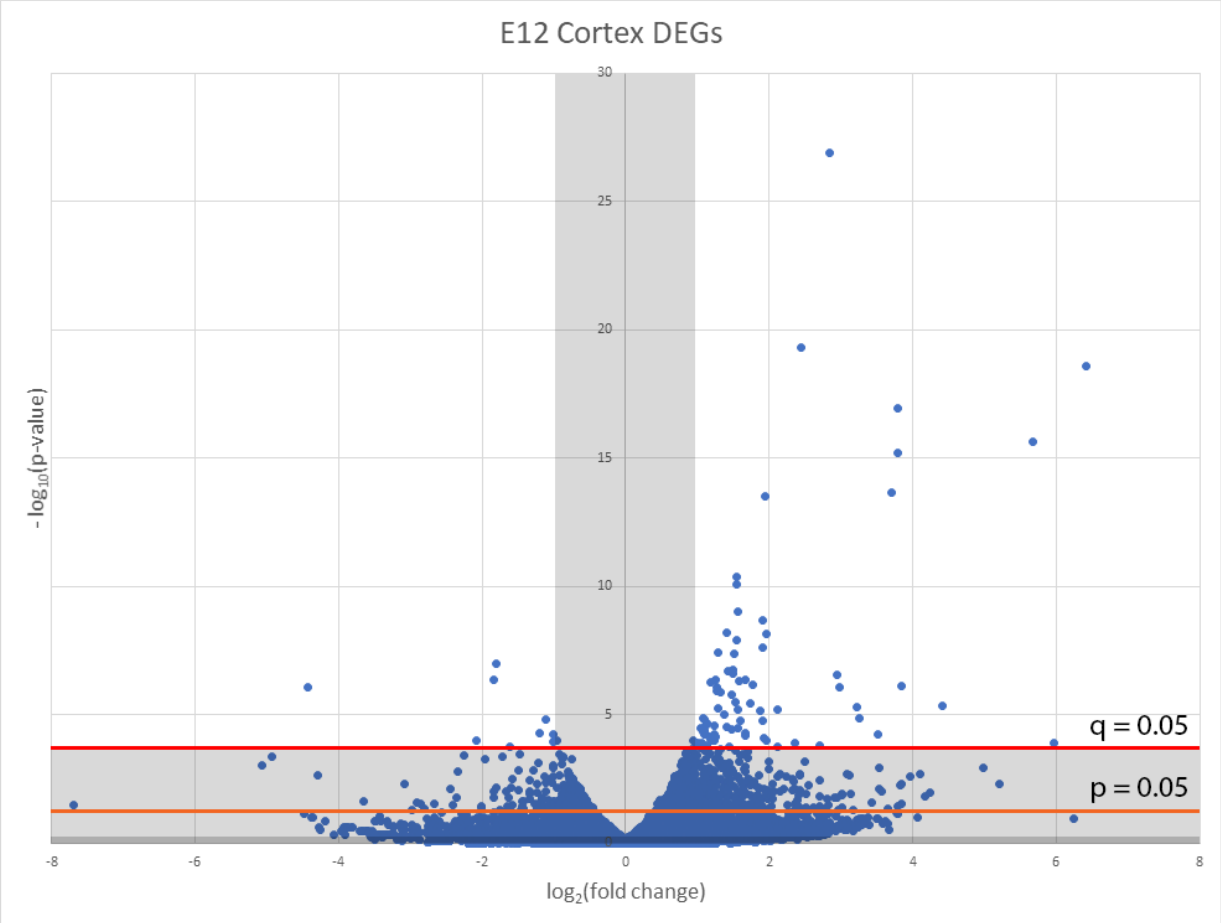

Figure 2: Volcano plot for all quantifiable expressional changes in the cortex of *Rbm8a* hcKO mice at E12.
28,117 genes' transcripts were detected in the RNA-seq data. 1,073 genes were expressed differently with $p < 0.05$, 430 genes were expressed differently with $p < 0.01$, and 87 genes were expressed differently with $q < 0.05$. 84 DEGs with significant q-values were up- or downregulated at least two-fold. The p and q cutoffs are shown.

Figure 3: Heat map of RNA transcript readings for all known genes with significant expressional changes in the E12 hcKO mouse cortex (q < 0.05).
From the RNA-seq data, the transcript counts of each gene were compared between the six mice. Of the 87 DEGs, 14 remain uncharacterized and have been excluded from the figure.

Table 2: CNS-related DEGs observed in the cortex of E12 *Rbm8a* hcKO mice.
20 out of 87 DEGs in the E12 cortex were identified to be relevant to CNS development or function and have fold changes of two or greater. Their names, fold changes, and pathways are listed.

Gene ID	Gene name	Fold change	Pathway
Abcd2	ATP-binding cassette D2	0.462	ABC transporter; imports very long fatty acyl-CoA chains into peroxisomes. Likely involved in maintaining the myelin sheath. [15]
Top2a	Topoisomerase II alpha	0.518	A DNA topoisomerase highly expressed in embryonic CNS, especially in rapidly-proliferating cells. [16]
Pcdh11x	Protocadherin 11 X-linked	1.912	The X chromosome copy of *Pcdh11*. The protocadherin pair *Pcdh11x/y* appears to influence asymmetrical development of the human cerebrum. [24]
Caly	Calcyon neuron-specific vesicular protein	2.059	Binds clathrin, localized in synapses and other membranes, regulated endo/exocytosis. Likely affects neurotransmitter release. [26]
Sst	Somatostatin	2.139	A metabolic hormone. In interneurons, *Sst* regulates synaptic plasticity and excitability. [26]
Hpcal4	Hippocalcin-like 4	2.143	Binds Ca^{2+}; extremely biased expression in the adult cortex and frontal lobe. [22]
Etv4	Ets variant 4	2.235	Necessary for dendritic development in some neuronal populations. [27]
Mal2	Mal, T cell differentiation protein 2	2.300	A proteolipid in myelin that regulates protein distribution in the membrane. [17]
Fam19a1	TAFA chemokine like family 1	2.361	Function uncertain. High, biased expression throughout the CNS.[28]
Sncb	Synuclein beta	2.374	Synucleins participate in neurotransmitter turnover processes at synapses. Deficiencies can cause decreased dopamine levels and loss of motor control in older mice.[29]
Diras2	DIRAS family, GTP-binding Ras-like 2	2.415	A Ras GTPase, highly expressed in adult cerebellum [20]
Fam212b (Inka2)	Inka box actin regulator 2	2.426	Associated with proliferating NPCs in the embryonic brain and with some immature neurons of the cerebral cortex, hippocampus, and cerebellum in the postnatal brain.[23]
Vsnl1	Visinin-like 1	2.433	A neuron-specific calcium sensor protein that associates with the membrane [30]
Vgf	VGF nerve growth factor inducible	2.653	Overexpression of this neuropeptide causes hyperactivity, memory deficits, and other mental symptoms in mice. [31]
Caln1	Calneuron 1	2.674	Similar to the calmodulin family; localizes to the Golgi membrane and acts as a calcium sensor in the cytoplasm.[21]
Thy1	Thymus cell antigen 1, theta	2.814	In the CNS, *Thy1* marks a subpopulation of neurons in the basolateral amygdala that moderates fear response. [32]
Zfp365	Zinc finger protein 365	2.863	Proposed to be a surveyor of telomere dysfunction that prevents chromosomal recombination when telomeres are critically short. [33]
Nrgn	Neurogranin	2.986	Binds calmodulin and *CaMKII*, active at synapses, participates in synaptic plasticity. [22]
Kcnc4	K^+ voltage gated channel, Shaw-related subfamily 4	3.340	A voltage-gated K^+ channel; rectifier channel that repolarizes the neuronal axon after an action potential. Subunit composition determines its effects on synaptic excitability. [18]
Gda	Guanine deaminase	3.776	Promotes microtubule assembly; promotes dendritic branching in neurons. [19]

3.3 DEG Analysis in the E12 Hindbrain

The same ~50,000 transcripts were assayed with RNA-seq in the E12 hindbrain. We were interested in whether different groups of genes would be affected by *Rbm8a* hcKO in the different brain regions. As before, a volcano plot was generated to display all genes that had quantifiable readings in both the WT and KO hindbrains (Figure 4). Once again, about 28,000 genes were plotted in the graph. 25 DEGs had significant q-values, and 23 of them had expressional changes of twofold or more in either direction. The original readings for these 23 DEGs are compared between the WT and KO mice (Figure 5).

