

**ABNORMAL LIPID SIGNALLING IN THE DEVELOPING MOUSE BRAIN:
IMPLICATIONS IN AUTISM SPECTRUM DISORDER**

RAVNEET RAI

A DISSERTATION SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

GRADUATE PROGRAM IN BIOLOGY
YORK UNIVERSITY,
TORONTO, ONTARIO

APRIL 2021

© Ravneet Rai, 2021

ABSTRACT

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, currently characterized by difficulties in social communication, delayed speech and language, and appearance of restricted and repetitive sensory-motor behaviours. Currently, there are many etiological factors, both genetic and environmental, that have been involved in its manifestation, including the lipid signaling pathway. The brain is rich in lipids, and the plasma membrane acts as a reservoir of bioactive molecules such as Prostaglandin E₂ (PGE₂), a lipid signaling molecule important for normal brain development and function. PGE₂ is converted by cyclooxygenase-1 and -2 (COX-1 and -2) enzymes, a downstream action following release of arachidonic acid from the plasma membrane. Various genetic and environmental factors can influence the level of PGE₂ and have been identified for their involvement in increased ASD-risk. However, the exact mechanisms behind this relationship are not well known, and thus the current dissertation aims to contribute to this gap in knowledge with four studies *in vivo* in the mouse brain. Using mouse models, we investigated the effects of maternal PGE₂ administration (PGE₂-injected) and gene knockdown of COX-1 and COX-2 enzymes (COX-1^{-/-} and COX-2^{-/-}) at E16 and E19, on sex- and age-dependent expression of developmental genes and proteins, global analyses of biological pathways, and characterization and analysis of dendritic spines morphology at P25. These studies established that aberrations in the COX/PGE₂ signalling pathway result in differential expression of many genes and pathways involved in neurodevelopment and ASD etiology. We found abnormal age- (i.e. *Mmp7* and *Wnt2*, *Spn*, *Actb*) and sex-dependent (i.e. *Spn*, *Actb*) gene and protein expression of developmental and synaptic functions, sex-specific enlargement of mushroom spines in cerebellar neurons, and ASD genes that are dysregulated throughout development (i.e. *Glo1*, *Gabra2*, *Grm5*). Interestingly, novel *in vivo* findings in mouse models of

PGE₂-administration and COX-2^{-/-} (high and low levels) both confirmed an interaction between COX-2/PGE₂ and Wnt pathway particularly at the E16 stage of development. The current findings strengthen the involvement of abnormal COX/PGE₂ signalling during early brain development, and thus, many genes and environmental exposures during gestation can act on different constituents of the COX/PGE₂ pathway, and result in neurological conditions such as ASD.

DEDICATION

This dissertation is dedicated to my beautiful family: my husband Jas, my son Gavin, and my daughter Evani. My PhD journey started with the encouragement and support from my husband, and I was able to reach the finish line because of the motivation I got from my children. This dissertation represents strength, perseverance, and hopefully demonstrates to my kids that they can reach for the stars. I love you.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisor, Dr. Dorota Crawford, for giving me the opportunity to work in such a wonderful lab environment. Your support, guidance and understanding through the years has been essential in reaching all my goals. You always pushed me forward, allowed opportunities for critical thinking, and moulded me into a better researcher and scientist. A special thank you to my current and former supervisory committee members: Dr. Terrance Kubiseski, Dr. Michael Scheid, and Dr. Suraj Unniappan. You have always provided invaluable feedback and insightful comments that helped shape my research.

I would also like to thank my lab family – Christine T. Wong, Cora Sin, Ashby Kissoondoyal, Denis Adigamov, Isabel Bestard Lorigados, Jennilee Davidson, Sarah Wheeler, Josee Smith, and Eizaaz Ahmad - our friendship over the years is unforgettable. I would also like to give a special thanks to Dr. Hongyan Li for all the technical knowledge that you instilled in me – I was able to navigate many aspects in the lab with ease because of your teachings.

I would like to thank everyone in the Bhogal family, especially my parents Amarjit and Sukhwinder Bhogal for giving me the courage to pursue all my goals and dreams. A special thank you to my parents, my in-laws Karamjit and Kulwant Rai, and Jashan Gill for giving up so much of your time to get me through the last mile of this journey. I am so grateful for the love and attention you gave to my kids when I was busy writing.

A special thank you to my husband Jas for persevering through this journey alongside me – helping with analysis, providing companionship through all the tough times, and bringing me countless snacks and Iced capps. An out-of-this world thank you to my children, Gavin & Evani, because of whom I never gave up. Your smiles, hugs, and kisses, got me through each and every day.

Table of Contents

ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS.....	xvii
CHAPTER 1. INTRODUCTION	1
1.1 Introduction to Autism Spectrum Disorder (ASD).....	1
1.2 Sex Differences	2
1.3 Genetic and environmental factors of autism	4
1.3.1 Genetic Factors	4
1.3.2 Environmental Factors	6
1.3.2.1 Maternal Risk Factors	7
1.3.2.2 Air pollution, heavy metals, and pesticides.....	8
1.3.2.3 Oxidative stress	10
1.4 Mouse Models of ASD	12
1.4.1 Cellular and molecular outcomes of ASD-risk genes	13
1.4.1.1 SHANK genes.....	13
1.4.1.2 Neurexins (Nrxns)/Neurologins (Nlgn).....	14
1.4.1.3 Fragile X Mental Retardation 1 (FMR1) Gene.....	16
1.4.2 Naturally Occurring ASD Models – Inbred Strains	17
1.4.2.1 BALB/c Mouse Strain.....	17
1.4.2.2 BTBR T+tf/J.....	18
1.4.3 Environmental factors associated with ASD	20
1.4.3.1 Valproic Acid	20
1.4.3.2 Maternal Infection.....	22
1.5 Early Brain Development	23
1.5.1 Lipids in the Brain	25

1.5.2	<i>Abnormal Brain Lipids in ASD</i>	29
1.6	Prostaglandin Pathway – Implications in ASD.....	30
1.6.1	<i>Phospholipase A2 (PLA₂)</i>	30
1.6.2	<i>Cyclooxygenase Enzymes</i>	30
1.6.2.1	<i>Cyclooxygenase-1 (COX-1)</i>	30
1.6.2.2	<i>Cyclooxygenase-2 (COX-2)</i>	32
1.6.3	<i>Prostaglandin E₂ (PGE₂)</i>	35
1.6.4	<i>EP Receptors</i>	39
1.6.5	<i>PGE₂ – Wnt Interaction</i>	41
1.7	Conclusion statement.....	43
CHAPTER 2. MATERIALS AND METHODS		44
2.1	Cell Model (Results in Appendix A)	44
2.1.1	<i>Cell differentiation</i>	44
2.1.2	<i>PGE₂ treatment</i>	44
2.2	Mouse Model	44
2.2.1	<i>Breeding</i>	45
2.2.2	<i>Study 1 – PGE₂-administered Mouse Model</i>	45
2.2.3	<i>Study 2 – COX-1^{-/-} and COX-2^{-/-} Mouse Models</i>	46
2.2.4	<i>Studies 3 and 4 – COX-2^{-/-} Mouse Model</i>	47
2.3	Genotyping Analysis.....	47
2.3.1	<i>Sample collection</i>	47
2.3.2	<i>Genomic DNA extraction</i>	48
2.3.3	<i>Polymerase Chain Reaction (PCR)</i>	48
2.4	Collection of brain tissue samples	51
2.4.1	<i>Study 1</i>	52
2.4.2	<i>Studies 2-4</i>	52
2.5	RNA/Protein Isolation	52
2.6	Microarray analysis.....	53
2.6.1	<i>Study 2</i>	53
2.6.2	<i>Study 3</i>	54
2.6.3	<i>Bioinformatics and identification of differentially expressed genes</i>	54
2.6.4	<i>Analysis of biological processes</i>	54

2.6.5	<i>Identification of ASD-risk genes and protein-interaction networks</i>	55
2.6.6	<i>Comparison between Microarray Platforms</i>	55
2.7	Reverse transcription	57
2.8	Quantitative real-time PCR	57
2.8.1	<i>Study 1</i>	57
2.8.2	<i>Study 2</i>	58
2.8.3	<i>Study 4</i>	58
2.8.4	<i>Selection of Optimal Housekeeping Genes</i>	60
2.9	Western Blot	61
2.9.1	<i>Study 1</i>	62
2.9.2	<i>Study 4</i>	63
2.10	Microscopy	64
2.10.1	<i>Cell Model (Results in Appendix A)</i>	64
2.10.2	<i>Study 4</i>	64
2.11	Spine classification and quantification	65
2.12	Statistics	66
2.12.1	<i>Studies 1-4</i>	66
2.12.2	<i>Study 4</i>	67
CHAPTER 3.	RESULTS – STUDY 1	68
3.1	Manuscript Introduction and Rationale	69
3.2	Objectives and Hypothesis	71
3.3	Experimental Model Systems	72
	Results	72
3.4	Expression of <i>E-Prostanoid (EP)</i> Receptors in the Normally Developing and Adult Mouse Brain	72
3.5	Preliminary Screening of <i>Wnt</i> and <i>Wnt</i> -target Genes following Maternal Exposure to PGE ₂	75
3.6	Expression of <i>Mmp7</i> and <i>Wnt2</i> during Normal Mouse Brain Development	76
3.7	mRNA Expression of <i>Mmp7</i> in the Brain of Individual Pups at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE ₂	78
3.8	mRNA Expression of <i>Wnt2</i> in the Brain of Individual Pups at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE ₂	83

3.9 Protein Expression of Mmp7 and Wnt2 in Mouse Brain at Developmental Stages E16 and E19 following Maternal Exposure to PGE ₂	87
3.10 Protein Expression of Active β -catenin Forms in Mouse Brain at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE ₂	89
 CHAPTER 4. RESULTS – STUDY 2.....	93
4.1 Manuscript Introduction and Rationale	94
4.2 Objectives and Hypothesis.....	96
4.3 Experimental model systems	96
Results.....	97
4.4 Differential Gene Expression across E16 and E19 in COX-1 ^{-/-} and -2 ^{-/-} Male Mouse Brain	97
4.5 Identification of Affected Biological Processes in the Male COX-1 ^{-/-} Mouse Brain	101
4.6 Identification of Affected Biological Processes in the Male COX-2 ^{-/-} Mouse Brain	105
4.7 COX-2/PGE ₂ Interaction with Wnt Signalling Pathway	107
4.8 Protein Interaction Networks of ASD genes in the COX-1 ^{-/-} and -2 ^{-/-} Male Mouse Brain	109
4.9 Identification of Differentially Expressed Genes from COX-1 ^{-/-} and -2 ^{-/-} Male Mouse Brain, Involved in Co-morbid Neurological Disorders.	111
4.10 Summary of Results.....	115
 CHAPTER 5. RESULTS – STUDY 3.....	116
5.1 Introduction and Rationale.....	117
5.2 Objectives and Hypothesis.....	118
5.3 Experimental Model System.....	119
Results.....	119
5.4 Differential Gene Expression across E16 and E19 in COX-2 ^{-/-} Female Mouse Brain	119
5.5 Identification of Affected Biological Processes in the Female COX-2 ^{-/-} Mouse Brain.....	122
5.6 ASD-Risk Genes and Biological Pathways in the COX-2 ^{-/-} Female Mouse Brain	123
5.6 Protein interaction networks of ASD genes in the COX-2 ^{-/-} female mouse brain.	126
5.7 Summary of Results.....	126
 CHAPTER 6. RESULTS – STUDY 4.....	128
6.1 Introduction and Rationale.....	129
6.2 Objective and Hypotheses.....	130

6.3 Experimental Model System.....	132
RESULTS	132
6.4 mRNA Expression of <i>SPN</i> and β - <i>actin</i> in the Normally Developing Male and Female Mouse Brain.....	132
6.5 mRNA Expression of <i>SPN</i> and β - <i>actin</i> in COX-2 ^{-/-} Males and Females at E19 and P25..	134
6.6 Protein Expression of SPN and β -actin in COX-2 ^{-/-} Males and Females at E19 and P25..	136
6.7 Dendritic Spine Characteristics of Cerebellar Neurons in WT and COX-2 ^{-/-} Male and Female Mice at P25	138
6.7.1 Spine Density	138
6.7.2 Dendritic Spine Types	140
6.7.3 Spine Area	143
6.7.4 Spine Volume	145
6.7.5 Spine Length	147
6.7.7 Spine Head Diameter	150
6.7.8 Spine Straightness	151
6.8 Summary of Results.....	152
 CHAPTER 7. DISCUSSION.....	154
7.1 Study 1 – Maternal Exposure to Prostaglandin E ₂ Modifies Expression of Wnt Genes in Mouse Brain – An Autism Connection.....	154
7.2 Study 2 – Global Microarray Analysis in the Cyclooxygenase-1 and -2 Male Knockout Mice	161
7.3 Study 3 – Global Microarray Analysis in the Cyclooxygenase-2 Female Knockout Mice	172
7.3.1 Comparisons between Global Microarray Analyses in the COX-2^{-/-} Male and Female Mouse Brain	176
7.4 Study 4 – Sex Differences in Dendritic Spine Morphology of Cerebellar Neurons in the COX-2 ^{-/-} Mouse	179
 CHAPTER 8. CONCLUSION	185
8.1 Key Findings and Future Directions.....	185
8.2 Study Limitations.....	189
8.3 Final Conclusions.....	190
 REFERENCES	191

APPENDICES	270
Appendix A. Effect of 1 μ M PGE ₂ on Neuroectodermal (NE-4C) Stem Cells.....	270
Appendix B. Supplementary Tables	272
<i>B1. Microarray Data – Differential Expression of All Genes in the COX-1 and -2 KO Male Mouse Brain</i>	272
<i>B2. Microarray Data – Differential Expression of All Genes in the COX-2 KO Female Mouse Brain</i>	279
<i>B3. Differential Expression of Common Genes between E16 and E19 in the COX-1 and -2 KO Male Mouse Brain</i>	285
<i>B4. Differential Expression of Common Genes between E16 and E19 in the COX-2 KO Female Mouse Brain</i>	288
Appendix C. Manuscripts	292
<i>C1. Study 1</i>	292
<i>C2. Study 2</i>	293
Appendix D. Copyright Permissions for Published Work.....	294
<i>C1. Study 1</i>	294
<i>C2. Study 2</i>	296

List of Tables

Table 2-1. Forward and reverse primer sequences for PCR	51
Table 2-2. Forward and Reverse Primer Sequences for qRT-PCR	59
Table 4-1. Biological Processes Affected in the COX-1 ^{-/-} E16 and E19 Male Mouse Brain	102
Table 4-2. Biological Processes Affected in the COX-2 ^{-/-} E16 and E19 Male Mouse Brain	106
Table 5-1. Biological Processes Affected in the COX-2 ^{-/-} E16 and E19 Female Mouse Brain .	124
Table 5-2. ASD-associated Genes Differentially Expressed in the COX-2 ^{-/-} E16 and E19 Female Mouse Brain.....	125