Overall, less DEGs were significant at any level in the E12 hindbrain than in the cortex. Ten DEGs overlapped between those detected in the cortex and hindbrain; all of these were upregulated. Their names and functions are presented in Table 3. Of note, six of these ten common, upregulated DEGs are known to directly influence cellular proliferation. These were: *Cdkn1a*, *Ccng1*, and *Phlda3*, which are known to slow or arrest cell cycle; [34,35,36] *Sesn2*, which protects cells from programmed death during stress;[37] *Eda2r*, which increases programmed cell death,[38] and *Fam212b*, which is highly expressed in rapidly-proliferating NPCs in the embryonic mouse brain.[23]

Based on the overlapping DEGs between the cortex and hindbrain, we conclude that *Rbm8a* may influence on the population and turnover of cells during early brain development.

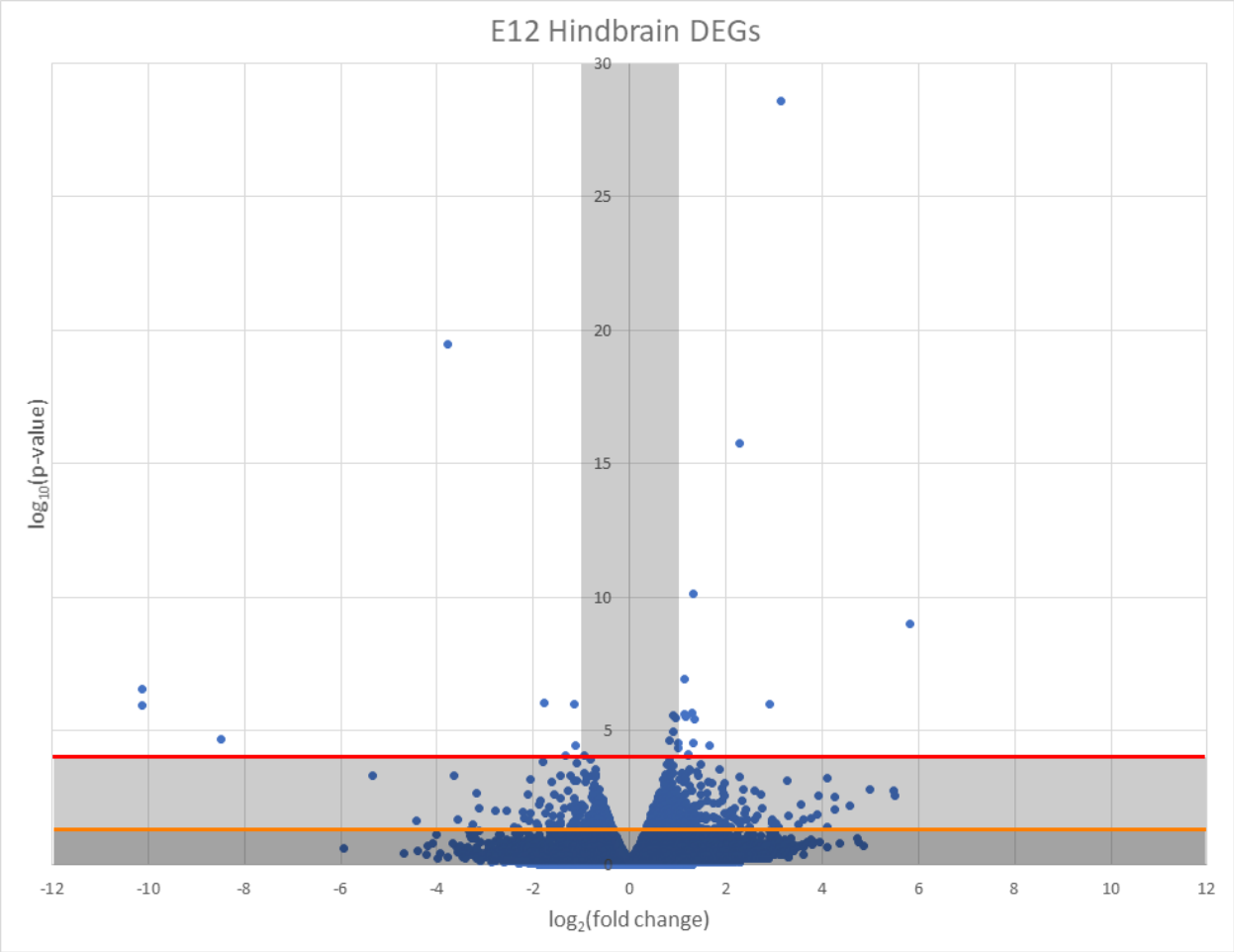

Figure 4: Volcano plot for all quantifiable expressional changes in the hindbrain of *Rbm8a* hcKO mice at E12.
28,117 genes' transcripts were detected in the RNA-seq data. 847 genes were expressed differently with $p < 0.05$, 281 genes were expressed differently with $p < 0.01$, and 25 genes were expressed differently with $q < 0.05$. 23 DEGs with significant q-values were up- or downregulated at least two-fold. The p and q cutoffs are shown.

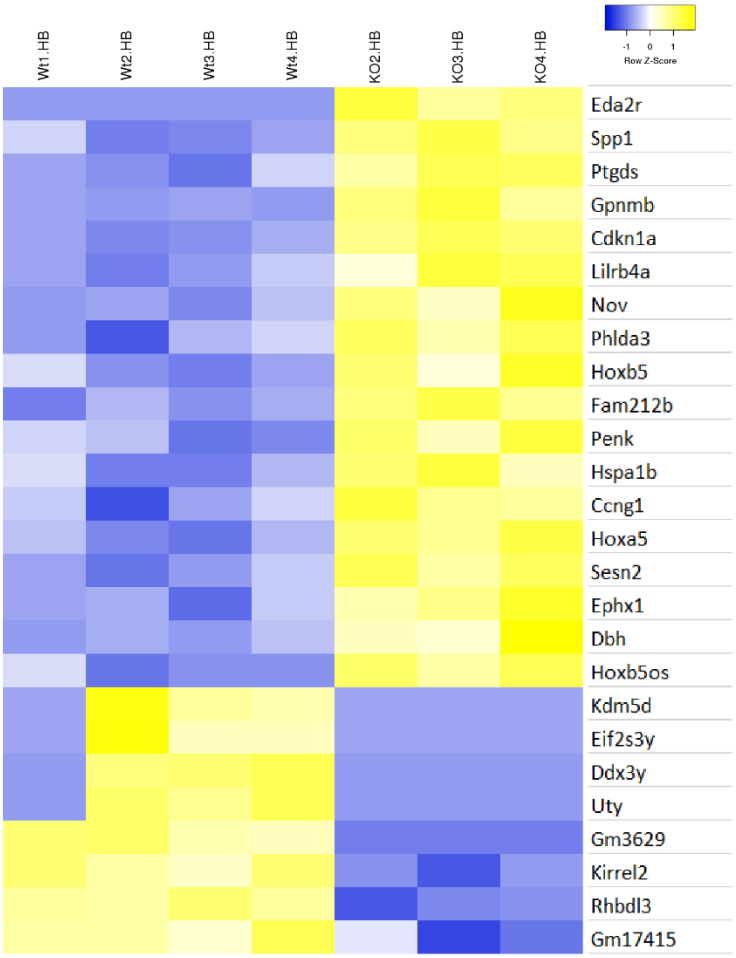

Figure 5: Heat map of RNA transcript readings for all genes with significant expressional changes in the E12 hcKO mouse hindbrain ($q < 0.05$).
From the RNA-seq data, the transcript counts of each gene were compared between the six mice.

Table 3: Common upregulated DEGs between the cortex and hindbrain regions at E12.
The 10 genes that were upregulated in both the cortex and hindbrain are described briefly. Highlighted rows represent genes that are known to influence cellular proliferation.

Gene ID	Gene Name	Pathway and function
Fam212b (Ink2a)	Inka box actin regulator 2	Associated with rapidly-proliferating NPCs in the embryonic brain and with some immature neurons of the cerebral cortex, hippocampus, and cerebellum in the postnatal brain .[23]
Cdkn1a (p21)	Cyclin-dependent kinase inhibitor 1A	Binds a wide range of Cdks to negatively regulate cell cycle progression. Overexpression of *Cdkn1a* causes decreased cell proliferation. [34]
Ccng1	Cyclin G1	Participates in G2/M cell cycle arrest, damage recovery, and resumption of cell division. [37]
Ephx1 (mEH)	Microsomal epoxide hydrolase 1	Detoxifies xenobiotic compounds throughout the body; also may hydrolyze certain endogenous fatty acid epoxides. [39]
Sesn2	Sestrin 2	Protects cells from death during stress, especially during glucose starvation. Predicted to regulate mitochondrial metabolic processes. [37]
Phlda3	Pleckstrin homology like domain A3	Competitively binds the PH domain of *Akt* to negatively regulate cell proliferation. May also interact positively with *p53*. [36]
Eda2r	Ectodysplasin A2 receptor	Activated downstream of *p53*; may contribute to cell death pathways. [38]
Lilrb4a	Leukocyte immunoglobulin-like receptor B4A	Inhibitory receptor that suppresses LPS-mediated and T_H2-dependent inflammation in the immune response. [40]
Spp1	Secreted phosphoprotein 1 (Osteopontin)	An anti-inflammatory cytokine that participates in tissue remodeling, bone homeostasis, and wound repair.[41]
Gpnmb	Glycoprotein nmb (Osteoactivin)	Negative regulator of osteoclast differentiation. May speed up cancer progression when overexpressed.[42]

3.4 DEG Analysis of the Whole Brain at P17

In the P17 whole brain, 22,750 genes had quantifiable transcript readings and were plotted in a volcano plot (Figure 6). 251 DEGs had significant q-values, and 140 of them had expressional changes of twofold or more in either direction.

At P17, upregulated and downregulated CNS-related DEGs were identified with the GO Enrichment Analysis tool. Among the upregulated group, two transcription factors stood out: *Neuronal differentiation 1* (*Neurod1*) and *Engrailed 2* (*En2*). *Neurod1* is a transcription factor that

promotes neuronal cell phenotypes when overexpressed in stem cells, and in neurons, it induces terminal differentiation.[43] *En2* also promotes differentiation of neuronal subtypes.[44] With these observations, it is possible that *Rbm8a* suppresses the activity neural transcription factors, which allows more NPCs to remain in the progenitor pool and proliferate. If neuronal differentiation is impeded by *Rbm8a*, then the competing process of progenitor cell renewal would occur more frequently in the cell population.

Downregulated CNS-related DEGs included several genes of interest. *Folate receptor 1* (*Folr1*) is a cell surface marker of midbrain dopaminergic neuron precursor cells and immature neurons of the same type.[45] The decrease of this marker means less of these immature cells were present. Reduced expression of *glial fibrillary acidic protein* (*Gfap*), mainly expressed in the CNS by astrocytes,[46] could be due to astrocytes having less cellular outgrowths, or due to a decreased population of astrocytes in the *Rbm8a* hcKO brain. *Orthodenticle homeobox 2* (*Otx2*) acts postnatally as a messenger that promotes critical period plasticity, during which the brain drastically modifies its neural circuitry based on sensory input. Previous studies have shown that knockdown of *Otx2* delays the critical period.[47] *LIM homeobox transcription factor 1a* (*Lmx1a*) is most highly expressed in the embryonic brain. After birth, its expression is maintained only in a few small regions, while it disappears from the rest of the brain in the first month after birth.[48] An early decline in Lmx1a might suggest to an early end in CNS development. Finally, *LIM homeobox protein 8* (*Lhx8*) is a transcription factor that regulates several differential pathways in the body. In the CNS, it controls the balance of neuronal subtypes, such as between GABAergic and cholinergic neurons in the basal forebrain.[49] Decreased *Lhx8* could indicate altered makeup of neuronal subtypes in the *Rbm8a* hcKO brain. Considering all of these changes together, it appears

that both the relative proportions of neuronal subtypes and the pace of differentiation into neuronal subtypes were disrupted by *Rbm8a* hcKO.

Compared to the E12 time point, even with hindbrain and cortex DEGs combined, many more genes showed significant expressional changes at P17. However, less genes overlapped between the P17 whole brain and the E12 brain regions than between the two E12 regions (Figure 7). In the P17 whole brain and the E12 cortex, *Nrgn* and *Anoctamin 3* (*Ano3*) were upregulated at both time points. *Ano3* is a calcium-dependent phospholipid scramblase highly expressed in the brain and skin.[50] Meanwhile, *Top2a* was downregulated at E12 and then upregulated at P17, and *Spp1* and *Gpnmb* were upregulated at E12 and then downregulated at P17. These findings suggest that some downstream effects of *Rbm8a* hcKO are temporally distinct, while others may underlie a long period of development in the brain.

In all time points and brain regions, *Fam212b* was upregulated. As mentioned earlier, the exact pathways implicating *Fam212b* are not yet known. In the embryonic brain, *Fam212b* is expressed by rapidly-proliferating NPCs, while in the postnatal brain it is expressed in limited, immature neuronal subtypes.[23] This increase in *Fam212b* could indicate a larger population of proliferating NPCs, contradicting our other findings, but it could also be the product of a compensatory mechanism among a dwindling pool of NPCs.

Table 4: CNS-related DEGs in the *Rbm8a* hcKO brain at P17.
CNS-related DEGs significant at q < 0.05 are shown categorized by the direction of change. GO Enrichment Analysis was used to identify CNS-related genes from among the P17 DEGs.