List of Figures

Figure 1-1. Autism prevalence as reported by Centers for Disease Control and Prevention (CDC).....	2
Figure 1-2. Critical periods of vulnerability in human embryonic development	6
Figure 1-3. Timing of neurodevelopmental events of the human brain	25
Figure 1-4. Intake of dietary fats are metabolized into essential fatty acids that are incorporated into the phospholipid membrane.....	27
Figure 1-5. Metabolism of arachidonic acid to inflammatory prostaglandins.....	28
Figure 1-6. Schematic diagram of genetic and environmental perturbances of the COX/PGE2 signalling pathway in ASD	31
Figure 2-1. The number of genes that overlap between the two microarray platforms.....	56
Figure 2-2. Optimal housekeeping genes in the mouse brain across developmental time points E16, E19, P8, and adult.....	61
Figure 3-1. mRNA (left) and protein (right) expression of EP receptors during mouse brain development.....	74
Figure 3-2. Screening of Wnt and Wnt-target Genes in the PGE ₂ -exposed embryo brain.....	75
Figure 3-3. Mmp7 and Wnt2 mRNA and protein expression throughout normal mouse brain development.....	77
Figure 3-4. Expression of Mmp7 mRNA in developing mouse brain following maternal exposure to PGE ₂	81

Figure 3-5. Sex differences in expression of Mmp7 in brains of PGE2-exposed animals throughout development	82
Figure 3-6. Expression of Wnt2 mRNA in developing mouse brain following maternal exposure to PGE2	85
Figure 3-7. Sex differences in expression of Wnt2 in brains of PGE2-exposed animals throughout development	86
Figure 3-8. Protein expression of Mmp7 and Wnt2 at developmental stages E16 and E19 following maternal exposure to PGE ₂	88
Figure 3-9. Protein expression of phosphorylated β -catenin (Ser-552) and non-phosphorylated β -catenin (Ser33/37/Thr41) in developing mouse brain following maternal exposure to PGE ₂	91
Figure 3-10. A proposed model of the interaction between PGE2 and Wnt pathways in mouse brain	92
Figure 4-1. Number of differentially expressed genes in the brain of COX-1 ^{-/-} or COX-2 ^{-/-} males at E16 and E19	99
Figure 4-2. Commonly affected genes between the E16 and E19 developmental stages in each COX-1 ^{-/-} and COX-2 ^{-/-} male mouse	100
Figure 4-3. Protein interaction network affected in the male COX-1 ^{-/-} model	104
Figure 4-4. Protein interaction networks affected in the COX-2 ^{-/-} male mouse model	108
Figure 4-5. Protein interaction networks of ASD-risk genes in the COX-1 ^{-/-} and COX-2 ^{-/-} male mouse model	110
Figure 4-6. Number of genes differentially expressed in COX-1 ^{-/-} and COX-2 ^{-/-} male mouse at E16 and E19 involved in various neurological disorders	113

Fig 4-7. Differentially expressed genes from the COX-1 ^{-/-} and COX-2 ^{-/-} male mice have implications in multiple disorders.....	114
Figure 5-1. Number of genes differentially expressed in the brain of COX-2 ^{-/-} females at embryonic stages E16 and E19	120
Figure 5-2. Commonly affected genes between the E16 and E19 developmental stages in the COX-2 ^{-/-} female mouse	121
Figure 5-3. Protein interaction networks of ASD-risk genes in the female COX-2 ^{-/-} model	127
Figure 6-1. mRNA Expression of <i>SPN</i> and <i>β-actin</i> in the brains of normally developing males and females at E19 and P25	133
Figure 6-2. mRNA Expression of <i>SPN</i> and <i>β-actin</i> in the COX-2 ^{-/-} male and female mouse brains at E19 and P25	135
Figure 6-3. Protein expression of SPN and β-actin in the COX-2 ^{-/-} male and female mouse brains at E19 and P25	137
Figure 6-4. Dendritic spine density in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	139
Figure 6-5. Types of dendritic spines	140
Figure 6-6. Dendritic spine types in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	142
Figure 6-7. Spine area measurement of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	144
Figure 6-8. Spine volume measurement of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	146

Figure 6-9. Spine length measurement of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	148
Figure 6-10. Spine neck length measurement of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25.....	149
Figure 6-11. Spine head diameter measurement of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25.....	151
Figure 6.12. Spine straightness of dendritic spines in the cerebellum of WT and COX-2 ^{-/-} males and females at P25	153

List of Abbreviations

AA	Arachidonic Acid
AD	Alzheimer's Disease
ALA	α -Linolenic Acid
ASD	Autism spectrum disorder
BBB	Blood Brain Barrier
cAMP	Cyclic AMP
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DHA	Docasohexaenoic Acid
E11	Embryonic Day 11
E16	Embryonic Day 16
E19	Embryonic Day 19
EP	E-prostanoid
FC	Fold-Change
FMR1	Fragile X Mental Retardation 1
FMRP	Fragile X Mental Retardation Protein
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GRM5	Metabolic Glutamate Receptor 5
HPRT	Hypoxanthine-Guanine Phosphoribosyltransferase
ID	Intellectual Disability
KO	Knockout
LA	Linoleic Acid

Nlgn	Neuroligins
Nrxn	Neurexin
P25	Postnatal Day 25
P8	Postnatal Day 8
PCR	Polymerase Chain Reaction
PG	Prostaglandin
PGE2	Prostaglandin E2
PGES	Prostaglandin E Synthase
PGK1	Phosphoglycerate Kinase 1
PI3K	phosphatidylinositol 3-kinase
PKC	Protein Kinase C
PLA2	Phospholipase A2
PLC	Phospholipase C
Ptgs1	Prostaglandin-Endoperoxide Synthase 1
Ptgs2	Prostaglandin-Endoperoxide Synthase 2
qRT-PCR	Quantitative Real-Time PCR
RQ	Relative Quantity
SPN	Spinophilin
SZ	Schizophrenia
VPA	Valproic Acid
Wnt	Wingless-Related Integration Site
WT	Wild-Type

CHAPTER 1. INTRODUCTION

1.1 Introduction to Autism Spectrum Disorder (ASD)

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, currently characterized by difficulties in social communication and appearance of restricted and repetitive sensory-motor behaviours. Symptoms of ASD include but are not limited to impaired social interactions, delayed receptive and expressive speech, rigid or repetitive behaviours, avoidance of eye contact, and lack of understanding emotions and others (Lai *et al.*, 2014; Lord *et al.*, 2018; Sharma *et al.*, 2018; Thabtah & Peebles, 2019). Every individual with ASD is different, as there are many associated symptoms and a wide range in degree of severity of those behaviours.

There has been an evolution of the diagnostic criteria and the term ASD itself. In the past, the spectrum included classic autism, Asperger's syndrome, Childhood disintegrative disorder, Pervasive Developmental Disorder-Not Otherwise Specified and Rett's Syndrome. Over the years, Childhood disintegrative disorder and Rett's Syndrome were removed from the ASD umbrella, and the diagnostic criteria (DSM-5) was updated to combine the remaining sub-categories under one established term – autism spectrum disorder. According to the CDC (Centers for Disease Control and Prevention), ASD currently affects 1 in 54 children – four times more boys than girls (Maenner *et al.*, 2020). The number of children diagnosed with ASD has substantially increased from just 20 years ago, when the CDC incidence rate reported was 1 in 150 (Sharma *et al.*, 2018) (Figure 1-1). One contributing factor for the increase is attributed to greater awareness and better diagnostic tools. However, a vast amount of research has confirmed the involvement of many genetic and environmental factors, which will be addressed below.

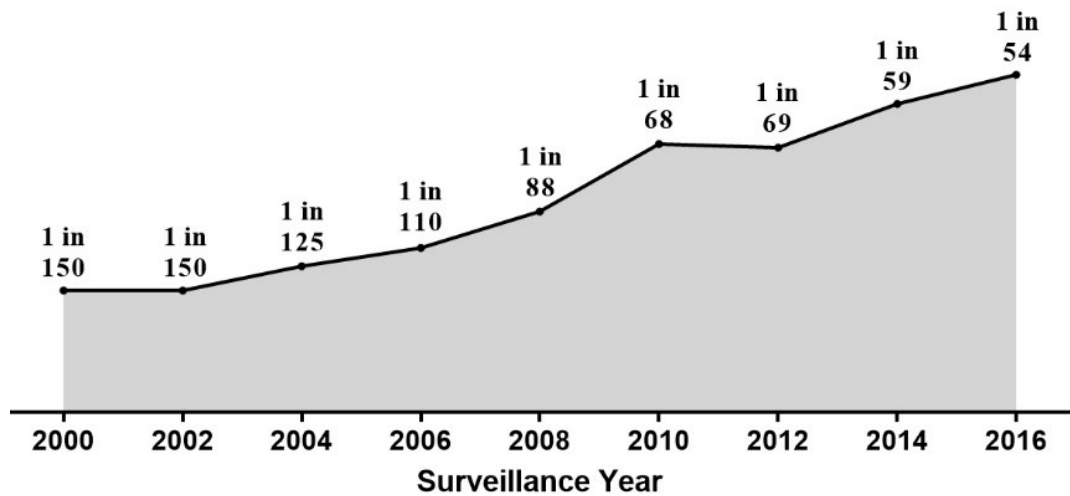


Figure 1-1. Autism prevalence as reported by Centers for Disease Control and Prevention (CDC).

1.2 Sex Differences

Although the male to female ratio is widely accepted as being 4:1, data from recent research suggests that the male to female ratio may actually be closer to 3:1 (Loomes *et al.*, 2017). This discrepancy may be explained by a few possible reasons. One major difference in studies that continue to show the 4:1 ratio, is that they only include individuals that have already been formally diagnosed. This produces an inaccurate ratio because it seems that girls with ASD symptoms are actually going unnoticed, even if they have the same severity of symptoms as boys (Russell *et al.*, 2011). Furthermore, the ratio of receiving an ASD diagnosis is 5.45:1 boys to girls in toddlers, and decreases to 1:3.21 at the preschool age, indicating that boys get diagnosed

earlier than girls (Ros-Demarize *et al.*, 2020). There is a clear gender bias in which even professionals are more inclined to provide diagnoses to boys rather than girls.

Second, the disorder presents itself slightly differently in females than their male counterparts. Females tend to have less rigid repetitive behaviours and fixed interests that are hallmark symptoms in males (Frazier *et al.*, 2014). The nature of their fixed interests are also not usually the stereotypical ones observed in males (Ros-Demarize *et al.*, 2020). Thus far, there has been conflicting evidence on the degree of social communication deficits during the school-age years, between males and females that have ASD. However, adult females with ASD exhibit better first impressions, when comparing likeability, awkwardness, and initiation of conversations in a social setting (Cage & Burton, 2019).

Although the actual ratio may be debatable, there is clearly a higher incidence of ASD in males compared to females, which has been a perplexing challenge for researchers, as ASD is a heterogeneous disorder with most implicated genes not located on the sex chromosomes. One theory suggests that the female brain may somehow be protected from the consequences of ASD-causing mutations. The female autistic brain expresses more severe mutations than males, suggesting that a greater number or severity in mutations may be necessary to result in the manifestation of ASD in females (Jacquemont *et al.*, 2014). Another theory suggests that since females are typically the more social and communicative sex, deficits of these traits in ASD females don't hinder their social-communication skills as much as in males with ASD. There is currently a significant gap in knowledge in understanding the exact mechanisms through which the male brain is more susceptible to developing ASD. A vast amount of research is focused on understanding the male brain and its pathology. However, to gain a more holistic perspective, it is necessary to study the female brain as well.

1.3 Genetic and environmental factors of autism

Though the etiology of autism is currently unknown, extensive research indicates that the causative factors may be genetic (Cook, 2009; Mitchell *et al.*, 2009; Rosenberg *et al.*, 2009; Tamiji & Crawford, 2010b; Kumar *et al.*, 2019) environmental (Wong *et al.*, 2015a; Sealey *et al.*, 2016), or a combination of both. The type and timing of insult(s) – genetic or environmental – will likely determine which brain processes/regions have been affected, and subsequently result in variable symptom profiles, giving it a very complex etiology. Thus far, numerous genes and environmental factors have been identified for their involvement in the pathogenesis of ASD.

1.3.1 Genetic Factors

Genetics play a substantial role in the development of ASD. Early evidence to support the involvement of genetics came in 1977, when it was discovered that the likelihood of autism occurrence is much greater in monozygotic twins compared to dizygotic twins (Folstein & Rutter, 1977). Over the years, many investigations have confirmed the incidence of autism to be greater in monozygotic twins (Bohm & Stewart, 2009; Rosenberg *et al.*, 2009). Furthermore, the occurrence of a sibling being diagnosed with autism or having milder ASD-like symptoms is higher than in half-siblings (Constantino *et al.*, 2013) and even more so than the general population (Greenberg *et al.*, 2001; Constantino *et al.*, 2010; Bohm *et al.*, 2013). These twin and sibling studies provide strong evidence towards the genetic contribution towards ASD occurrence.

Thus far, over 100 genes have been implicated in the etiology of ASD. Typically, defects in chromosomes, copy number variations, and epigenetic regulation through methylation and histone modifications abnormalities are present and affect multiple genes or processes

(Talkowski *et al.*, 2012; Ghasemi Firouzabadi *et al.*, 2016; Wisniowiecka-Kowalnik & Nowakowska, 2019). In few cases where the causative factor is monogenic, the abnormally expressed gene is usually responsible for transcription or regulation of a large number of genes (Ascano *et al.*, 2012; Shpileva *et al.*, 2018), many of which are involved in critical neuronal and synaptic development and functions (Bourgeron, 2015; Zatkova *et al.*, 2016; Kumar *et al.*, 2019). However, a greater volume of evidence points to a polygenic etiology of ASD (Lee *et al.*, 2018; Pohl *et al.*, 2019).

There are also cases of de novo mutations, which are not inherited but arise in individuals without familial risk. Numerous genome-wide studies have identified and categorized de novo mutations in ASD individuals. Many implicated genes were involved in gene expression regulation and neuronal communication, and highly enriched in the brain during mid-to-late gestation period of the fetus (Satterstrom *et al.*, 2020). De novo mutations that cause gene disruption were more likely to result in ASD with high IQ, specifically in males (Iossifov *et al.*, 2014). Clusters of genes involved in development of the cytoskeleton and circuitry of the neural system, as well as those encoding protein receptors, ion channels, and involvement in synaptic function, have been identified. An enrichment of de novo mutations in ASD genes was also found in developing and mature excitatory and inhibitory neurons during gestation, (Polioudakis *et al.*, 2019; Ruzzo *et al.*, 2019; Satterstrom *et al.*, 2020), supporting the excitatory-inhibitory imbalance theory of synaptic transmission in ASD (Gao & Penzes, 2015; Uzunova *et al.*, 2016; Corradini *et al.*, 2018; Wang *et al.*, 2018). Therefore, it is evident that genetics play a significant role in the etiology of ASD.

1.3.2 Environmental Factors

The environment can pose a threat to the growing fetus by interfering with developmental events of all organs including the central nervous system (CNS) (Figure 1-2). Consequences of fetal exposure to teratogens are dependent on the dose of substance and timing of exposure. Over the last few decades, an increase in ASD research has led to the discovery of many environmental factors that can alter various brain functions.