Upregulated	**Downregulated**
Cbln1	*Bmp7*
Egr2	*Folr1*
Adora2a	*Ngfr*
Neurod1	*Aldh1a2*
En2	*Gfap*
Nr4a3	*Otx2*
Insm1	*Lmx1a*
Robo3	*Lhx8*

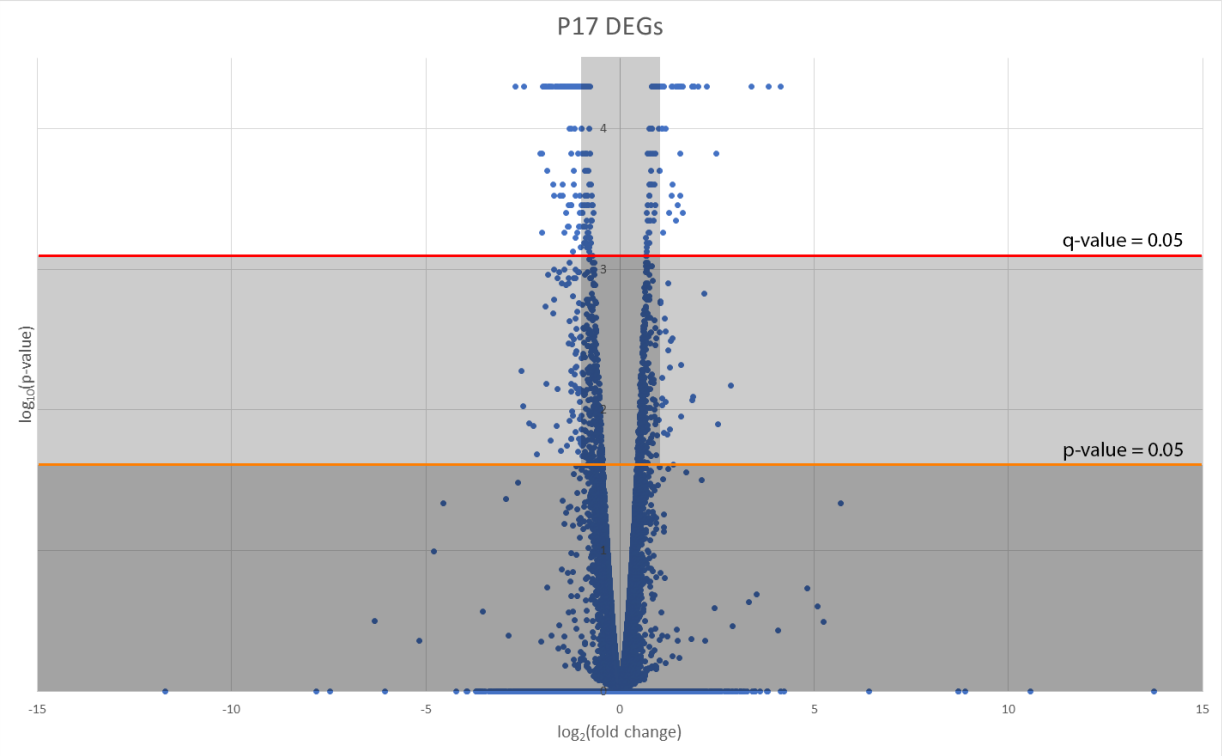

Figure 6: Volcano plot for all quantifiable expressional changes in a whole-brain comparison between WT and *Rbm8a* hcKO mice at P17.
When referenced against 23,992 genes in the mouse genome, 1227 genes were expressed differently with p < 0.05 , 597 genes were expressed differently with p < 0.01 , and 251 genes were expressed differently with q < 0.05 . 140 DEGs with significant q-values were up- or downregulated at least twofold. The p and q cutoffs are shown.

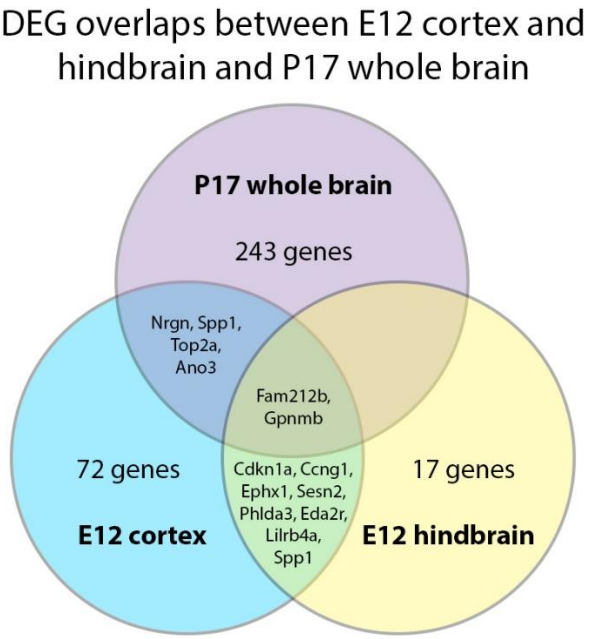

Figure 7: Overlaps between significant DEGs of the E12 cortex, E12 hindbrain, ad P17 whole brain. DEGs with significant q-values were compared across the three RNA-seq data sets. Very few DEGs overlapped between the P17 and E12 time points. *Fam212b* was upregulated in all three conditions. *Gpnmb* was upregulated at E12 and downregulated at P17.

3.5 Analysis of Enriched Pathways and Gene Clusters

Enrichment analysis was performed separately for the E12 cortex, E12 hindbrain, and P17 whole brain. In the E12 data sets, the gene pool was expanded to include all DEGs that were significant at $p < 0.01$ because there were not enough DEGs significant at $q < 0.05$ for the software to detect enrichment of any gene clusters. The P17 data contained enough DEGs with significant q-values for the analysis to be performed on only those DEGs. Selected, gene clusters that were enriched more than two-fold are shown in Figures 8 and 9 for the E12 and P17 time points. GO

accession IDs are listed on the left side of the graphs for easy reference to the GO database. The more a gene cluster is enriched, the more we suspect it to be regulated or influenced downstream of *Rbm8a* activity.

In the E12 cortex, several upregulated gene clusters seemed to revolve around trafficking of cell components, with endocytosis, exocytosis, and other secretions affected (Figure 8A). Immune activity and synaptic plasticity also appeared to be affected (Figure 8A). Meanwhile, in the hindbrain, a variety of gene clusters were upregulated. The most highly enriched clusters were involved in catecholamine synthesis (GO:0042423) and behavioral fear response (GO:2000822) (Figure 8C).

At E12, downregulated genes generally showed greater enrichment than upregulated genes. Enriched clusters of downregulated genes would correspond to *depletions* of those gene clusters in the hcKO mice. In the cortex, most of the notable enrichments pertained to cell division or differentiation. Enrichment of the Notch signaling pathway (GO: 0007219), neuron fate specification (GO:0048665), and oligodendrocyte differentiation (GO:0048709) clusters would affect differentiation of progenitor cells (Figure 8B). Additionally, genes for the positive regulation of neural precursors (GO:2000179) were depleted (Figure 8B).

In the hindbrain, a general theme among the depleted gene clusters was cellular differentiation. In the brain, cellular differentiation could be affected by: the Notch signaling pathway (GO:0007219), positive regulation of neural precursor proliferation (GO:2000179), oligodendrocyte differentiation (GO:0048709), neuron fate specification (GO:0048665), and neural nucleus development (GO:0048857) (Figure 8D). Axon guidance (GO:0007411) is also a process that would be observed during the growth and maturation of neurons (Figure 8D). Interestingly, the gene cluster named “positive regulation of transcription by RNA polymerase II”

(GO:0045944) was enriched in both the upregulated and downregulated DEGs of the hindbrain (Figure 8C-D).

Although not shown in Figure 10, four enriched clusters from the downregulated hindbrain genes had calculated enrichment values of over 100. These were: auditory receptor cell fate commitment (GO:0009912), forebrain neuron fate commitment (GO:0021877), cerebral cortex GABAergic interneuron differentiation (GO:0021892), and negative regulation of photoreceptor cell differentiation (GO:0046533). These groups of genes directly control neuronal differentiation into specific subtypes.

At P17, genes involved in neuron development (GO:0048666), learning or memory (GO:0007611), regulation of synaptic plasticity (GO:0048167), and hindbrain development (GO:0030902) were enriched in *Rbm8a* hcKO mice (Figure 9A). Positive regulation of behavior (GO:0048520), defined by the GO database as a group of genes contributing to the development of a behavior, was also enriched (Figure 9A).

The enriched gene clusters in the downregulated DEGs of the P17 whole brain formed a less coherent list. Most notably, osteoblast differentiation (GO:0001649) genes were depleted at P17, while at E12, the same category was overrepresented among upregulated DEGs (Figures 8C & 9B). This pattern matches the relative upregulation, then downregulation of *Spp1* and *Gpnmb*, the two bone-remodeling DEGs we noted earlier.

In summary, a variety of gene clusters and pathways were affected by *Rbm8a* hcKO, potentially altering cell secretions, neuronal subtype ratios, timing of neuronal differentiation, neurotransmitter production, synaptic modifications, and more.

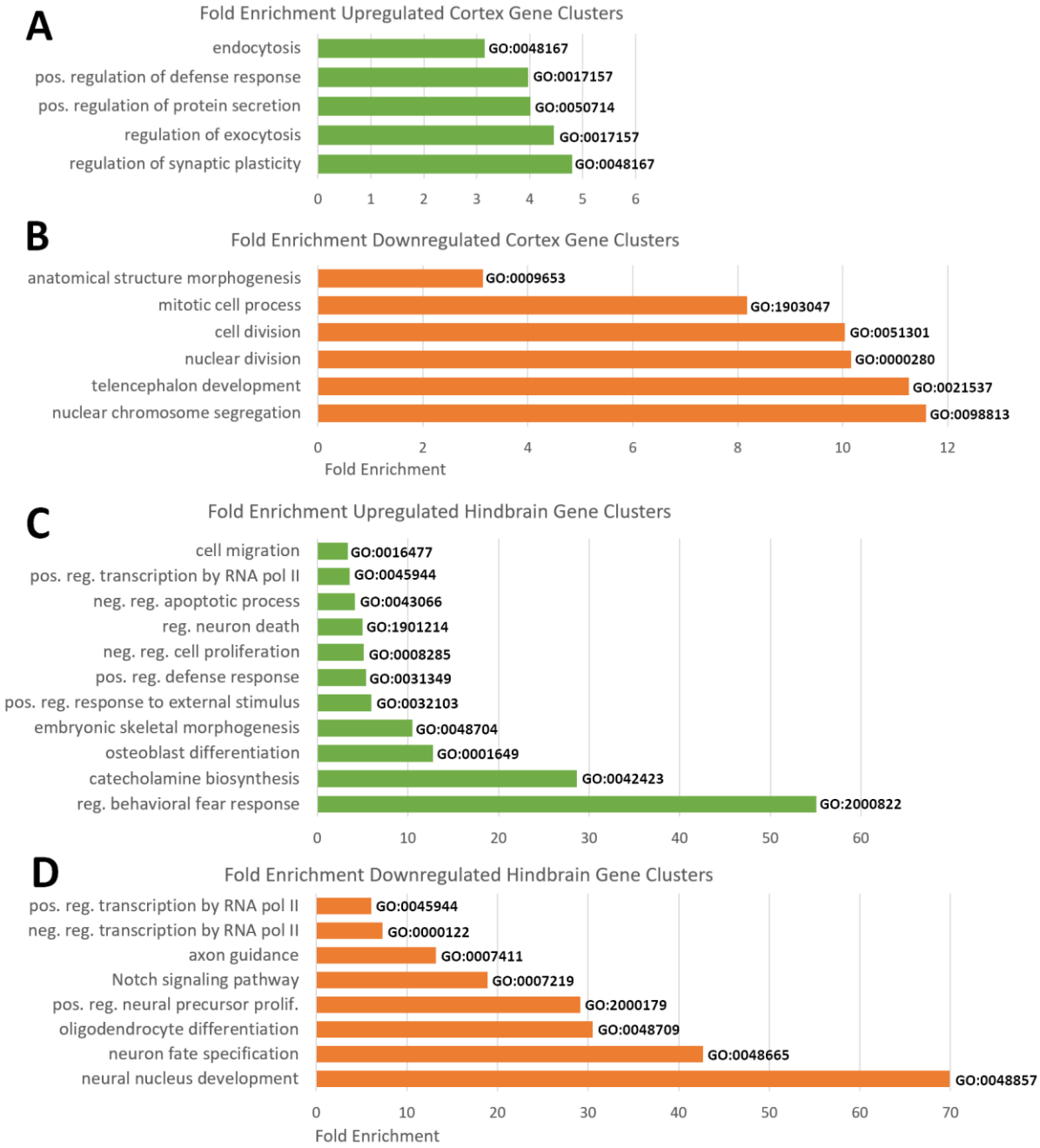

Figure 8: Upregulated and downregulated gene clusters in *Rbm8a* hcKO mice at E12.
Enriched gene clusters were identified among significantly upregulated and downregulated genes at E12. Selected clusters with more than two-fold enrichment are shown with their GO accession numbers to the left of the bars. Note that downregulated clusters were *depleted* rather than enriched, though the statistical test for overrepresentation is the same. (A) Enriched gene clusters of the E12 cortex regulated endocytosis, immune responses, cell secretions, and synaptic plasticity. (B) Depleted gene clusters of the E12 cortex were mainly involved in cell cycle processes. (C) Enriched gene clusters of the E12 hindbrain regulated a variety of processes including cell death, bone development, proliferation, neurotransmitter synthesis, and behavior. (D) Depleted gene clusters of the E12 hindbrain regulated proliferation of NPCs, neuronal differentiation, axon guidance, and other pathways critical to early nervous system development.