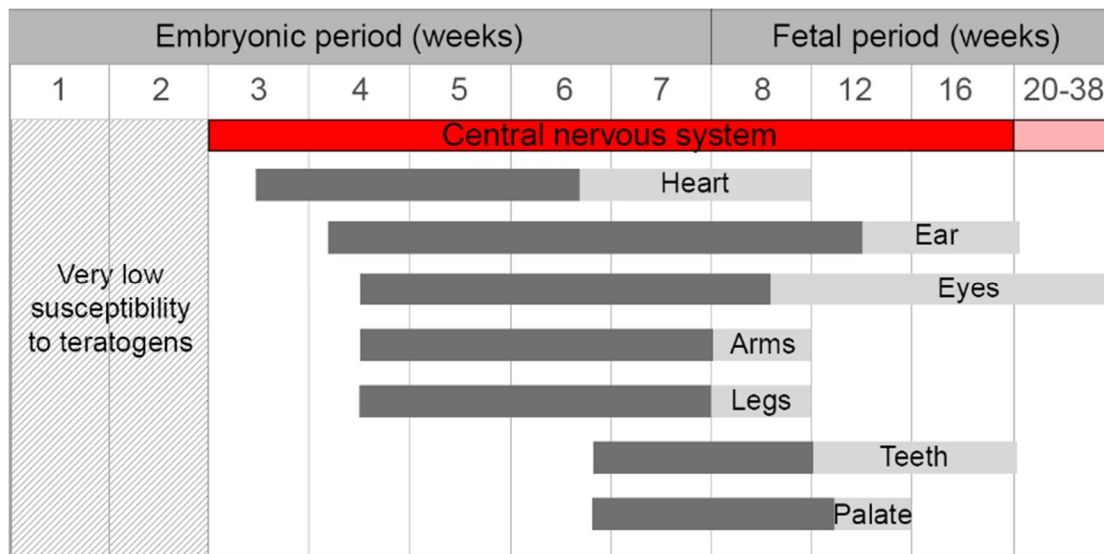


Figure 1-2. Critical periods of vulnerability in human embryonic development. The central nervous system is highly susceptible to teratogens during the first trimester (3-20 weeks; red). The time period between 20-38 weeks is less sensitive to congenital anomalies, but still particularly sensitive to various functional outcomes. The dark and light bars represent high-sensitive and low-sensitive time periods of susceptibility to teratogens of other tissues, respectively. Image adapted from Selevan et al, 2000.

1.3.2.1 Maternal Risk Factors

Healthy fetal development, in part is dependent on maternal health during the gestation period. External influences such as maternal infections and inflammation, drug use during pregnancy, stress, older age, and nutritional deficiencies, can impose risks on development, and lead to an array of abnormalities. Contraction of severe viral infections requiring hospitalization during the first trimester of pregnancy were associated to ASD occurrence in that cohort of children (Atladottir *et al.*, 2010). Thus far, various types of maternal viral infections have been identified to increase the risk of ASD – rubella (Libbey *et al.*, 2005; Mawson & Croft, 2019), cytomegalovirus (Yamashita *et al.*, 2003; Sweeten *et al.*, 2004; Libbey *et al.*, 2005; Engman *et al.*, 2015; Slawinski *et al.*, 2018), Epstein-Barr virus (Valayi *et al.*, 2017), and influenza (Atladottir *et al.*, 2010; Atladottir *et al.*, 2012). In addition, infections that co-occur with a fever during pregnancy also increase risk of ASD to the children (Atladottir *et al.*, 2012; Hornig *et al.*, 2018; Croen *et al.*, 2019).

Intake of various drugs for illness or medical health issues can also prove to be detrimental during pregnancy. The drug thalidomide, prescribed for pregnancy symptoms such as nausea and morning sickness, caused defects and deformities in the fetus, as well as increased incidence of ASD (Stromland *et al.*, 1994). Similarly, improper intake of the drug misoprostol – which must be used in conjunction with mifepristone for successful termination of pregnancy – resulted in fetal malformations, Möbius Syndrome, as well as ASD (Bandim *et al.*, 2003). The use of antidepressants that act to inhibit serotonin re-uptake, and anti-epileptic drug valproic acid, are also strongly associated with ASD incidence (Williams *et al.*, 2001; Christensen *et al.*, 2013; Boukhris *et al.*, 2016). A more frequently used drug that has been targeted for its involvement in ASD following prenatal or postnatal exposure, is acetaminophen – commonly

sold under major brands such as Tylenol and Paracetamol. Current evidence suggests that acetaminophen use may contribute to motor delays, hyperactivity, and attention deficits, leading to potential outcomes of ASD, attention-deficit hyperactivity disorder, or hyperkinetic disorder (Brandlistuen *et al.*, 2013; Andrade, 2016; Avella-Garcia *et al.*, 2016; Liew *et al.*, 2016; Schultz & Gould, 2016; Bauer *et al.*, 2018; Bittker & Bell, 2018; 2020).

Maternal stress as a result of common life changes and events, such as divorce, financial stress, etc., can also lead to severe ASD outcomes with particular deficits in communication (Varcin *et al.*, 2017; Manzari *et al.*, 2019). Other contributors include advanced parental age (Durkin *et al.*, 2008; Wu *et al.*, 2017b), maternal diet (Peretti *et al.*, 2019), and maternal conditions such as diabetes (Xiang *et al.*, 2015; Li *et al.*, 2016; Wan *et al.*, 2018), obesity (Wang *et al.*, 2016), hypertension (Cordero *et al.*, 2019) and depression (Rai *et al.*, 2013). In addition, maternal depression may play a role in deficits of motor functions, language, behavioural difficulties such as anxiety and depression, aggression and emotional dysregulation, and inability to focus and sleep (Wiggins *et al.*, 2019).

1.3.2.2 Air pollution, heavy metals, and pesticides

Various prenatal exposures in the environment such as air pollution, heavy metals, chemicals, and pesticides, have also been implicated in ASD (Wong *et al.*, 2015a). Prenatal exposure to air pollutants such as nitrogen oxides and particulate matter – products of combustion and fuel emissions – has gained tremendous interest due to the higher incidence of ASD in many heavily polluted geographical areas around the world (Sram *et al.*, 2017; Oudin *et al.*, 2019; Pagalan *et al.*, 2019). Postnatal and adult exposure to these toxins also disrupt the blood brain barrier (BBB) to cause brain inflammation and adult-onset neurological conditions (Calderon-Garciduenas *et al.*, 2008).

Maternal exposure to heavy metals such as mercury, lead, manganese, methylene chloride, nickel, and cadmium (Windham *et al.*, 2006; Roberts *et al.*, 2013; Wong *et al.*, 2015a), also pose a toxic environment for proper fetal brain growth. Detection of higher levels of heavy metals in amniotic fluid (Long *et al.*, 2019), phthalates in urine of pregnant mothers (Miodovnik *et al.*, 2011), and workplace exposures to chemicals (McCanlies *et al.*, 2012), are indicative that parental exposures to toxic substances can be harmful to the growing fetus.

Bisphenol A (BPA) is a chemical that was widely used to make plastics and lining of canned food items, including baby bottles and formula. However, after decades of use, its properties of leaching into food and liquids proved to have adverse effects on various health conditions (Rochester, 2013; Jalal *et al.*, 2018; Nomiri *et al.*, 2019). It is alarming to note that gestational exposure to BPA persisted in urine samples collected from children aged 1-5 years old. Higher BPA levels correlated with ASD occurrence (de Cock *et al.*, 2012; Stein *et al.*, 2015), and impediments in development of language, display of aggressive and hyperactive behaviours, as well as increased anxiety and depression (Braun *et al.*, 2009; Braun *et al.*, 2011; Perera *et al.*, 2012; Jensen *et al.*, 2019).

Lastly, current research shows that exposure and ingestion of pesticides are responsible for various neurological defects. Although pesticides are widely used as a form of pest control and salvation of crops, their exposure over time presents many possible health concerns. Pesticides of a persistent nature such as organochlorines have been banned in North America due to their long half-lives and bioaccumulation risk, but continue to be present in the environment. Persistent pesticides are also still used in most countries around the world. Exposure to or accumulation of these chemicals in pregnant women can be especially detrimental to the developing fetus. Persistent pesticides have been detected in maternal urine (Agay-Shay *et al.*,

2015), maternal serum (Rodriguez-Dozal *et al.*, 2012; Cheslack-Postava *et al.*, 2013; DiPietro *et al.*, 2014; Vafeiadi *et al.*, 2014; Wang *et al.*, 2014a), placenta (Freire *et al.*, 2011), amniotic fluid (Luzardo *et al.*, 2009), fetal meconium (Ostrea *et al.*, 2009; Veyhe *et al.*, 2013), umbilical cord (Koppen *et al.*, 2009; Neta *et al.*, 2010; Vizcaino *et al.*, 2010; Silver *et al.*, 2015; Sturza *et al.*, 2016), and breast milk (Hoover, 1999; Lu *et al.*, 2015; Aerts *et al.*, 2019; Komprda *et al.*, 2019).

Due to the negative effects of persistent pesticides, non-persistent pesticides – such as organophosphates and pyrethroids – that have shorter half-lives and are currently being used in North America. However, numerous studies show that non-persistent pesticides are also detected in pregnant women and potentially affect the fetus (Bradman *et al.*, 2003; Ye *et al.*, 2009; Bouchard *et al.*, 2011; Spaan *et al.*, 2015; Woskie *et al.*, 2017; Philippat *et al.*, 2018; van den Dries *et al.*, 2018; van den Dries *et al.*, 2019). There is a definite correlation between maternal pesticide exposure and various developmental issues in the fetus, such as lower birth weight (Weisskopf *et al.*, 2005; Wolff *et al.*, 2007; Dewan *et al.*, 2013; Vafeiadi *et al.*, 2014), abnormal newborn reflexes (Engel *et al.*, 2007; Woskie *et al.*, 2017), abnormal head circumference (Eskenazi *et al.*, 2004; Wolff *et al.*, 2007; Liu *et al.*, 2016a), cognitive deficits (Rohlman *et al.*, 2005; Marks *et al.*, 2010; Bouchard *et al.*, 2011), developmental delays (motor, social, language) (Liu *et al.*, 2016a; Sagiv *et al.*, 2018), and ASD (Roberts *et al.*, 2007; Brown *et al.*, 2018; Philippat *et al.*, 2018; Sagiv *et al.*, 2018; von Ehrenstein *et al.*, 2019; Ongono *et al.*, 2020).

1.3.2.3 Oxidative stress

Many of the aforementioned environmental conditions including air pollution, metal, and pesticide exposures can also lead to oxidative stress, which is caused by a state of imbalance between free radicals and antioxidants (Nagiah *et al.*, 2015; Jablonska-Trypuc *et al.*, 2017; Kim *et al.*, 2019). The brain is particularly susceptible to oxidative stress due to the following reasons:

1) Requirement of increased oxidative phosphorylation to fulfill a high demand of oxygen and energy, which results in increased production of reactive oxygen species or oxygen free radicals;

2) The brain is rich in lipids and fatty acids, which are known targets of lipid peroxidation (Koudelova & Mourek, 1994; Leutner *et al.*, 2001). Prenatal stress factors that increase oxidative stress alter neurons and cause DNA damage in the brain (Zhu *et al.*, 2004; Song *et al.*, 2009), leading to abnormal behaviours (Lante *et al.*, 2007; Neri *et al.*, 2015; Bharadwaj *et al.*, 2018). Increased levels of biomarkers for oxidative stress have been found in blood and urine samples of individuals with ASD (Damodaran & Arumugam, 2011; El-Ansary *et al.*, 2017; Oshodi *et al.*, 2017; Osredkar *et al.*, 2019; Bjorklund *et al.*, 2020). Thus, it is evident that environmental factors play a significant role in the manifestation of pathologic conditions during development of the brain.

Many genes and environmental factors have been discovered in the etiology of ASD, and an interaction between the two may result in severe outcomes of the condition (Ehninger *et al.*, 2012; Schaafsma *et al.*, 2017). Some individuals with ASD may have a genetic profile which is more susceptible to environmental triggers. Various groupings of genes – copy number variants and single nucleotide variants – have been identified as being more sensitive to factors such as exposure to valproic acid (Furnari *et al.*, 2014), bisphenol A (Kundakovic 2015, Singh 2012), air pollution (Wei *et al.*, 2016a), and heavy metals (Stamova *et al.*, 2011; Tian *et al.*, 2011; Rahbar *et al.*, 2016). Epigenetic mechanisms, such as DNA methylation, chromatin remodelling, and histone acetylation (Chaste & Leboyer, 2012; Tordjman *et al.*, 2014), influence the expression of many genes leading to abnormalities and diverse behavioural profiles. Examples of well known epigenetic factors implicated in ASD are CHD8 (chromodomain helicase DNA binding protein 8) (Cotney *et al.*, 2015; Wilkinson *et al.*, 2015), HDAC (histone deacetylases) (Moldrich *et al.*,

2013), and MECP2 (methyl CpG binding protein 2) (Shibayama *et al.*, 2004). Therefore, individuals with a genetic predisposition may be more easily influenced by environmental factors that cause perturbation to the development of the brain.

1.4 Mouse Models of ASD

The multifactorial nature of ASD in addition to a long sensitive window during development, makes it a complex disorder to study. Moreover, cellular and molecular changes resulting from genetic or environmental triggers, can only be studied in deceased human samples. There are limitations to these studies as the sample size and characteristics of the individuals is variable. It is also impossible to understand the ongoing mechanisms during the gestational period. Due to these shortcomings, the last few decades have relied extensively on animal models to understand the possible occurrences in the brain, and translate the knowledge to humans.

Mouse models are especially favourable for research because of their short reproductive time compared to humans. Deficits in genes or environmental exposures can be evaluated at a rapid rate, and assessments can include experiments that are beyond ethical limits to conduct on humans. Overall, there are three categories that are studied in animal models: 1) Cellular and molecular outcomes of ASD-risk genes; 2) Naturally occurring ASD models – Inbred strains that display autism symptoms without external influences; and 3) Environmental factors associated with ASD. Validation of animal models as reliable representatives of ASD is required through stringent and analogous behavioural assays that can be translated to human symptoms. This review will focus on a few current well established rodent models in the field of autism research, particularly those exhibiting synaptic defects.

1.4.1 Cellular and molecular outcomes of ASD-risk genes

Hundreds of ASD-risk genes are currently associated with ASD, inclusive of a subset of genes that have strong associations. Using animal models is critical in characterization of these genes and understanding the consequences of their abnormal expression. One of the major functional deficits in ASD etiology are modifications in synaptic operations, which may be caused by several gene groupings.

1.4.1.1 SHANK genes

SHANK genes (*SHANK1*, *SHANK2*, and *SHANK3*) encode for scaffolding proteins expressed in the post-synaptic density of excitatory neurons, and have been repeatedly reported in individuals with ASD (Durand *et al.*, 2007; Moessner *et al.*, 2007; Gauthier *et al.*, 2009; Berkel *et al.*, 2010; Leblond *et al.*, 2012; Sato *et al.*, 2012; Boccuto *et al.*, 2013; Soorya *et al.*, 2013). Deletion of *SHANK1* or *SHANK2* in mice causes a reduction in the thickness of the post-synaptic density (PSD), and number and size of dendritic spines, leading to decreased synaptic transmission (Hung *et al.*, 2008; Schmeisser *et al.*, 2012). These mice also exhibit fewer social interactions, decreased communication, and motor difficulties such as increased repetitiveness in anxious behaviours like self-grooming (Silverman *et al.*, 2011; Wohr *et al.*, 2011b; Sungur *et al.*, 2014; Wohr, 2014). In addition, *SHANK2* deletion may also affect transmission in GABAergic neurons (Lim *et al.*, 2017), and excitation in cerebellar Purkinje cells leading to deficits in various motor-associated behaviours (Ha *et al.*, 2016; Peter *et al.*, 2016). *SHANK3* is more largely recognized as an ASD-risk gene, and its depletion has been achieved in many mouse models to show deficits in connectivity of the cortex and striatum, as well as a reduction in spine density and PSD thickness similar to the effects of other *SHANK* proteins. Behaviourally, they exhibit characteristic ASD-like symptoms such as deficits in socialization, communication, and

motor functions. Overall, it has been determined that decreased expression of *SHANK* genes alter some morphological characteristics of dendritic spines and affect connectivity and transmission between neurons.