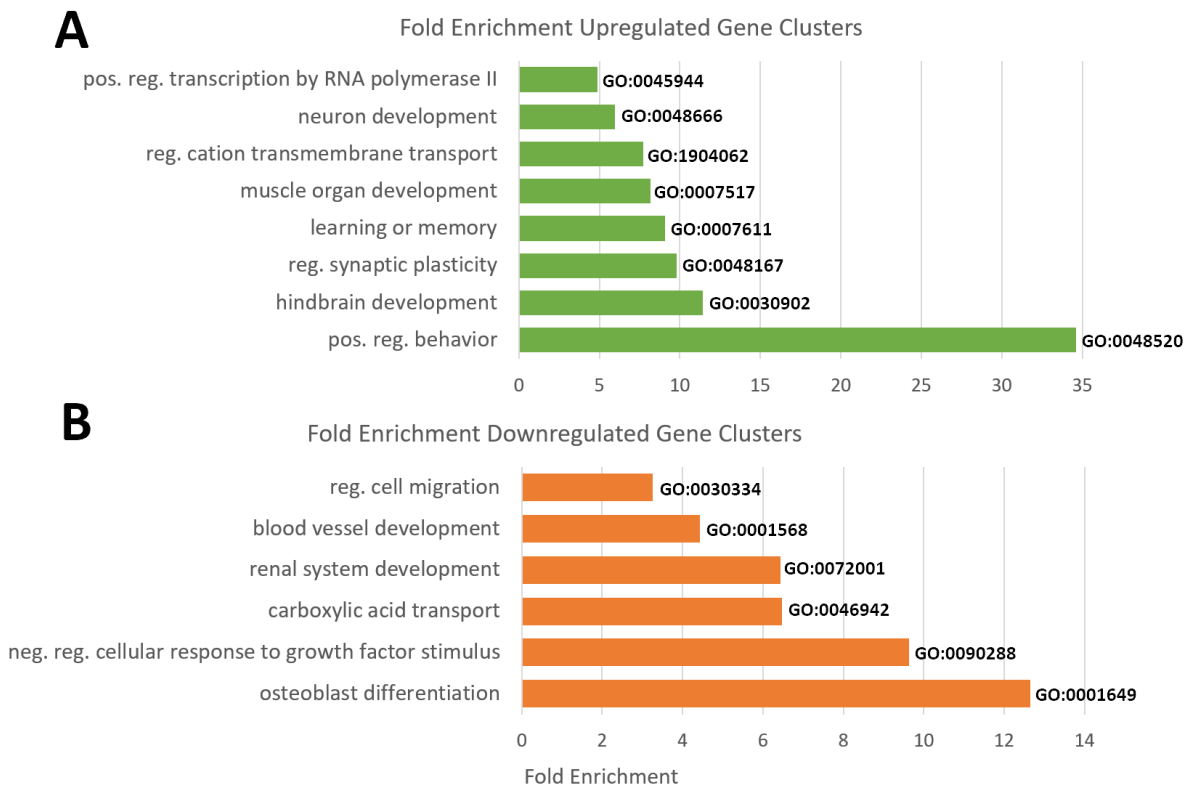

Figure 9: Upregulated and downregulated gene clusters in *Rbm8a* hcKO mice at P17.
Enriched gene clusters were identified among significantly upregulated and downregulated genes at P17. Selected clusters with more than two-fold enrichment are shown, with GO accession numbers to the left of the bars. (A) Enriched gene clusters in the P17 hcKO brain regulated neuronal development, synaptic plasticity, learning processes, behavior, and more. (B) Depleted gene clusters in the P17 hcKO brain regulated several, seemingly unrelated processes, including carboxylic acid transport, growth factor signaling, and bone development.

Chapter 4

Discussion

4.1 DEG Analysis

In this study, three RNA-seq datasets were analyzed to explore the altered transcriptome of *Rbm8a* hcKO mice. Transcriptomes were assessed at the E12 and P17, and at E12, the brain was split into cortex and hindbrain for separate sequencing. The results showed that the different brain regions and time points had many expressional changes, with little overlap between them. Therefore, loss of *Rbm8a* has temporally and spatially restricted effects during CNS development.

At E12, in the cortex, 20 DEGs significant at $q < 0.05$ were known to be implicated in the CNS. They affect many aspects of nervous system development ranging from cell proliferation to myelin maintenance to calcium signaling. The hindbrain at E12 shared ten upregulated DEGs with the cortex, more than half of which could modulate the rate of cell proliferation and turnover. Some of them were pro-apoptotic and some were anti-apoptotic, while others regulated the progression of the cell cycle. Based on this data alone, it is not possible to conclude whether cell populations increased or decreased. However, the small body size and microcephaly of the mice suggests that the cells were less proliferative or more prone to dying. A direct comparison of apoptotic rates between WT and *Rbm8a* hcKO brains will confirm whether or not increased apoptosis was a major cause of the underdevelopment. It would also be interesting to see if knocking down anti-proliferative genes could restore some brain mass. For example, *Cdkn1a, Phlda3,* and *Eda2r* were upregulated in both the cortex and hindbrain, and are known to oppose cell proliferation. Inhibiting or disrupting their gene products might allow the brain to expand at a faster pace. However, simply disinhibiting cell proliferation could also predispose a living host to tumors.

At P17, a much different set of CNS-related DEGs were identified. (Ideally, we would have divided the P17 brains into cortex and hindbrain as well, but the P17 brains were harvested without this comparison in mind.) Significant *Neurod1* and *En2* upregulation at P17, as well as downregulation of several genes associated with the immature CNS, indicates that neurons were possibly reaching terminal differentiation long before the CNS should have stopped developing. There was also evidence that the distribution of cell types was abnormal in the *Rbm8a* hcKO brains, based on the decrease in *Lhx8* expression, which regulates the NPC's decision of differentiating into a GABAergic versus a cholinergic neuron.[49] These results correlate with our previous findings that *Rbm8a* generally suppresses NPC differentiation. Apparently, loss of *Rbm8a* may also disrupt the ratios of NPCs that differentiate into each neuronal subtype.

A few of the significant DEGs from E12 reappeared in the P17 hcKO brains. Notably, three of them had changed significantly in opposite directions between E12 and P17. *Spp1* and *Gpnmb* were upregulated at E12 but downregulated at P17, while *Top2a* was downregulated at E12 and upregulated at P17. This could indicate that some pathways are not continuously overactive or underactive, but rather may become active on different timelines. On this assumption, *Spp1* and *Gpnmb* expression would peak early and *Top2a* expression would peak late in the *Rbm8a*-deficient brain. *Spp1* participates in bone and tissue remodeling, as does *Gpnmb*. *Top2a* participates in DNA replication. Both could influence the speed or extent of development of regions where they are expressed. However, their expressional changes could also be compensatory for other disruptions in the CNS. For example, if brain volume is expanding too slowly, then less supporting bone and tissue will need to be produced to encase it, explaining the early declines of *Spp1* and *Gpnmb*.

Fam212b was the only two gene that recurred as an upregulated DEG ($q < 0.05$) in all three analyses. According to previous explorative studies, *Fam212b* is expressed by highly proliferative

NPCs, immature neurons in the postnatal developing brain, and very specific subtypes of mature neurons in the adult forebrain.[23] Unfortunate to the purpose of this study, the exact pathways that this protein participates in are unknown. Further investigation is necessary to elucidate the role of *Fam212b* in CNS development, and its relation to *Rbm8a*.

4.2 GO Enrichment Analysis

Enrichment analysis showed that several pathways were affected by *Rbm8a* hcKO in the brain. A few patterns that appeared across the three RNA-seq data sets were enrichments in genes related to cellular differentiation, regulation of RNA transcription, proliferation, and cell death.

Changes in differentiation pathways can result in delayed differentiation, premature differentiation, or an unbalanced distribution of cell types at maturity. Among enriched and depleted pathways, cell fates including oligodendrocytes, osteoblasts, neurons, and specific neuronal subtypes were named. Considering that several genes expected to be expressed in the adult brain were upregulated in the embryonic cortex, and considering that negative regulation of photoreceptor differentiation was *depleted*, we hypothesize that the *Rbm8a* hcKO mouse nervous system differentiates prematurely, resulting in underdevelopment of nervous system tissues.

Closely tied to differentiation is the renewal of progenitor cell populations, regulated by signals for cell cycle progression versus arrest, and survival versus apoptosis. In the E12 cortex, genes for cell division process were depleted; likewise in the hindbrain, *negative* regulation of proliferation was increased and neural precursor proliferation was specifically determined to be

depleted. This falls in line with our previous observations that *Rbm8a* promotes the renewal of NPCs and inhibits differentiation of neuronal subtypes.[6]

In the P17 brain, at first glance, it appears that the nervous system is getting a head start and developing quickly in *Rbm8a* hcKO mice: Neuronal development genes are enriched, and pathways pertaining to synaptic plasticity and behavior are more active. However, these could also be the results of premature differentiation of neurons. At a stage when the nervous system should still be expanding, the neurons are settling into their mature roles, approaching terminal differentiation. Furthermore, synaptic plasticity and behavior changes are observed in both juvenile and adult animals. Increased activity of these pathways is not necessarily an advantage for animals at such an early developmental stage.