1.4.1.2 Neurexins (*Nrxns*)/Neuroligins (*Nlgn*)

Neurexins (*Nrxns*) and neuroligins (*Nlgns*) are cell-adhesion molecules involved in binding of the synaptic cleft, and important for synapse formation and differentiation in synaptic signalling (Craig & Kang, 2007). *Nrxns* are localized on the presynaptic membrane and bind to *Nlgns* on the postsynaptic membrane of neurons. Although there are three *Nrxn* genes, *Nrxn1* has been most frequently associated with ASD (Kim *et al.*, 2008; Wisniewiecka-Kowalnik *et al.*, 2010; Liu *et al.*, 2012b; Yangngam *et al.*, 2014; Onay *et al.*, 2016). Mouse/rat models with a deficiency in the *Nrxn1* gene exhibit classic ASD symptoms such as increase in repetitive behaviours (Etherton *et al.*, 2009; Rabaneda *et al.*, 2014), decrease in social interactions (Grayton *et al.*, 2013; Rabaneda *et al.*, 2014), cognitive defects, hyperactivity (Esclassan *et al.*, 2015), and aggressive behaviours (Grayton *et al.*, 2013). Such animal studies successfully show that a defect in the *Nrxn1* gene can lead to abnormal excitatory current frequency in hippocampal neurons and glutamatergic synaptic transmission in cortical neurons (Etherton *et al.*, 2009; Rabaneda *et al.*, 2014).

Of the Neuroligin (*Nlgn*) proteins, the variants *Nlgn3* and *Nlgn4* are associated with increased ASD risk (Jamain *et al.*, 2003; Laumonnier *et al.*, 2004; Yan *et al.*, 2005). A specific mutation of *Nlgn3*, a substitution from arginine to cysteine at position 451 (R451C) is extensively studied in mouse models. Mice with this particular mutation have difficulties in social behaviours (Cao 2018, Tabuchi 2007), but outperform their wild-type (WT) counterparts in spatial learning tasks due to enhanced spatial memory (Tabuchi 2007). Expression of the

R451C mutation in cells causes the resulting protein product to be largely confined in the ER, with a small proportion being able to translocate to the surface of the membrane (Chih *et al.*, 2004; Comoletti *et al.*, 2004). In addition, the proteins that do successfully localize to the cell membrane (Comoletti *et al.*, 2004), have reduced ability of binding to neurexin, a connection which is critical to relay signals during a synapse. Studies on synaptic properties show that mice expressing the R451C mutation exhibit greater frequency of inhibitory synapses (Tabuchi 2007), less excitation in interneurons, and inefficient gamma oscillations in the prefrontal cortex (Cao 2018). An increase in inhibitory synapses was also observed in the somatosensory cortex, whereas there was an increase in excitatory synapses and more complex dendritic branching in the hippocampus (Etherton *et al.*, 2011). The *Nlgn3* (R451C) mutation is also associated with gastrointestinal issues, which is a common co-morbid condition of ASD. An increase in transit time in the small intestine and altered levels of gut microbiota were both found to be probable causes of the gastrointestinal symptoms (Hosie *et al.*, 2019). Therefore, the *Nlgn3* (R451C) mutation in mouse models identified various changes in synaptic connections in the brain, as well as ASD-like gastrointestinal conditions.

Nlgn4 is expressed throughout the brain, and its knockout in mice results in the classic triad of ASD symptoms – deficits in social interactions, reduction in ultrasonic vocalizations (speech), and an increase in repetitive behaviours (Jamain *et al.*, 2008; Ey *et al.*, 2012; El-Kordi *et al.*, 2013; Ju *et al.*, 2014). Similar to the effects of *Nlgn3* (R451C) mutation, *Nlgn4* knockout mice also exhibit defects in inhibitory synaptic transmission – GABAergic and glycinergic, and have altered gamma oscillations in cells of the hippocampus (Hammer *et al.*, 2015; Zhang *et al.*, 2018) (Hammer 2015, Zhang 2018). It is interesting to note that *Nlgn4* mutation also causes

impairments in social interactions of *Drosophila* (Corthals *et al.*, 2017). Thus, its function and involvement in symptoms of ASD is apparent across species.

1.4.1.3 *Fragile X Mental Retardation 1 (FMR1) Gene*

The *FMR1* gene encodes for FMRP (Fragile X Mental Retardation Protein), which causes Fragile X Syndrome (FXS). Over 90% of children that have FXS also display symptoms of ASD, making it the most common monogenetic known cause of ASD. FMRP is localized in dendritic spines, and its depletion can cause havoc on various functions of synaptic plasticity. Disruption of *FMR1* causes interruption in the normal role of FMRP, which includes the regulation of dendritic mRNA translation – critical for LTP and LTD functions, and development and function of synapses (Garber *et al.*, 2006; Bassell & Warren, 2008). High throughput sequencing of RNAs isolated by crosslinking immunoprecipitation was used to uncover more than 800 genes that interact with FMRP, with enrichments in various synaptic functions and many targets revealed as previously known ASD-risk genes (Darnell *et al.*, 2011).

Knockout of the *FMR1* gene in mice has revealed analogous behavioural outcomes to humans with ASD. These mice exhibit social deficits (Spencer *et al.*, 2005; Mineur *et al.*, 2006; McNaughton *et al.*, 2008; Sare *et al.*, 2019), repetitive behaviours indicated by increased self-grooming, and marble burying (Pietropaolo *et al.*, 2011; Sare *et al.*, 2019), difficulties in learning and memory (Mineur *et al.*, 2002; Spencer *et al.*, 2005), fewer ultrasonic vocalizations (Toledo *et al.*, 2019), hyperactivity (Peier *et al.*, 2000; Mineur *et al.*, 2002; Spencer *et al.*, 2005; Pietropaolo *et al.*, 2011), and unsettled sleep (Sare *et al.*, 2017).

As a result of the *FMR1*-KO condition, mice exhibit larger brain volume than their WT counterparts (Lai *et al.*, 2016), which may be due to an increase in the number of oligodendrocytes and hence myelination in the cortex (Lee *et al.*, 2019). The *FMR1* mutation

also resulted in fewer neurons in the cingulate cortex – part of the limbic system and involved in learning and memory as well as regulation of emotions.

As FMRP is localized in dendritic spines, its abnormal expression results in changes of spine characteristics. Various brain structures of *FMR1*-KO mice exhibit an increase in spine density (Comery *et al.*, 1997; Nimchinsky *et al.*, 2001; Galvez & Greenough, 2005; McKinney *et al.*, 2005; Liu *et al.*, 2011), longer dendritic spines (Nimchinsky *et al.*, 2001; Liu *et al.*, 2011), and greater number of spines with immature morphology (Comery *et al.*, 1997; Grossman *et al.*, 2006; Cruz-Martin *et al.*, 2010; Liu *et al.*, 2011; Su *et al.*, 2011). Neonatal mouse brains deficient in FMRP show an abnormal reorganization of F-actin which impacts the morphology of dendritic spines (Lee *et al.*, 2019). Abnormalities in dendritic spines also contribute to altered synaptic plasticity such as amplified LTD as a result of increased metabotropic glutamate receptor activity (Huber *et al.*, 2002; Chatterjee *et al.*, 2018). Therefore, it is evident that alteration or ablation of *FMR1* leads to brain abnormalities that contribute to the behavioural outcome of ASD.

1.4.2 Naturally Occurring ASD Models – Inbred Strains

There are a few known inbred strains of mice that exhibit classic symptoms of ASD, similar to the genetically engineered mouse models as indicated in the previous section. Most common examples of these are the BALB/c and BTBR T+tf/J mouse strains.

1.4.2.1 BALB/c Mouse Strain

The Balb/c mouse strain is well known in exhibiting reduced sociability. When compared to other outbred strains, there were significant differences in social behaviours such as increased avoidance, hence fewer social and reciprocal interactions with other mouse subjects (Brodkin *et al.*, 2004; Sankoorikal *et al.*, 2006; Moy *et al.*, 2007; Panksepp *et al.*, 2007; Jacome *et al.*, 2011).

Example of interaction and avoidance behaviours can be measurements of active sniffing periods of the novel mouse, and freeze and withdraw events near the novel mouse, respectively. Balb/c mice also display fewer sexual behaviours such as pursuing, mounting, and sniffing genital areas of a novel mouse (Brodkin, 2007; Jacome *et al.*, 2011). These mice also exhibit a reduction in vocalizations during same-sex interactions (Panksepp *et al.*, 2007).

Research studies have shown that abnormal social behaviours in the Balb/c mouse may be due to a reduction in size of the corpus callosum (Fairless *et al.*, 2008) or the entire brain as a whole (Fairless *et al.*, 2012). More microscopic investigations suggest that transmission by NMDA receptors is altered in these mice, and also likely associated with the decreased social behaviours (Deutsch *et al.*, 1997; Deutsch *et al.*, 1998; Burket *et al.*, 2010).

1.4.2.2 BTBR T+tf/J

The BTBR T+tf/J mouse is another inbred strain which displays many classic symptoms of ASD. Various measures and tests revealed stark differences between the BTBR T+tf/J mouse and other highly sociable mouse strains, as well as comparisons of other ASD behaviours (Moy *et al.*, 2007; McFarlane *et al.*, 2008; Silverman *et al.*, 2010). The BTBR T+tf/J mouse strain is naturally less social and spends less time sniffing around the chamber with a novel mouse in contrast to the C57BL/6J strain, which show a lot more interest in the novel mouse (Moy *et al.*, 2007; Yang *et al.*, 2007; McFarlane *et al.*, 2008). These mice also display fewer reciprocal social interactions such as less time spent interacting with each other, reduction in social grooming, and overall an increased amount of time spent being inactive (Bolivar *et al.*, 2007; McFarlane *et al.*, 2008). The BTBR T+tf/J mice at juvenile stages display a substantial increase in self-grooming which is indicative of repetitive behaviours (Yang *et al.*, 2007; McFarlane *et al.*, 2008; Silverman *et al.*, 2010) that may be sex-specific in males only (Amodeo *et al.*, 2019). In

addition, the adult BTBR T+tf/J mice produce fewer ultrasonic vocalizations, also observed in the BALB/c mice (Wohr *et al.*, 2011a). Interestingly, at earlier development ages, pups produced a greater number of vocalizations, but of a more unusual nature (Scattoni *et al.*, 2008; Scattoni *et al.*, 2011). Studying the mechanisms behind these abnormal vocalizations may help in getting further in understanding why individuals with ASD talk in atypical pitches and tones.

Deeper anatomical, cellular, and molecular investigations reveal the mechanisms that are responsible for the behaviours of the BTBR T+tf/J mouse strain, allowing for researchers to draw potential parallels with clinical studies. Anatomical analysis of the brain indicated severe differences in the BTBR T+tf/J strain compared to C57BL/6 (Mercier *et al.*, 2012) (23). The hippocampi on both sides were set further apart than normal, and the separation comprised of brain meninges in place of the corpus callosum. The corpus callosum separates and connects the right and left hemispheres of the brain, and is missing in BTBR T+tf/J mice (Wahlsten *et al.*, 2003; Mercier *et al.*, 2012). The amygdala and amygdalar nuclei – responsible for emotions – are also reduced in size, similar to what is observed in human individuals with ASD (Mercier *et al.*, 2012).

The BTBR T+tf/J mouse strain also display increased oxidative stress in the cerebellum, which was shown to be associated with analogous repetitive behaviours of ASD (Nadeem *et al.*, 2019). They have increased levels of oxidative stress markers and oxidation of lipids and proteins in the cerebellum, along with decreased levels of antioxidants resulting in inefficiency of neutralizing harmful oxidants. Supplementation of folic acid to BTBR T+tf/J mice improved levels of oxidants to reduce oxidative stress, and resulted in a correction of repetitive behaviours as well as other social and memory deficits (Zhang *et al.*, 2019).

Several protein and immunoglobulin levels are also altered in the BTBR T+tf/J mouse brain (Hwang *et al.*, 2015). A reduction of Syntaxin-1a – a presynaptic protein involved in docking synaptic vesicles to the plasma membrane – alongside a reduction of the neurotransmitter, glutamate, was found in the cerebral cortex of these mice (Wei *et al.*, 2016b). In the hippocampus, there were defects in excitatory synapses as well as an increase in inhibitory transmission through the GABA neurotransmitter in newborn BTBR T+tf/J mice (Cellot *et al.*, 2016). Altogether, there is compelling evidence that the BTBR T+tf/J mice possess defective mechanisms of synaptic functions in the brain.

1.4.3 Environmental factors associated with ASD

1.4.3.1 Valproic Acid

Valproic acid (VPA) is an anticonvulsant drug which is most commonly prescribed for epileptic seizures, migraines and bipolar disorders (Johannessen & Johannessen, 2003). It has been established as a teratogen that can cause various embryonic deformities such as spina bifida, dysmorphic structures and features, as well as cognitive deficits (Phiel *et al.*, 2001; Ornoy, 2009; Tomson *et al.*, 2016; Nicolini & Fahnestock, 2018). Human studies of maternal VPA use can result in fetal valproate syndrome, with a substantial risk of comorbidity with ASD (Ardinger *et al.*, 1988; Williams *et al.*, 2001; Dean *et al.*, 2002; Rasalam *et al.*, 2005).

Similar outcomes have been observed in animal models that were subjected to gestational exposure to VPA. Administration of VPA to pregnant monkeys lead to behavioural and molecular changes in their offspring. They exhibited ASD-like behaviours such as fewer social interactions, repetitive walking, and unfocused eye attention to social stimuli (images with faces) (Zhao *et al.*, 2019). In the brain, there were fewer mature neurons, but an increase in astrocyte numbers in the prefrontal cortex, and a decrease in neuron proliferation in the cerebellum – all

indicative of abnormal neurogenesis. Furthermore, genetic analyses showed disruptions in various neurodevelopmental and signalling genes and pathways.

In mice, gestational exposure to VPA also leads to abnormal social behaviours (Roullet *et al.*, 2010), differential regulation of gene expression (Roullet *et al.*, 2010; Weinstein-Fudim *et al.*, 2019), and a decrease in immune factors (Baronio *et al.*, 2018). Assessments of social behaviour show that VPA-exposed mice were indifferent to the addition of a novel mouse subject, whereas their control counterparts spent more time exploring the side with the novel mouse, implying decreased sociability (Roullet *et al.*, 2010). Nasal administration of oxytocin in the VPA-induced ASD mice improved social interactions for approximately 2 hours (Hara *et al.*, 2017). Processing of olfactory cues may also be affected in these mice, as they spent significantly more time finding their bedding (Roullet *et al.*, 2010). Genetic analysis revealed decreased cortical levels of the ASD-risk gene, brain-derived neurotrophic factor (BDNF), important in the organization and plasticity of neurons. VPA exposure to neuro-2A cells, acts to inhibit histone deacetylase and β -catenin, causing hyper-acetylation and activation of the Wnt pathway respectively – both events that cause increased transcription of genes.

Postnatal exposure to VPA in mice also causes behavioural and molecular changes. There were significant decreases in behaviours such as natural sniffing of the genital area, grooming, motor activity, and frequency of crawling over and under each other, in VPA-exposed mice (Yochum *et al.*, 2008). Staining of granule cells in the cerebellum and hippocampus showed increased numbers of apoptotic cells in these brain regions, which would potentially explain the delay in behaviours. Studies on VPA exposure have been helpful in understanding modifications to the brain that may be translated to human clinical studies of ASD.

1.4.3.2 Maternal Infection

Maternal infection is a significant risk factor associated with the occurrence of ASD in human offspring (Yamashita *et al.*, 2003; Libbey *et al.*, 2005; Atladottir *et al.*, 2010; Atladottir *et al.*, 2012; Engman *et al.*, 2015; Valayi *et al.*, 2017; Slawinski *et al.*, 2018; Mawson & Croft, 2019). Many studies using mouse models have confirmed the presentation of classic ASD symptoms in the offspring – social deficits such as decreased preference for a novel mouse, decreased frequency of sniffing (Malkova *et al.*, 2012; Schwartz *et al.*, 2013; Fernandez de Cossio *et al.*, 2017); repetitive behaviours such as increased marble burying and grooming (Malkova *et al.*, 2012; Schwartz *et al.*, 2013; Fernandez de Cossio *et al.*, 2017); and abnormalities in ultrasonic vocalizations (Malkova *et al.*, 2012; Yee *et al.*, 2012; Schwartz *et al.*, 2013; Choi *et al.*, 2016; Fernandez de Cossio *et al.*, 2017).

Genetic analysis following maternal infection with Influenza A virus resulted in differential expression of ASD-risk genes (*i.e.* *Auts2*, *Foxp2*, *Myt1l*, *Nrcam*, *Sox2*, etc) (Fatemi *et al.*, 2008; Fatemi *et al.*, 2009b), myelination genes (Fatemi *et al.*, 2009a), hormones (Miller *et al.*, 2013), and neurotransmitters (*i.e.* Serotonin) (Fatemi *et al.*, 2009a; Miller *et al.*, 2013). Viral infections can also cause anatomical changes in the brain, such as a decrease in hippocampal volume (Fatemi *et al.*, 2009b), cerebellar volume, total brain volume (Fatemi *et al.*, 2009a) due to a decrease in neurons (Fatemi *et al.*, 2008), and thickening of white matter in the brain regions such as the corpus callosum (Fatemi *et al.*, 2009a). The structure of the cerebellum in neonatal mice had an atypical wide shape and increase in the number of Purkinje cells (Aavani *et al.*, 2015). Furthermore, Purkinje cells were found in lobules VI and VII, when they are normally localized to the Purkinje layer of the cerebellum. This may suggest incomplete migration of Purkinje cells in the cerebellum during development. (Shi *et al.*, 2009).

Maternal infection can also lead to changes in synaptic functions in the offspring. There was increased excitation of cells in the anterior cingulate cortex, which is responsible for cognition, emotions, empathy and decision-making (Okamoto *et al.*, 2018). Inhibition of these cells improved social behavioural symptoms in the mouse offspring. A balance in neuronal excitation in the brain is important for healthy brain growth and maintenance. Hyper-excitation may be a result of faulty inhibitory mechanisms by inhibitory GABA neurotransmitters. In prenatal brain development, GABA plays an excitatory role, and switches to its well-known inhibitory role following birth – this developmental event is termed the GABA switch. This switch is critical to the excitatory-inhibitory balance in the brain, and is found to be delayed in offspring exposed to maternal infection (Corradini *et al.*, 2018). Therefore, activation of the immune system upon infections can wreak havoc on the developing brain of the fetus.

Mouse models of ASD have provided an array of insight into the potential molecular mechanisms of the disorder, and allowed for research that is out of reach or unethical in human subjects. Ongoing studies can continue to enhance the current repertoire of knowledge on early development of the brain and its pathology.

1.5 Early Brain Development

The brain is the most complex organ, responsible for a wide array of functions, making its proper development and organization critical to functioning of the entire nervous system. In humans, development of the CNS is initiated shortly after conception at approximately 3 weeks of gestational age and continues until after birth. The CNS is derived from the ectoderm layer during embryogenesis with the formation of the neural tube.

During neurogenesis, there are numerous critical events that take place such as proliferation of neural stem cells (Hemond & Glover, 1993), migration (Choi, 1979),

differentiation of progenitor cells (Buse & Krisch, 1987), synaptogenesis (Seeds & Vatter, 1971), myelination of axons (Meier, 1976), and apoptosis of excessive neurons (Homma *et al.*, 1994). There are critical windows during these events within which various environmental insults/agents can interfere in development, and cause a cascade of abnormal regulation (Figure 1-3). Timing of the insult determines the type and magnitude of deficit that can occur, and area of brain that may be affected (Rice & Barone, 2000; Selevan *et al.*, 2000; LeBlanc & Fagiolini, 2011). For example, exposure to a teratogen very early on can affect all subsequent developmental events, in contrast to a later exposure at which time many developmental events are already complete and no longer sensitive to changes. The brain is vulnerable to different toxins and drugs during particular windows of opportunity and can cause consistent defects and delays in the fetus (Heyer & Meredith, 2017). Interestingly, various environmental exposures during gestation can disturb brain lipids, which serve many diverse roles during early neurodevelopment (Wong *et al.*, 2015a).

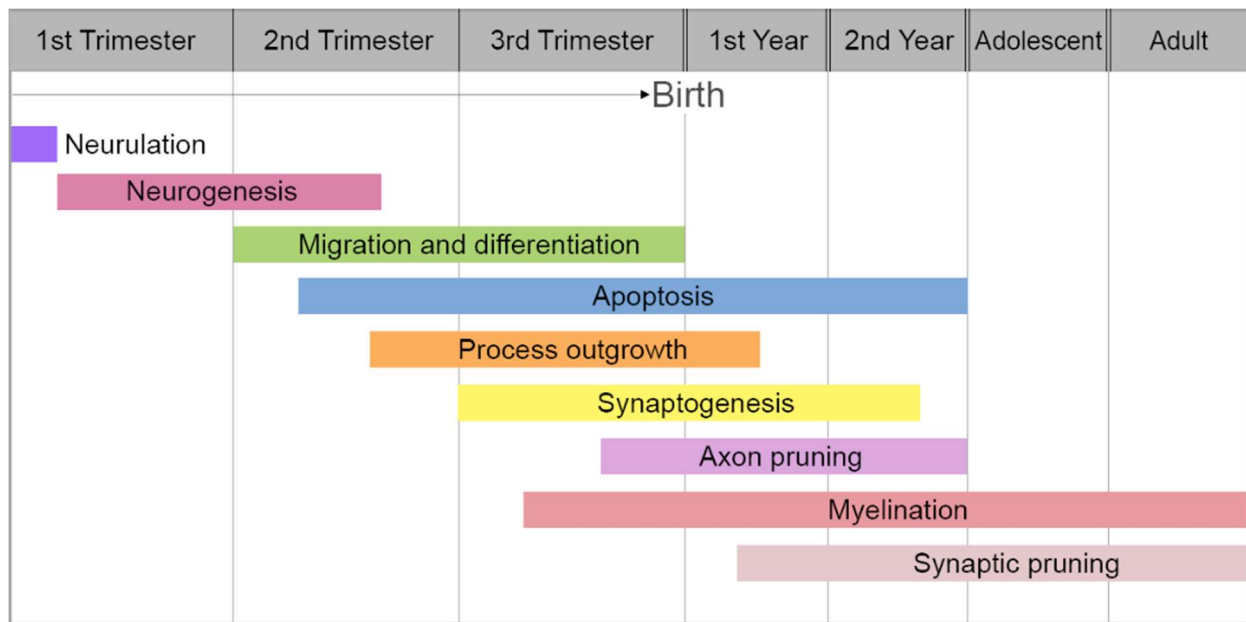


Figure 1-3. Timing of neurodevelopmental events of the human brain. From conception, the central nervous system undergoes many timed events that are sensitive to genetic and environmental perturbances. Timing of insult determines which processes could be affected. Image adapted from (Loth *et al.*, 2016).

1.5.1 Lipids in the Brain

Lipids are a key constituent of the human brain and make up 50-60% of its dry weight. The complexity of lipids in the brain particularly have evolved faster, and have a unique profile distinct from other organs and tissues in the body (Bozek *et al.*, 2015). In the neocortex region of the human brain, the concentration of lipids is more advanced compared to rodents and other primate species, defining a role in complex higher-order functions expressed exclusively by the human species. In the first few years of life, during stages of rapid neurodevelopment, the

differences in lipid composition between humans and other species are the most pronounced (Li *et al.*, 2017).

Of the brain lipids, more than 20% are polyunsaturated fatty acids. The major essential fatty acids are omega-6 linoleic acid (LA), and omega-3 α -linolenic acid (ALA), which are acquired through diet and serve as structural components and signalling molecules throughout life. LA and ALA are metabolized to arachidonic acid (AA) and docosahexaenoic acid (DHA), respectively (Figure 1-4). There is an abundance of DHA and AA in the brain, especially during early brain development, as the brain is rapidly growing with significant increases in white and grey matter (Svennerholm, 1968; Clandinin *et al.*, 1980; Martinez, 1992). These fatty acids serve as key constituents of the phospholipid membrane, and are broken down into metabolites for various functions such as maintenance of the membrane, gene transcription, synaptic transmission, neuronal plasticity, apoptosis, and inflammation (Massicotte, 2000; Carlson, 2001; Sun *et al.*, 2004; Chen & Bazan, 2005; Burke & Dennis, 2009; Poorani *et al.*, 2016).

The downstream pathway of EPA/DHA exhibit anti-inflammatory roles, whereas AA is responsible for the release of inflammatory signalling molecules. AA is readily released from the phospholipid membrane by phospholipase-2 (PLA₂) (Burke 2009). It is then metabolized by the enzymes cyclooxygenases-1 and -2 (COX-1,-2), to prostaglandin G₂ (PGG₂), which is converted to prostaglandin H₂ (PGH₂). Prostaglandin synthases (PGS) metabolize PGH₂ into various eicosanoids such as prostaglandin D₂ (PGD₂), prostaglandin F_{2 α} (PGF_{2 α}), prostacyclin I₂ (PGI₂), thromboxane A₂ (TXA₂) and prostaglandin E₂ (PGE₂) (Wang *et al.*, 2007) (Figure 1-5). These eicosanoids exert their effects through binding to multiple receptors. The recycling of lipids as important signalling molecules allow for continuous maintenance of the phospholipid membrane, and is crucial for proper development of the brain.

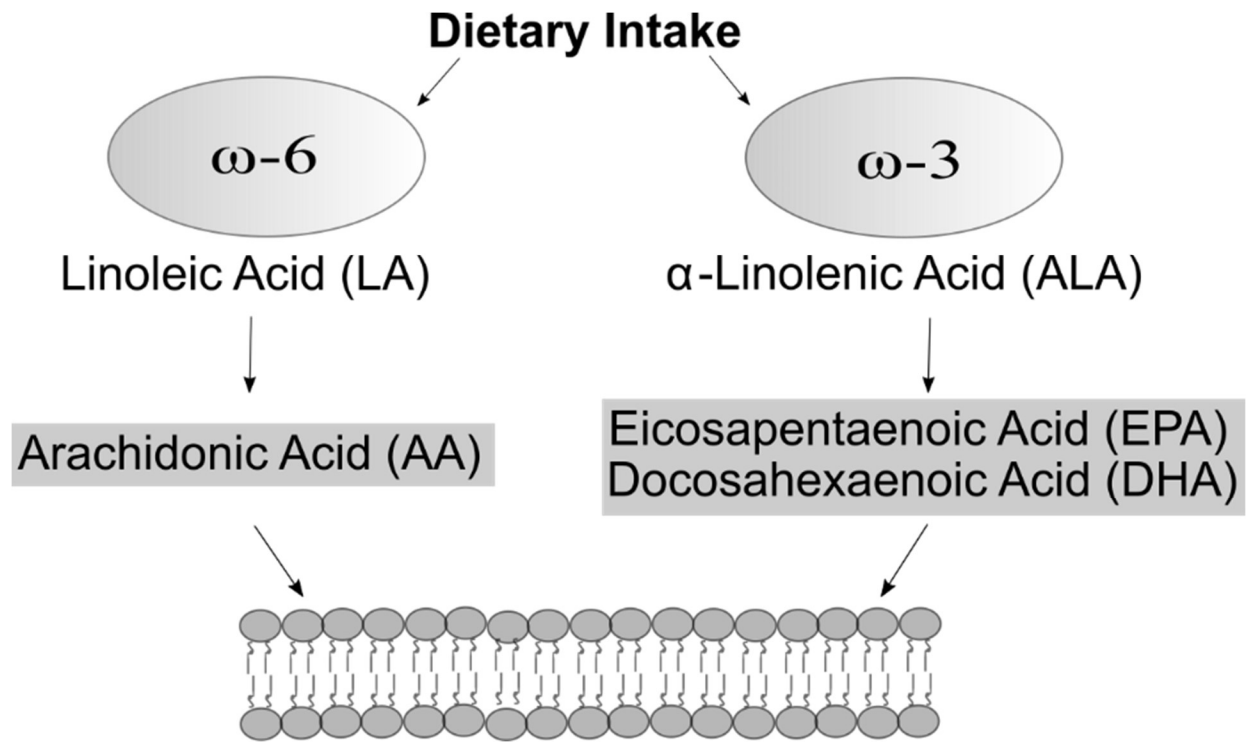


Figure 1-4. Intake of dietary fats are metabolized into essential fatty acids that are incorporated into the phospholipid membrane.

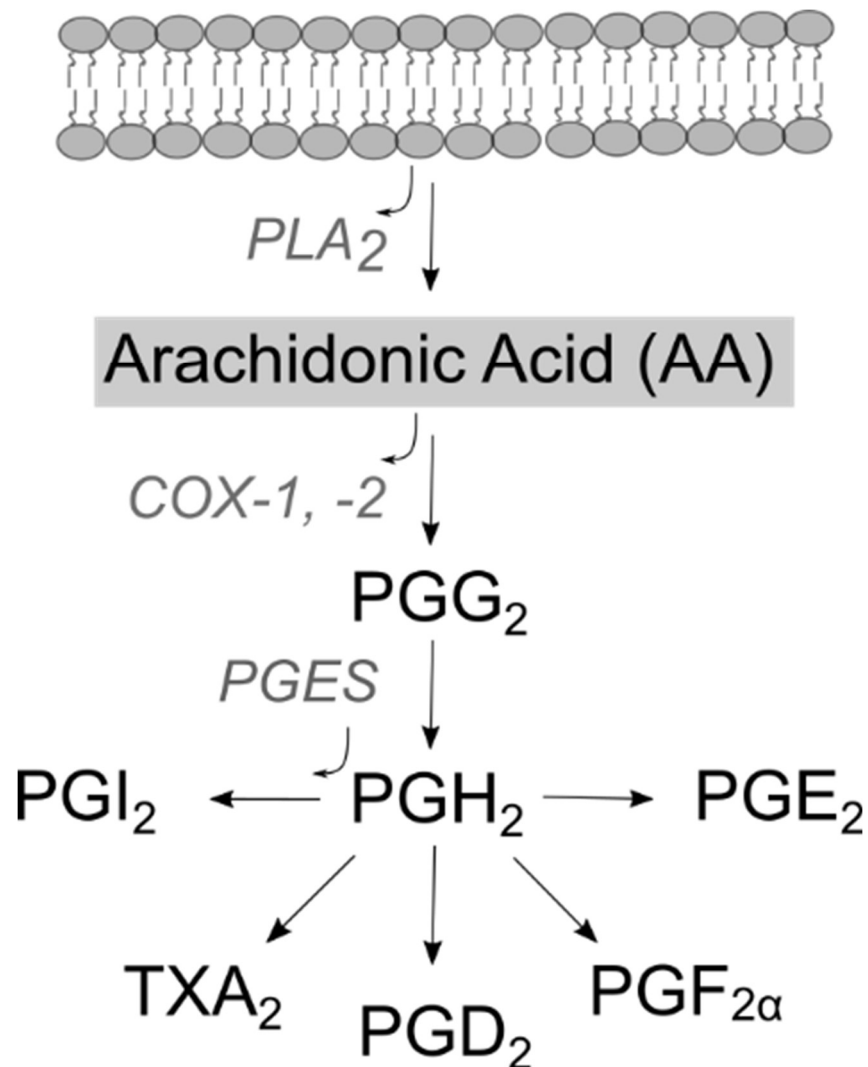


Figure 1-5. Metabolism of arachidonic acid to inflammatory prostaglandins. Arachidonic acid is released from the phospholipid membrane by phospholipase A₂ (PLA₂), and metabolized to Prostaglandin G₂ (PGG₂) by cyclooxygenase enzymes 1 and 2 (COX-1, -2). Prostaglandin synthase (PGES) converts Prostaglandin H₂ (PGH₂) to prostacyclin I₂ (PGI₂), thromboxane A₂ (TXA₂), prostaglandin D₂ (PGD₂), prostaglandin F_{2α} (PGF_{2α}) and prostaglandin E₂ (PGE₂).