Taken together, the DEG analysis and GO Enrichment analysis support our hypothesis that *Rbm8a* maintains renewal of the neural precursor population and inhibits differentiation. Additionally, we uncovered specific genes and pathways for further investigation that may be critical to early CNS development. We are interested in whether the direct downstream targets of *Rbm8a* are regulators of the cell cycle, such as *Cdkn1a* or *Ccng1*, and how promotion of the cell cycle in NPCs may compete with differentiation. Perhaps stimulating cell proliferation or inhibiting neural transcription factors could reverse the early depletion of NPCs in the *Rbm8a* hcKO brain. Finally, our RNA-seq analysis featured several genes whose functions have not been elucidated in the context of early brain development, including *Spp1*, *Gpnmb*, and *Fam212b*. We hope that this exploratory analysis will provide useful leads for further studies of brain development in mice and other mammalian models.

BIBLIOGRAPHY

1. Lamond AI. Nuclear RNA Processing. Curr Opin Cell Biol. 1991 June; 3(3):493-501
2. Roundtree IA, Evans ME, Pan T, He C. Dynamic RNA Modifications in Gene Expression Regulation. Cell. 2017 June 15;169(7):1187-1200.
3. Salicioni AM, Xi M, Vanderveer LA, Balsara B, Testa JR, Dunbrack RL Jr, Godwin AK. Identification and structural analysis of human RBM8A and RBM8B: two highly conserved RNA-binding motif proteins that interact with OVCA1, a candidate tumor suppressor. Genomics. 2000 Oct 1; 69(1):54-62
4. Le Hir H, Saulière J, Wang Z. The exon junction complex as a node of post-transcriptional networks. Nat Rev Mol Cell Biol. 2016 Dec 16; 17:41-54
5. Choudhury SR, Singh AK, McLeod T, Blanchette M, Jang B, Badenhorst P, Kanhere A, Brogna S. Exon junction complex proteins bind nascent transcripts independently of pre-mRNA splicing in *Drosophila melanogaster*. eLife. 2016 Nov 23; 5: e19881.
6. Zou D, McSweeney C, Sebastian A, Reynolds DJ, Dong F, Zhou Y. A critical role of RBM8a in proliferation and differentiation of embryonic neural progenitors. Neural Dev. 2015 Jun 21;10:18.
7. Shoura MJ, Vetcher AA, Giovan SM, Bardai F, Bharadwaj A, Kesinger MR, Levene SD. Measurements of DNA-loop formation via Cre-mediated recombination. Nucleic Acids Res. 2012 Aug; 40(15):7452-7464.
8. McSweeney, C. Rbm8a is required for normal cortical development. PhD Thesis, Penn State University. 2013.
9. Reuter JA, Spacek DV, Snyder MP. High-Throughput Sequencing Technologies. Mol Cell. 2015 May 21; 58(4):586-597.
10. "An Introduction to Next-Generation Sequencing Technology." *Illumina*. Illumina, Inc., 2015. Web. 25 March 2019. <https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf>
11. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expressional analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012; 7:562-578.Robinson MD, Smyth GK. Small-sample estimation of negative binomial dispersion, with applications to SAGE data. Biostatistics. 2008 Apr; 9(2):321-332.
12. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. May 2000; 25(1):25-9.

13. The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res. Jan 2019; 47(D1):D330-D338.
14. Mi H, Huang X, Muruganujan A, Tang H, Mills C, Kang D, Thomas PD. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. Jan 2017; 45(D1):D183-D189.
15. Fourcade S, Savary S, Gondcaille C, Berger J, Netik A, Cadepond E, et al. Thyroid hormone induction of the adrenoleukodystrophy-related gene (ABCD2). Mol Pharmacol. 2003 Jun; 63(6):1296-303.
16. Harkin LF, Gerrelli D, Diaz DCG, Santos C, Alzu'bi A, Austin CA, Clowry GJ. Distinct expression patterns for type II topoisomerases IIA and IIB in the early fetal human telencephalon. J Anat. 2016 Mar; 228(3):452-463.
17. Bijlard M, de Jong JC, Klunder B, Nomden A, Hoekstra D, Baron W. MAL Is a Regulator of the Recruitment of Myelin Protein PLP to Membrane Microdomains. PLoS One. 2016 May 12; 11(5):e0155317.
18. Rowan MJM, Christie JM. Rapid State-Dependent Alteration in Kv3 Channel Availability Drives Flexible Synaptic Signaling Dependent on Somatic Subthreshold Depolarization. Cell Rep. 2017 Feb 21; 18(8):2018-2029.
19. Akum BF, Chen M, Gunderson SI, Riefler GM, Scerri-Hensen MM, Firestein BL. Cypin regulates dendrite patterning in hippocampal neurons by promoting microtubule assembly. Nat Neurosci. 2004 Feb; 7(2):145-152.
20. Koscielny G, Yaikhom G, Iyer V, Meehan TF, Morgan H, Atienza-Herrero J, et al. The International Mouse Phenotyping Consortium Web Portal, a unified point of access for knockout mice and related phenotyping data. Nucleic Acids Res. 2014 Jan;42(Database issue):D802-9.
21. McCue HV, Burgoyne RD, Haynes LP. Membrane targeting of the EF-hand containing calcium-sensing proteins CaBP7 and CaBP8 . Biochem Biophys Res Commun. 2009 Mar 20; 380(4): 825–831.
22. Dies-Guerra FJ. Neurogranin, a link between calcium/calmodulin and protein kinase C signaling in synaptic plasticity. IUBMB Life. 2010 Aug; 62(8):597-606.
23. Iwasaki Y, Yumoto T, Sakakibara S. Expression profiles of inka2 in the murine nervous system. Gene Expr Patterns. 2015 Sep-Nov;19(1-2):83-97.
24. Priddle TH, Crow TJ. The protocadherin 11X/Y (PCDH11X/Y) gene pair as determinant of cerebral asymmetry in modern *Homo sapiens*. Ann N Y Acad Sci. 2013 Jun; 1288(1):36-47.
25. Muthusamy N, Ahmed SA, Rana BK, Navarre S, Kozlowski DJ, Liberles DA, and Bergson C. Phylogenetic Analysis of the NEEP21/Calcyon/P19 Family of Endocytic Proteins: Evidence for Functional Evolution in the Vertebrate CNS. J Mol Evol. 2009 Oct; 69(4):319-332.