1.5.2 Abnormal Brain Lipids in ASD

Lipid composition and concentration are essential during early brain development, especially of essential fatty acids (omega-3 and omega-6), and their metabolites. Prenatal and postnatal nutritional deficiencies of these fatty acids have been linked to increased risk of ASD (Schultz *et al.*, 2006). ASD prevalence was significantly higher in children who were formula-fed without supplementation of fatty acids, compared to children who were breast-fed or formula-fed with supplementation of DHA and AA. ASD individuals may also have an imbalance in the plasma levels of DHA:AA and EPA:AA, caused by increased DHA/EPA and decreased AA (Yui *et al.*, 2016a; Yui *et al.*, 2016b), which leads to the downregulation of its downstream signalling molecules (Yui *et al.*, 2016b). A Canadian study found that children with ASD had an imbalanced ratio between Omega-3 and Omega-6, and consequently reduced levels of DHA, EPA, and AA in blood cells and serum fatty acid concentrations (Jory, 2016). Many studies provide additional evidence to show that children with ASD have inadequate levels of fatty acids compared to typically developing children (Vancassel *et al.*, 2001; Meguid *et al.*, 2008; Wiest *et al.*, 2009; Parellada *et al.*, 2017). Research studies also show that ASD children who were supplemented with fatty acids, showed a remarkable improvement in symptoms of ASD as measured by CARS (Childhood Autism Rating Scale) (Meguid *et al.*, 2008), and SRS (Social Responsiveness Scale) assessment scores (Parellada *et al.*, 2017). There is sufficient evidence to validate the effects of abnormal lipid levels in ASD, as well as fatty acid supplementation as treatment to improve behavioural outcomes. It is then expected that an imbalance in fatty acids would affect their downstream metabolites and dysregulate essential signalling mechanisms.

1.6 Prostaglandin Pathway – Implications in ASD

1.6.1 *Phospholipase A2 (PLA₂)*

Phospholipase A2 (PLA₂) is an important mediator of inflammation, with a key responsibility to release AA from the plasma membrane and initiate the cascade of events leading to eicosanoid production. Previous studies have provided evidence for increased levels (Bell *et al.*, 2004; Hayek *et al.*, 2017; Qasem *et al.*, 2017) and activity of PLA₂ (Tostes *et al.*, 2013; Hayek *et al.*, 2017) to be associated with ASD. In addition, altered gene expression via a gene polymorphism of PLA₂ gene (Liu *et al.*, 2016b), and dysregulation of various PLA₂ genes (Ning *et al.*, 2015) have also been associated with ASD. These studies suggest that PLA₂ defects may be considered as a potential biomarker for ASD diagnosis (Hayek *et al.*, 2017; Qasem *et al.*, 2017).

1.6.2 *Cyclooxygenase Enzymes*

Upon the release of AA from the plasma membrane, the enzymes cyclooxygenase-1 and -2 (COX-1, -2), metabolize it to prostaglandin G₂ (PGG₂), which is converted to prostaglandin H₂ (PGH₂), to initiate the formation of prostaglandins (PGs) (Figure 1-6).

1.6.2.1 *Cyclooxygenase-1 (COX-1)*

COX-1 is constitutively expressed throughout the brain (O'Neill & Ford-Hutchinson, 1993; Amateau & McCarthy, 2004; Kirkby *et al.*, 2012), and is commonly known as the housekeeping enzyme responsible for the production of regulatory PGs. However, emerging evidence is identifying its role in development, inflammatory functions, and disease (Hoozemans *et al.*, 2001; Loftin *et al.*, 2002). COX-1 is primarily expressed by macrophages (Deininger *et al.*, 1999) and microglia, and its intracellular enzymatic activity is predominantly in the endoplasmic reticulum of the cytoplasm (Morita *et al.*, 1995).

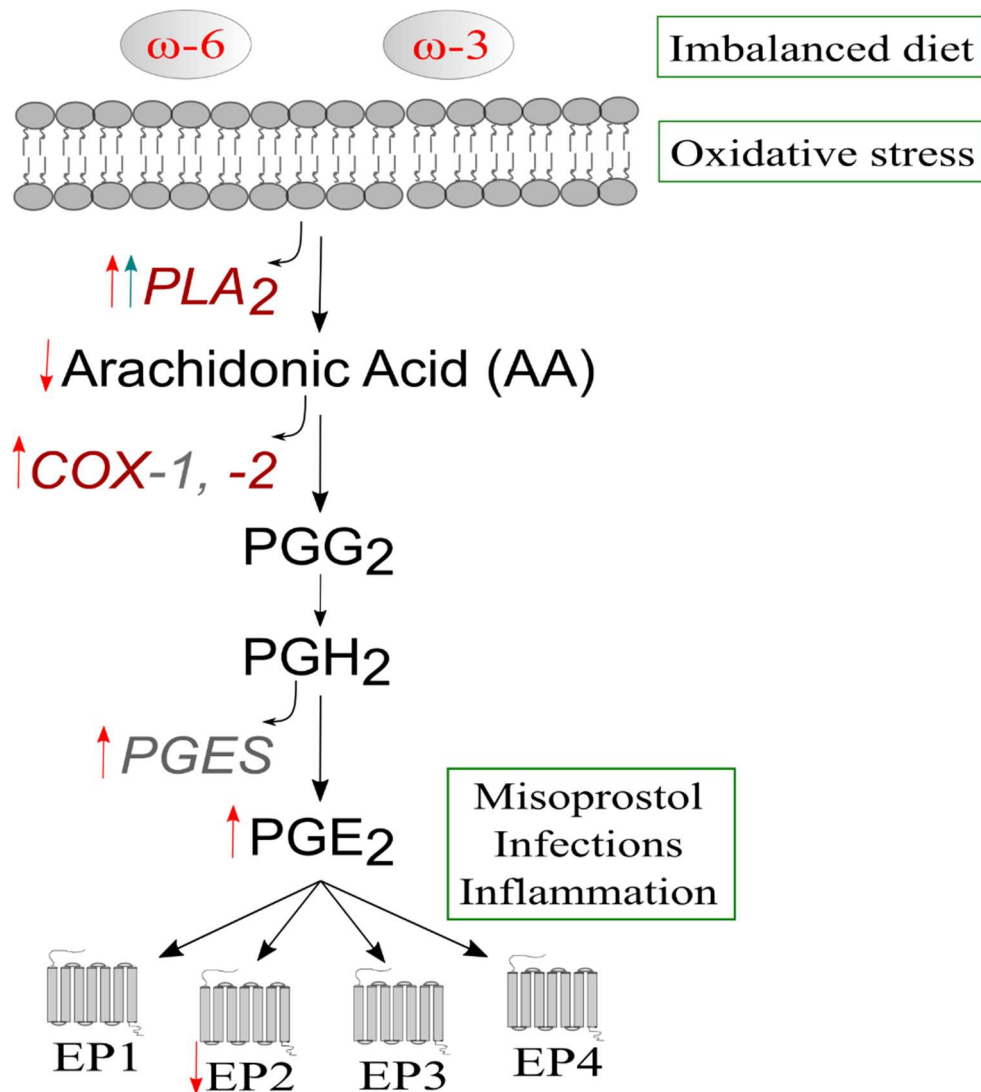


Figure 1-6. Schematic diagram of genetic and environmental perturbances of the COX/PGE₂ signalling pathway in ASD. Environmental influences or genetic defects can cause abnormal function of the pathway at various points of metabolism, which can lead to changes in gene expression and cell function, and contribute to risk of ASD. Red arrows = increased/decreased levels; blue arrow = enzymatic activity; Deep red font colour = Gene defect; Green rectangles = Environmental factors

It is evident that COX-1 plays a role in development of the central nervous system through its expression during the gestation period (Gersting *et al.*, 2009). In the ovine fetal brain, there was substantial expression of COX-1 in all areas of the brain throughout the second half of the gestation period. There is typically an exponential increase in microglial cells during mid-late gestation (Reemst *et al.*, 2016), and as COX-1 expression is predominantly in microglia (Hoozemans *et al.*, 2001; Shukuri *et al.*, 2011), it may be involved in normal apoptosis necessary for proper development. There is still a gap in knowledge of what roles COX-1 executes during early brain development, as COX-2 is more widely studied for early neurodevelopment.

1.6.2.2 Cyclooxygenase-2 (COX-2)

COX-2 is an inducible enzyme (Chen & Bazan, 2005) that is expressed in most tissues in response to stimuli, such as growth factors, inflammatory molecules (Hinz & Brune, 2002; Minghetti, 2004) and acute stress (Yamagata *et al.*, 1993). COX-2 plays a versatile pathophysiological role in regulating an inflammatory response during illness (Mancini & Di Battista, 2011), and injuries (Chikazu *et al.*, 2011), exposure to air pollution (Calderon-Garciduenas *et al.*, 2008), diseases such as osteoarthritis and rheumatoid arthritis (Fan *et al.*, 2015), pulmonary (Hsu *et al.*, 2019), kidney (Rangarajan *et al.*, 2019) and inflammatory bowel disease (Baumgart & Carding, 2007), and various tumor and cancer conditions (Deininger *et al.*, 1999; Garrido *et al.*, 2019; Wyatt *et al.*, 2019; Zhu *et al.*, 2019b). Its expression in gliomas is typically found in surrounding macrophage and microglial cells (Deininger *et al.*, 1999). Its intracellular localization in the nucleus (Morita *et al.*, 1995) is responsible for regulation of gene expression, and in microsomes to facilitate inflammatory responses (Vazquez-Tello *et al.*, 2004).

Induction of COX-2 during reproductive events such as ovulation, fertilization, implantation, and decidualization, is imperative to successful gestation (Lim *et al.*, 1997; Saint-

Dizier *et al.*, 2011). Female mice that are deficient for the *Ptgs2* gene (COX-2^{-/-}) have impairments in all these reproductive events, and are infertile (Lim *et al.*, 1997). There is high expression of COX-2 in the morulae and blastula stages of bovine embryo and therefore indicative of its role in fertilization (Saint-Dizier *et al.*, 2011). It is also highly expressed during labour (Fuentes *et al.*, 1996; Lu *et al.*, 2019), where a deficiency can lead to delayed parturition resulting in fetal death. Other complications such as abnormalities in development of the ductus arteriosus due to a lack of COX-2 also resulted in higher rates of neonatal mortality (Loftin *et al.*, 2001).

In early human brain development, COX-2 was found to be expressed throughout the developmental period, from 12 week onwards of fetal age in the choroid plexus (Maslinska *et al.*, 1999), and during the third trimester in glial cells (Shiow *et al.*, 2017). COX-2 is also involved during postnatal neurodevelopment in rats, as levels of mRNA gradually increase from day 8 to week 3 of postnatal age (Yamagata *et al.*, 1993; Kaufmann *et al.*, 1996; Kaufmann *et al.*, 1997). The newborn rat's developing brain undergoes extensive synapse formation during postnatal weeks 2-3 (Blue & Parnavelas, 1983), which overlaps with the time period during which COX-2 mRNA is at its highest. Irregularities in COX-2 signalling during early brain development and its consequences, will be further discussed below.

Although COX-2 plays an inducible role throughout the body, it is constitutively expressed in many regions of the brain. Its expression has been found in glutamatergic neurons of the forebrain, cerebral cortex, hippocampus (Yamagata *et al.*, 1993; Adams *et al.*, 1996; Kaufmann *et al.*, 1996), cerebellum, and spinal cord (Hewett *et al.*, 2016), to facilitate the release of the neurotransmitter glutamate (Sang *et al.*, 2005; Tian *et al.*, 2008; Lin *et al.*, 2014). Its

expression is highly enriched in the cerebral cortex and hippocampus, which are areas of the brain that are involved in higher complex functions such as learning and memory, and emotions.

COX-2 is primarily localized in dendrites and dendritic spines of excitatory postsynaptic neurons (Kaufmann *et al.*, 1996), playing a critical role in synaptic transmission, plasticity (Chen *et al.*, 2002; Sang *et al.*, 2005; Sang & Chen, 2006), and long-term depression (Le *et al.*, 2010). In fact, administration of COX-2 inhibitors to the cerebellum during postnatal development, caused an increase in dendrite length, and number of spines in Purkinje cells (Dean *et al.*, 2012a). Disruptions or abnormalities in the expression of COX-2, and the resulting changes to synaptic function have been implicated in several neurological disorders such as of epilepsy and seizures (Tu & Bazan, 2003; Kim & Jang, 2006; Oliveira *et al.*, 2008), multiple sclerosis (Rose *et al.*, 2004; Palumbo & Bosetti, 2013), schizophrenia, depression (Muller & Schwarz, 2008), Alzheimer's disease (Guan *et al.*, 2019a; Guan & Wang, 2019; Guan *et al.*, 2019b), and ASD.

COX-2 gained attention as a gene of interest in ASD, after a polymorphism of the *Prostaglandin-Endoperoxide Synthase 2 (Ptgs-2)* gene encoding COX-2 was found in ASD individuals who were exhibiting behaviours of hyperactivity and atypical communication (Yoo *et al.*, 2008). In addition, increased levels of COX-2 and PGE₂ was found in blood plasma of individuals with ASD correlated with atypical sensory behaviours (El-Ansary *et al.*, 2016) and difficulties in sensory processing (Qasem *et al.*, 2018). Interestingly, two different mouse models of autism, induced by propionic acid and valproic acid, also exhibited cells with higher COX-2 expression (Watanabe *et al.*, 2017; Alfawaz *et al.*, 2018). Therefore, previous studies have provided sufficient evidence for an association between COX-2 and ASD. Our lab confirmed for the first time that the COX-2-deficient mouse displays ASD-like behaviours such as decreased

social skills, motor deficits, and hyperactive and anxious behaviours (Wong *et al.*, 2019). The possible role of COX-2 in manifestation of ASD will be further explored in this dissertation.

1.6.3 Prostaglandin E₂ (PGE₂)

Prostaglandin E₂ (PGE₂) is one of the major lipid metabolites of AA, converted through the action of COX-1 and -2 (Funk, 2001), and PGES. It is a well-known inflammatory and immune response molecule (Park *et al.*, 2006; Rodriguez *et al.*, 2014; Kawahara *et al.*, 2015; Loynes *et al.*, 2018; Shimizu *et al.*, 2020), and exerts its physiological signalling functions through four G-protein coupled E-prostanoid receptors (EP1-4) (Jones *et al.*, 2015; Leclerc *et al.*, 2015a; Sreeramkumar *et al.*, 2016)

In the brain, PGE₂ is the most predominantly expressed PG (Vazquez-Tello *et al.*, 2004). It plays an important role in neuronal functions such as dendritic spine formation (Burks *et al.*, 2007), neurotransmitter release (Nishihara *et al.*, 1995), synaptic transmission (Mizutani *et al.*, 2015), and synaptic plasticity (Chen *et al.*, 2002). Due to PGE₂'s ability to cross the BBB, its expression in response to environmental agents may alter the normal levels in the brain during prenatal or postnatal development.

The association between PGE₂ and brain abnormalities was brought to light by clinical studies in which ingestion of the drug misoprostol (prostaglandin E analogue) during the first trimester of pregnancy, lead to the occurrence of Mobius syndrome and ASD (Bandim *et al.*, 2003). Misoprostol is commonly used for gastric ulcers (Vonkeman & van de Laar, 2010), labour induction (Davies *et al.*, 2001), and termination of pregnancy in conjunction with the drug mifepristone (Gemzell-Danielsson & Lalitkumar, 2008). Independent use of misoprostol without mifepristone is insufficient to terminate pregnancy, and resulted in various congenital malformations and abnormalities in a cohort of children in Brazil. *In vitro* studies demonstrated

similarities between the physiological effects of misoprostol and PGE₂. Both molecules act on EP receptors with similar affinities, cause changes in intracellular calcium levels (Tamiji & Crawford, 2010c; a), and a reduction in the number and length of neurite extensions in neuro-2a cells.

Induction of maternal inflammatory response caused by bacterial (Fang *et al.*, 2015) and viral infections have also been implicated in increased risk of ASD (Atladottir *et al.*, 2010; Atladottir *et al.*, 2012). Hospital records reveal associations between hospitalizations due to maternal infection and increased outcome of ASD in the children (Atladottir *et al.*, 2010). Viral infections that required hospitalization during the first trimester of pregnancy, in particular, were significantly associated with increased risk of developing ASD. In addition, an influenza viral infection, accompanied with prolonged fever, doubled and tripled the occurrence of ASD, respectively (Atladottir *et al.*, 2012). It is interesting that the influenza virus activates the COX-2/PGE₂-signalling pathway (Liu *et al.*, 2012a; Full & Gack, 2014), and PGE₂ has been used as a reliable biomarker for inflammation in urine samples of infants hospitalized due to viral infection and fever (Idborg *et al.*, 2014). Recent research has also successfully used PGE₂, COX-2, and PGES as markers of inflammation in individuals with ASD, and confirmed a strong association particularly with impairments in sensory functions (El-Ansary *et al.*, 2016; Qasem *et al.*, 2018).

Maternal infection and elicitation of the immune system in various animal models have been used to confirm ASD-like characteristics in the offspring (Parker-Athill & Tan, 2010; Carlezon *et al.*, 2019). Evidence from our lab revealed for the first time, that gestational exposure to PGE₂ during early prenatal neurodevelopment, caused age- and sex-dependent ASD-like social, repetitive, and anxious behaviours (Wong CT et al, unpublished). Maternal or postnatal immune activation in mouse models has shown alterations in development of

serotonergic neurons (Ohkawara *et al.*, 2015), abnormal synaptic transmission (Li *et al.*, 2018b), increased microglial activation and cell proliferation coupled with communication and cognition deficits (Pang *et al.*, 2016), and defective synaptic pruning process coupled with a reduction in microglial receptors (Fernandez de Cossio *et al.*, 2017) – all characteristic changes that have been observed in ASD.

Recently, our lab has also provided *in vivo* evidence that abnormal COX-2/PGE₂ in early brain development can lead to changes in the morphology, branching, and density of microglia – the neuro-immune cells responsible for facilitating an immune response and initiating phagocytosis to remove damaged cells from the brain (Wong CT et al, unpublished). This suggests a PGE₂-driven regulatory mechanism of microglial structure and function. Furthermore, direct evidence confirms that COX-2-inhibited PGE₂ leads to changes in phagocytic activity by microglia *in vitro* (He *et al.*, 2016), and *in vivo* in the rat cerebellum (Perez-Pouchoulen *et al.*, 2019). In turn, microglia not only act in response to PGE₂, but can also facilitate PGE₂ production in the preoptic area (Lenz *et al.*, 2013) and hippocampus (Qiu *et al.*, 2019b).

Microglia are well known for their involvement in the inflammatory response. Over the last decade, microglia have also been under investigation for playing an active role in sexual differentiation of the brain (Lenz *et al.*, 2013) and modulation of synaptic circuitry through pruning and apoptosis (Schafer *et al.*, 2012; Schafer *et al.*, 2013), in early brain development. The process of synaptic pruning is a critical event during neurodevelopment for remodeling and organizing synaptic circuits to ensure proper growth and functioning of the brain. Excessive or fewer synaptic connections have been linked to disorders such as schizophrenia and ASD. Interestingly, increased density of microglia has been noted in several cortical areas including cerebrum and cerebellum in individuals with ASD (Vargas *et al.*, 2005; Morgan *et al.*, 2010;

Tetreault *et al.*, 2012; Koyama & Ikegaya, 2015). Inflammatory stress can also initiate an increase of PGE₂ in a sex-dependent manner. Following an immune response, greater levels of both COX-2 and PGE₂ were expressed in neutrophil cells of males only (Pace *et al.*, 2017). This presents potential sex-specific mechanisms driven by PGE₂ that may contribute to the male-centric characteristics of ASD.

PG molecules are also well known to mediate hormones and events of the reproductive system. In particular, PGs have been shown to regulate synthesis of estradiol in the breast and ovary, through activation of aromatase (Brueggemeier *et al.*, 2001). Through a similar mechanism, increased levels of cerebellar PGE₂ cause higher activity of aromatase and elevate estradiol (Dean *et al.*, 2012b). It is interesting to note that the cerebellum is not involved in any reproductive events, however, during its critical development in the second postnatal week, there is an increase in expression of estrogen receptors and aromatase (Jakab *et al.*, 2001; Ikeda & Nagai, 2006; Lavaque *et al.*, 2006). Purkinje cells of the cerebellum produce steroids, and thus the synthesis of estradiol through enzymatic aromatase activity is local (Tsutsui *et al.*, 2003; Lavaque *et al.*, 2006). Coincidentally, COX-2 levels are also increased in the brain during this time period (Kaufmann *et al.*, 1996). Inhibition of systemic and cerebellar COX-2 during the second postnatal week in mice, has led to a decrease in play and sensory behaviours, and long-term changes in volume and dendritic spines of the cerebellum, in males (Dean *et al.*, 2012a). Furthermore, administration of PGE₂, and increasing PGE₂ levels via lipopolysaccharide (LPS)-induced inflammation, both resulted in increased aromatase activity and estrogen levels in the cerebellum, associated with abnormal social play behaviours in juvenile males only (Hoffman *et al.*, 2016). These consequences were exclusive to inflammatory exposures during the second postnatal week, of the three weeks investigated, supporting the importance of critical

developmental periods in the brain. The PGE₂-estradiol interaction in the brain may begin to provide an explanation for the heterogeneity of males to females in ASD.

1.6.4 EP Receptors

PGE₂ exerts its physiological signalling functions through four G-protein coupled E-prostanoid receptors (EP1-4) (Jones *et al.*, 2015; Leclerc *et al.*, 2015a; Sreeramkumar *et al.*, 2016), with binding affinities ranging from highest to lowest: EP3 > EP4 > EP2 > EP1 (Boie *et al.*, 1997; Abramovitz *et al.*, 2000). The ability of PGE₂ to bind to various EP receptors to initiate specific signalling pathways determines which biological function will be regulated.

EP receptors are expressed throughout the body, and the type and concentration of each is tissue and cell specific. Following are the primary functions and downstream signalling pathways of each EP receptor. When PGE₂ binds to EP1 receptors, it initiates the cascade by activating phospholipase C (PLC), which acts on protein kinase C (PKC) to hydrolyze phosphatidylinositol resulting in increased levels of intracellular calcium (Ca²⁺) (Tang *et al.*, 2005). In the brain, EP1 receptors are found at basal levels in the cerebral cortex, cerebellum and hippocampus (Candelario-Jalil *et al.*, 2005; Kawano *et al.*, 2006). Furthermore, this receptor is primarily expressed in oligodendrocytes (Zhang *et al.*, 2014a; Shiow *et al.*, 2017), which are responsible for the production of myelin sheath to insulate neuronal axons. Thus, it has been implicated in neonatal white matter injury (Shiow *et al.*, 2017). EP1 receptors are also involved in breakdown of the BBB in ischemic stroke injuries, (Frankowski *et al.*, 2015), and dopaminergic signaling in the striatum (Kitaoka *et al.*, 2007).

EP2 receptors are the most predominantly expressed in neurons (McCullough *et al.*, 2004; Lin *et al.*, 2014; Li *et al.*, 2018a) and astrocytes (Di Cesare *et al.*, 2006), and their activation leads to increased levels of cyclic AMP (cAMP) by adenylate cyclase (Di Cesare *et*

al., 2006; Chang *et al.*, 2016). The PGE₂-EP2 receptor complex has previously shown to increase inflammation in the brain through activation of microglial cells (Quan *et al.*, 2013), and may promote the development of neurodegenerative diseases such as Alzheimer's disease (Liang *et al.*, 2005; Shie *et al.*, 2005), amyotrophic lateral sclerosis (Liang *et al.*, 2008; Kosuge *et al.*, 2018), Parkinson's disease (Jin *et al.*, 2007), and growth of brain tumours (Qiu *et al.*, 2019a). However, its activation has also lead to neuroprotective effects in certain conditions. Studies have demonstrated EP2 receptor's involvement in causing a reduction in the neurotoxic effect of amyloid- β -peptide – hallmark of Alzheimer's disease – *in vitro* in cortical cell cultures (Echeverria *et al.*, 2005), and following a stroke (Leclerc *et al.*, 2015b). It is thought that these differing roles of EP receptors are highly dependent on the concentration of cAMP, and whether the downstream signaling is through PKA or other molecules such as Epac (exchange protein activated by cAMP). Research indicates that EP2-cAMP-PKA signaling pathway may be involved in the protective effect, whereas EP2-cAMP-Epac progresses the inflammatory response and pathological condition (Luo *et al.*, 2017).

The EP3 receptors (comprised of three isoforms in the mouse), play an inhibitory role by decreasing cAMP levels through inhibition of adenylate cyclase. The expression of EP3 is localized in subcortical regions, primarily in the hypothalamus (Vasilache *et al.*, 2007), and plays a major role in eliciting a febrile response upon infections and inflammation (Ushikubi *et al.*, 1998). Emerging evidence is strengthening its inflammatory role as well as involvement in synaptic modification in brain pathology, particularly in Alzheimer's disease (Shi *et al.*, 2012; Maingret *et al.*, 2017). There is also a noted increase in COX-PGE₂ signalling via EP3 receptors to regulate nitric oxide synthase, immediately before a burst of synaptogenesis during the

perinatal growth period (Dumont *et al.*, 1998). Thus the EP3 receptor may play a critical role in early postnatal brain development.

Lastly, similar to EP2, EP4 receptor is also expressed in neurons and increases cAMP through the action of adenylate cyclase. Furthermore, EP4 has also shown to activate other pathways such as the phosphatidylinositol 3-kinase (PI3K) and β -catenin pathways, and is involved in increasing the permeability of the BBB through the action of AA (Dalvi *et al.*, 2015). During inflamed condition of the brain following cerebral ischemia (stroke), activation through the EP4 receptor prevents substantial tissue damage, and hence prevents a severe behavioural outcome (Liang *et al.*, 2011). In addition, EP4 receptors also confirm their protective capability through suppression of neuroinflammatory responses upon infection (Shi *et al.*, 2010), and to decrease neurotoxic effects in Alzheimer's disease (Echeverria *et al.*, 2005). Therefore, depending on the downstream signaling cascade that's activated, EP4 may play a neuroprotective role in the brain.

The collective literature validates the functional diversity of PGE₂-EP receptor signaling throughout the body, and particularly in the normal development and functioning of the brain. Furthermore, it also plays a significant role during inflammation – exacerbation of diseased conditions, or exertion of a protective mechanism. These qualities make EP receptors a favourable therapeutical target for many brain diseases, using stimulation or inhibition to improve the functional and behavioural outcomes.

1.6.5 PGE₂ – Wnt Interaction

As is becoming clearly evident, PGE₂ incontestably has substantial involvement in brain functions and early neurodevelopment through various signaling mechanisms, and likely cross-talk with other developmental pathways. Previous studies have indicated that there is an

interaction between PGE₂ and an early developmental morphogen, Wntless-related integration site (Wnt), in various non-neuronal cell types (Goessling *et al.*, 2009; Liu *et al.*, 2010; Yoshida *et al.*, 2013; Li *et al.*, 2014; Hiyama *et al.*, 2015; Gupta *et al.*, 2019), and cancer cells (Huang *et al.*, 2017; Qinglin *et al.*, 2017; Wu *et al.*, 2017a). Furthermore, in our lab we have shown that PGE₂ and Wnt collaboratively increased the migration and proliferation of neuroectodermal (NE-4C) stem cells, verifying their interaction in the nervous system. This was accompanied by increased expression of the gene regulator β -catenin, and thus altered regulation of critical Wnt-target genes (Wong *et al.*, 2016). Wnt genes play key roles in early central nervous system developmental events such as neural tube formation, neuronal migration, and differentiation (Ciani & Salinas, 2005; Ille & Sommer, 2005). Furthermore, Wnts are involved in many neuronal functions that overlap with those of PGE₂, such as remodelling of growth cones through calcium, axon guidance, synapse formation, and synaptic plasticity (Sanchez-Camacho *et al.*, 2005). Therefore, we recognize that PGE₂ and Wnt signalling pathways converge and cross-talk *in vitro* in neuronal and non-neuronal cells. *In vivo*, the PGE₂-Wnt interaction has been observed in zebrafish and rats in injury and wound healing, respectively (North *et al.*, 2010; Zheng *et al.*, 2019). However, to date, this interaction has not been shown *in vivo* in the brain.

The Wnt/ β -catenin signalling pathway itself is extensively involved in the etiology of ASD (Medina *et al.*, 2018; Yao *et al.*, 2019). Numerous ASD-risk genes that have been identified across many studies are regulated by the Wnt/ β -catenin pathway (Caracci *et al.*, 2016; Bae & Hong, 2018; Medina *et al.*, 2018). Wnt signalling is also involved in the excitatory/inhibitory balance (Caracci *et al.*, 2016), interruption of which is implicated in ASD (Selimbeyoglu *et al.*, 2017; Davenport *et al.*, 2019; Dong *et al.*, 2020). As abnormal functioning of both the PGE₂ and Wnt signalling pathways are involved in ASD pathology, and their

interaction has been observed in some experimental models, its relationship will be further investigated in this dissertation.

1.7 Conclusion statement

In conclusion, it is evident through the literature that lipid signalling pathways are important for early brain development, as well as maintenance of homeostatic functions. Aberrant signalling as a result of genetic defects or environmental exposures can lead to oxidative stress, inflammation, and imbalances in the brain. Such insults, particularly during a period of rapid brain growth, can wreak havoc on the brain and lead to neurodevelopmental disorders such as ASD (Figure 1-6). ASD has been characterized by a definitive triad of symptoms, but there is great variance in behavioural profiles of affected individuals. This variability could be attributed to the type and timing of insult to the developing brain. Thus far, numerous genes and environmental factors have been identified in ASD etiology. However, it is essential to uncover pathways and functional groups that are affected, so the underlying mechanisms can be identified. For example, many ASD-risk genes and environmental factors lead to faulty synaptic events, mechanisms, and connections – as indicated by many human and animal studies. The current investigation aims to understand the role of abnormal COX/PGE₂ signalling during critical periods of brain development, with implications to ASD.