26. Liguz-Lecznar M, Urban-Ciecko J, Kossut M. Somatostatin and Somatostatin-Containing Neurons in Shaping Neuronal Activity and Plasticity. Front Neural Circuits. 2016; 10:48.
27. Fontanet PA, Ríos AS, Alsina FC, Paratcha G, Ledda F. Pea3 Transcription Factors, Etv4 and Etv5, Are Required for Proper Hippocampal Dendrite Development and Plasticity. Cereb Cortex. 2018 Jan 1; 28(1):236-249.
28. Yue F, Cheng Y, Breschi A, Vierstra J, Wu W, Ryba T, et al. A comparative encyclopedia of DNA elements in the mouse genome. Nature. 2014 Nov 20; 515(7527):355-64.
29. Connor-Robson N, Peters OM, Millership S, Ninkina N, Buchman VL. Combinational losses of synucleins reveal their differential requirements for compensating age-dependent alterations in motor behavior and dopamine metabolism. Neurobiol Aging. 2016 Oct; 46:107-112.
30. Spilker C, Richter K, Smalla KH, Manahan-Vaughan D, Gundelfinger ED, Braunewell KH. The neuronal EF-hand calcium-binding protein visinin-like protein-3 is expressed in cerebellar Purkinje cells and shows a calcium-dependent membrane association. Neuroscience. 2000; 96(1):121-129.
31. Mizoguchi T, Minakuchi H, Ishisaka M, Tsuruma K, Shimazawa M, Hara H. Behavioral abnormalities with disruption of brain structure in mice overexpressing VGF. Sci Rep. 2017 Jul 5; 7(1):4691.
32. McCullough KM, Choi D, Guo J, Zimmerman K, Walton J, Rainnie DG, Resslera KJ. Molecular characterization of Thy1 expressing fear-inhibiting neurons within the basolateral amygdala. Nat Commun. 2016; 7: 13149.
33. Zhang Y, Shin S, Liu D, Ivanova E, Foerster F, Ying H, et al. ZNF365 promotes stability of fragile sites and telomeres. Cancer Discov. 2013 Jul; 3(7): 798–811.
34. Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. Nature. 1993 Dec 16; 366(6456):701-704.
35. Kimura SH, Ikawa M, Ito A, Okabe M, Nojima H. Cyclin G1 is involved in G2/M arrest in response to DNA damage and in growth control after damage recovery. Oncogene. 2001 May 31; 20(25):3290-300.
36. Kawase T, Ohki R, Shibata T, Tsutsumi S, Kamimura N, Inazawa J, Ohta T, Ichikawa H, Aburatani H, Tashiro F, Taya Y. PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt. Cell. Cell. 2009 Feb 6; 136(3):535-550.
37. Ding B, Parmigiani A, Divakaruni AS, Archer K, Murphy AN, Budanov AV. Sestrin2 is induced by glucose starvation via the unfolded protein response and protects cells from non-canonical necroptotic cell death. Sci Rep. 2016; 6:22538.
38. Brosh R, Sarig R, Natan EB, Molchadsky A, Madar S, Bornstein C, Buganim Y, Shapira T, Goldfinger N, Paus R, Rotter V. p53-dependent transcriptional regulation of EDA2R and its involvement in chemotherapy-induced hair loss. FEBS Lett. 2010 Jun 3; 584(11):2473-2477.

39. Marowsky A, Meyer I, Erismann-Ebner K, Pellegrini G, Mule N, Arand M. Arch Toxicol. Beyond detoxification: a role for mouse mEH in the hepatic metabolism of endogenous lipids. 2017 Nov; 91(11):3571-3585.
40. Breslow RG, Rao JJ, Xing W, Hong DI, Barrett NA, Katz HR. Inhibition of Th2 adaptive immune responses and pulmonary inflammation by leukocyte Ig-like receptor B4 on dendritic cells. J Immunol. 2010 Jan 15; 184(2):1003-1013.
41. Denhart DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. J Clin Invest. 2001 May 1; 107(9):1055-1061.
42. Sondag GR, Mbimba TS, Moussa FM, Novak K, Yu B, Jaber FA, Abdelmagid SM, Geldenhuys WJ, Safadi FF. Osteoactivin inhibition of osteoclastogenesis is mediated through CD44-ERK signaling. Exp Mol Med. 2016 Sep 2; 48(9):e257.
43. Goparaju SK, Kohda K, Ibata K, Soma A, Nakatake Y, Akiyama T. Rapid differentiation of human pluripotent stem cells into functional neurons by mRNAs encoding transcription factors. Sci Rep. 2017 Feb 13; 7:42367.
44. Boschian C, Messina A, Bozza A, Castellini ME, Provenzano G, Bozzi Y, et al. Impaired Neuronal Differentiation of Neural Stem Cells Lacking the Engrailed-2 Gene. Neuroscience. 2018 Aug 21; 386:137-149.
45. Gennet N, Tamburini C, Nan X, Li M. FolR1: a novel cell surface marker for isolating midbrain dopamine neural progenitors and nascent dopamine neurons. Sci Rep. 2016 Sep 1; 6:32488.
46. Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci. 2015 Jun; 38(6):364-374.
47. Rebsam A, Mason CA. Otx's incredible journey. Cell. 2008 Aug 8; 134(3):386-387.
48. Zou H, Su C, Shi M, Zhao G, Li Z, Guo C, et al. Expression of the LIM-homeodomain gene Lmx1a in the postnatal mouse central nervous system. Brain Res Bull. 2009 Mar 30; 78(6):306-312.
49. Zhou C, Yang G, Chen M, He L, Xiang L, Ricupero C, et al. Lhx6 and Lhx8: cell fate regulators and beyond. FASEB J. 2015 Oct; 29(10): 4083–4091.
50. Suzuki J, Fujii T, Imao T, Ishihara K, Kuba H, Nagata S. Calcium-dependent Phospholipid Scramblase Activity of TMEM16 Protein Family Members. J Biol Chem. 2013 May 10; 288(19): 13305–13316.

ACADEMIC VITA

Miranda Chen

mic5117@psu.edu | msujingc@gmail.com

Summary

Senior undergraduate and aspiring medical student with life goal of becoming a volunteer physician under Médecins Sans Frontières. Adaptable to long work hours, irregular shifts, and scheduling changes. Efficient and enthusiastic in memorizing new material.

Education

B.S. in General Science with a Minor in Microbiology
Pennsylvania State University, University Park, PA
Schreyer Honors College, Class of 2019
Honors in Biology
Dean's List for all semesters thus far completed.

Study Abroad, BMB 460 (Cell Growth and Differentiation)
Fudan University, Shanghai, China
Led a group of six students on an independent trip to the Yellow Mountains.
Class was taught in English to a mixed class with PSU and Fudan students.

Research Experience

Undergraduate Research under Dr. Gong Chen, Penn State Department of Biology, Aug. 2015 – Dec. 2017

- Contributed IHC experiments and analyses, behavioral quantification data, and Golgi staining in the Stroke Project, which demonstrated reprogramming of reactive astrocytes into functional neurons in the mouse brain using a single transcription factor, NeuroD1.
- Mouse surgeries: Assisted with injections of viral vectors into the brain and carried out euthanizing perfusions independently.
- Cultured human astrocytes and performed *in vitro* drug assays to test drug-based alternatives to NeuroD1 injections. Quantified cellular morphology and protein expression using IHC.
- **Manuscript under review**: "Functional repair after ischemic injury through high efficiency *in situ* astrocyte-to-neuron conversion."
- **Manuscript in preparation:** "A chemical cocktail screened from FDA drugs converts human astrocytes directly into neurons."

Undergraduate Research under Dr. Yingwei Mao, Penn State Department of Biology, Jan. 2018 – (ongoing)

- Contributed IHC experiments to research projects on several genes of implicated in brain development, including Rbm8a, ZNF, and GIGYF.
- Completed an undergraduate honors thesis on RNA-seq analysis of Rmb8a heterozygous KO mice.

Professional Experience

Learning Assistant for STAT 250, 1 semester

In-class peer tutor in Introductory Biostatistics.
Was recommended for higher peer tutoring position by both the instructor and teaching assistant.

Guided Study Group Leader for STAT 250, 3 semesters

Peer tutor under Penn State Learning, a program that provides free studying resources and help sessions for students in introductory courses.
Spring 2018 to Spring 2019.

University Exam Proctor

Proctored exams for the Math Department, Fall 2018 to Spring 2019.

Volunteer at Mount Nittany Medical Center

- Café and Gift Shop worker, 1 year
- Patient Floors Volunteer, 4+ years

> 350 hours of service since 2014

Other Skills

Languages

- English (native)
- Chinese (fluent)
- French (basic conversation and reading)