CHAPTER 2. MATERIALS AND METHODS

2.1 Cell Model (Results in Appendix A)

Mouse NE-4C stem cells (ATCC CRL-2925) were grown in Gibco minimal essential medium (MEM) containing 10% fetal bovine serum, 2mM L-glutamine, and 1X penicillin streptomycin (Invitrogen #11965118). Cells were maintained in an incubator at a temperature of 37°C, 95% humidity, and 5% CO₂. Cells were cultured on plates coated with 0.01% poly-L-lysine (PLL; Sigma #P-9155). Supplemented MEM was changed every 2 days.

2.1.1 Cell differentiation

NE-4C cells were dissociated and plated onto PLL-coated plates, and differentiation was induced by using serum free media (SFM; neurobasal medium, 2 mM L-glutamate, 1X Pen Strep, and 1X B-27). This was considered as day 0 of differentiation, and SFM was replaced every other day.

2.1.2 PGE₂ treatment

1 µM PGE₂ (Cayman Chemical) was added to the freshly prepared SFM every other day. The plates were monitored every other day, and images were captured on days 6, 8, 9, 10, 11 and 12, for measurement of neurites.

2.2 Mouse Model

All protocols for animal procedures used in this study were approved by the Animal Care Committee (ACC). The mice were maintained at the animal facility at York University, kept at a 12 h light/dark cycle, and provided with food and water ad libitum. All mice were acclimated for at least a week prior to breeding.

2.2.1 Breeding

Individual male mice were allowed to habituate in their cage, while the females were housed together in groups of 3-4. Mice were bred at the fertile age of 7+ weeks old. The female(s) were introduced to the males' cage and left overnight. Females were checked every morning until a vaginal plug was observed by a trained animal care personnel at the animal facility. The day of a confirmed vaginal plug was considered as gestation or day 1 (G1), and the females were housed separately for the remainder of the gestation period.

2.2.2 Study 1 – PGE₂-administered Mouse Model

Male and female mice (C57BL/6NCrl; Strain Code: 027) were obtained from Charles River Laboratories. C57BL/6NCrl colonies have been inbred for generations and thus are considered as genetically identical based on testing of single nucleotide polymorphisms (SNPs) by the distributor.

On gestation day 11 (G11), the pregnant females were weighed, and injected subcutaneously under the loose skin between the shoulders with 0.2mg/kg concentration of 16,16-dimethyl prostaglandin E₂ (dmPGE₂; Cayman Chemical #14753) in saline as used in previous studies (Chung *et al.*, 1998; Tessner *et al.*, 2004), or saline vehicle only. Subcutaneous injections were chosen due to accuracy and reproducibility between subjects and to avoid injury to the embryos, as opposed to intraperitoneal injections which are administered to the abdomen and have previously been documented to result in a higher risk in errors even when administered by experienced scientists (Steward *et al.*, 1968; Levin-Arama *et al.*, 2016). Therefore, subcutaneous injections were the most suitable to avoid injury to the embryos, and maintain consistent exposure.

The compound dmPGE₂ was used as it has a slower metabolism rate than PGE₂, and therefore remains active for a longer period of time (Ohno *et al.*, 1978). Control animals were injected with saline only. Twelve pregnant females were administered with a single dose of saline, or with the dmPGE₂ compound, resulting in three litters for each condition and developmental stage tested. Embryonic day 11 (E11) was chosen as the day of PGE₂ exposure to the embryo as it is the onset of neurogenesis in the embryonic mouse brain (Rodier, 1980; Clancy *et al.*, 2001; Clancy *et al.*, 2007). This time period is also equivalent to the first trimester in humans, the timing during which the analogous drug misoprostol was consumed in human clinical studies that resulted in manifestation of Moebius syndrome and ASD (Pastuszak *et al.*, 1998; Bandim *et al.*, 2003).

The samples were collected from three stages of neuronal development: embryonic day 16 (E16) at the peak of neurogenesis in many brain regions, embryonic day 19 (E19) when critical development in most brain regions has been completed (Rodier, 1980; Rice & Barone, 2000) and postnatal day 8 (P8) (Semple *et al.*, 2013; Sengupta, 2013) during which the prefrontal cortex, hippocampus, and particularly the cerebellum are still undergoing postnatal development. (Rodier, 1980).

2.2.3 Study 2 – COX-1^{-/-} and COX-2^{-/-} Mouse Models

Timed-pregnant female mice of WT background (B6-TP; C57BL/6NTac) or with a disruption in either the *Ptgs1*^{+/-} (B6;129P2-*Ptgs1*^{tm1Unc}; COX-1) or *Ptgs2*^{+/-} (B6;129P2-*Ptgs2*^{tm1Unc}; COX-2) genes (Langenbach *et al.*, 1995), were obtained from Taconic Biosciences. Females that are homozygous null for *Ptgs1* have delayed parturition and therefore cannot be used for breeding (Reese *et al.*, 2000). Females that are homozygous null for *Ptgs2* are infertile (Dinchuk *et al.*, 1995; Wang *et al.*, 2010). Therefore, for either *Ptgs* gene knockout, it is critical

to breed females that possess a normal or heterozygous genotype, with one at least one normal copy of the *Ptgs* gene. Male *Ptgs1*^{-/-} or *Ptgs2*^{-/-} mice are fertile and are used for breeding. Homozygous null males were bred with females of a heterozygous genotype to produce four possible genotypes in the offspring: male *Ptgs*^{+/-}, female *Ptgs*^{+/-}, male *Ptgs*^{-/-}, and female *Ptgs*^{-/-}. For the purpose of this study, only homozygous null mutants were obtained.

2.2.4 Studies 3 and 4 – COX-2^{-/-} Mouse Model

Male mice homozygous for the mutated *Ptgs2* gene (*Ptgs2*^{-/-}; COX-2^{-/-}), and female mice with a heterozygous genotype (*Ptgs2*^{+/-}; COX-2^{+/-}) were obtained from Jackson laboratories (*Ptgs2*^{tm1.1Fun}; targeted mutation 1.1, Colin D Funk, JAX stock #008101) (Yu *et al.*, 2006). Females heterozygous for the *Ptgs2* gene were used for breeding, due to the fact that females with the homozygous knockout genotype are not fertile. The offspring from this pairing can have either a *Ptgs2*^{-/-} or *Ptgs2*^{+/-} genotype. The objective was to study the effects of the homozygous mutation. Mice of the WT strain 129S6/SvEvTac (Taconic model #129SVE-M (males); #129SVE-F (females)) were bred to obtain WT male and female offspring samples.

2.3 Genotyping Analysis

2.3.1 Sample collection

Tail samples from all fetuses/pups were collected at E16, E19 and P8. For the later stages of postnatal day 25 (P25) in study 4, ear tissue was collected via ear punches approximately at P21-P22, post weaning from the mother. All samples were immediately submerged in alkaline lysis reagent (25mM NaOH, 0.2mM EDTA, water; pH = 12) on ice, in preparation for crude genomic DNA extraction.

2.3.2 Genomic DNA extraction

Tail/ear samples in 75µl of alkaline lysis reagent were heated at 95°C for 30-60 minutes followed by cooling on ice. 75µl of neutralization buffer (40mM Tris-HCL, water; pH = 5) was added to the samples, followed by centrifugation at 3000 rpm for 3 minutes at 4°C. The supernatant containing genomic DNA was used for polymerase chain reaction (PCR) to distinguish between sex, and *Ptgs-1* or -2 knockout zygosity.

2.3.3 Polymerase Chain Reaction (PCR)

Sex was determined by choosing fragments from the *sex-determining region Y (SRY)* gene present only in males, and thus presence or absence of a band indicates male or female genotype, respectively (Patelia *et al.*, 2013). The PCR reaction consisted of 4µl of genomic DNA, 1x OneTaq[®] Standard Reaction Buffer (New England BioLabs #B9022S), 200µM of each dNTP (New England Biolabs #N0447), 0.625 units of OneTaq[®] Hot Start DNA Polymerase (New England BioLabs #M0481), and 0.2µM for each forward (*TCC CAG CAT GCA AAA TAC AGA GAT CAG C*) and reverse primers (*TTG GAG TAC AGG TGT GCA GCT CTA C*). The PCR reaction tubes were placed in the Eppendorf[®] Mastercycler Gradient thermal cycler using the following parameters for sex genotyping: denaturation at 94°C for 30 seconds, 30 cycles of denaturation at 94°C (30 seconds), annealing at 62°C (45 seconds) and extension at 68°C (1 minute), and final extension at 68°C for 5 minutes. The PCR product mixed with 6X Orange G dye (Sigma #O-1625), was loaded onto a 1.2% agarose gel in 1X TAE buffer (0.04M Tris-Acetate, 0.0001M EDTA) with SafeView, alongside a 100bp ladder (Biolabs #N323) to determine the size of the fragment at 297bp.

To test the COX-1 and COX-2 deficient mice obtained from Taconic Biosciences (Study 2), optimal PCR conditions and primer sequences for the knockout genes were acquired from

Taconic Biosciences. The PCR reaction for *Ptgs-1* consisted of 4μl of genomic DNA, 1x OneTaq[®] Standard Reaction Buffer (New England BioLabs #B9022S), 200μM of each dNTP (New England Biolabs #N0447), 0.625 units of OneTaq[®] Hot Start DNA Polymerase (New England BioLabs #M0481), and 0.2μM for each forward and reverse primers (COX1-KOF: *CAG CCT CTG TTC CAC ATA CAC*; COX1-R: *CTG ACT TTC TGA GTT GCC AAC*; COX1-WTF: *GAG ATG GCT GCT GAG TTG G*). Presence of one band at ~700bp or two bands at ~700 and ~600bp were indicative of *Ptgs-1*^{-/-} and *Ptgs-1*^{+/-} genotypes, respectively.

The PCR reaction for the COX-2 deficient mice consisted of 4μl of genomic DNA, 1x OneTaq[®] Standard Reaction Buffer, 0.625 units of OneTaq[®] Hot Start DNA Polymerase (New England BioLabs #M0481), 200μM of each dNTP (New England Biolabs #N0447), and 0.4μM for each forward and reverse primers (COX2-KOF: *ACG CGT CAC CTT AAT ATG CG*; COX2-R: *TCC CTT CAC TAA ATG CCC TC*; COX2-WTF: *ACA CAC TCT ATC ACT GGC ACC*). Presence of one band at ~950bp and two bands at ~950 and ~800bp were indicative of *Ptgs-2*^{-/-} and *Ptgs-2*^{+/-} genotypes, respectively.

The PCR reaction tubes were placed in the Eppendorf[®] Mastercycler Gradient thermal cycler using the following parameters for *Ptgs-1* and *-2* genotyping: denaturation at 94°C for 30 seconds, 30 cycles of denaturation at 94°C (30 seconds), annealing at 57°C (45 seconds) and extension at 68°C (1 minute), and final extension at 68°C for 5 minutes. The PCR product mixed with 6X Orange G loading buffer, was loaded onto a 2% agarose gel in 1X TAE buffer with SafeView. A 100bp ladder was used to determine the size of the fragments.

To test zygosity of the COX-2 deficient mice acquired from Jackson Laboratories (Studies 3 and 4), the PCR reaction consisted of 4μl of genomic DNA, 1x Taq Reaction Buffer, 2mM MgSO₄, 2 units of Taq DNA Polymerase (Bio Basic #B0089), 200μM of each dNTP (New

England Biolabs #N0447), and 0.5µM for each forward and reverse primers (F: *ACC AGT CTC TCA ATG AGT AC*; R: *AGA ATG GTG CTC CAA GCT CTA C*). One band at 585bp indicated the *Ptgs*^{-/-} mutation, and two bands at 493bp and 585bp confirmed the heterozygous genotype (*Ptgs*^{2+/-}).

The PCR reaction tubes were placed in the Eppendorf® Mastercycler Gradient thermal cycler using the following parameters of a touchdown method as per the Jackson Lab protocols: denaturation at 94°C for 30 seconds, 10 cycles of denaturation at 94°C (30 seconds), annealing at 65°C (45 seconds; -0.5°C per cycle decrease), extension at 60°C (1 minute); 28 cycles of denaturation at 94°C, annealing at 60°C, extension at 72°C; and final extension at 72°C. The PCR product mixed with 6X Orange G loading buffer, was loaded onto a 2% agarose gel in 1X TAE buffer with SafeView. A 100bp ladder was used to determine the size of the fragments.

The housekeeping gene GAPDH was used to verify validity of the samples. The PCR reaction consisted of 2µl of cDNA, 1x OneTaq® Standard Reaction Buffer, 0.625 units of OneTaq® DNA Polymerase (New England BioLabs #M0480) 200µM of each dNTP (New England Biolabs #N0447), and 0.2µM for each forward (*TTC ACC ACC ATG GAG AAG GC*) and reverse primers (*GGC ATG GAC TGT GGT CAT GA*). The PCR reaction tubes were placed in the Eppendorf® Mastercycler Gradient thermal cycler using these parameters for sex genotyping: denaturation at 94°C for 30 seconds, 30 cycles of denaturation at 94°C (30 seconds), annealing at 55°C (45 seconds) and extension at 68°C (1 minute), followed by final extension at 68°C for 5 minutes. The PCR product mixed with 6X Orange G loading buffer, was loaded onto a 1.2% agarose gel in 1X TAE buffer with SafeView. A 100bp ladder was used to determine the size of the fragment, approximately at 246bp. All PCR primers are listed in Table 1.

Table 2-1. Forward and reverse primer sequences for PCR

Gene	Primer Sequences (5'→3')	Amplicon size (base pairs)
<i>SRY</i>	F: <i>TCC CAG CAT GCA AAA TAC AGA GAT CAG C</i> R: <i>TTG GAG TAC AGG TGT GCA GCT CTA C</i>	297
<i>COX-1</i> (Taconic)	KOF: <i>CAG CCT CTG TTC CAC ATA CAC</i> WTF: <i>GAG ATG GCT GCT GAG TTG G</i> R: <i>CTG ACT TTC TGA GTT GCC AAC</i>	700 and 600
<i>COX-2</i> (Taconic)	KOF: <i>ACG CGT CAC CTT AAT ATG CG</i> WTF: <i>ACA CAC TCT ATC ACT GGC ACC</i> R: <i>TCC CTT CAC TAA ATG CCC TC</i>	950 and 800
<i>COX-2</i> (Jackson)	F: <i>ACC AGT CTC TCA ATG AGT AC</i> R: <i>AGA ATG GTG CTC CAA GCT CTA C</i>	585 and 493
<i>GAPDH</i>	F: <i>TTC ACC ACC ATG GAG AAG GC</i> R: <i>GGC ATG GAC TGT GGT CAT GA</i>	246

2.4 Collection of brain tissue samples

For collection of the embryonic stages E16 and E19, the pregnant dam was placed in an induction chamber maintaining 3-4% isoflurane concentration, until she appeared to be sedentary. Unconsciousness was confirmed by pedal reflex (toe pinch), followed by 1-2% isoflurane concentration delivered by face mask. The female was placed on her back to rinse the abdomen with 70% ethanol, and an incision was made along the midline to expose the uterus. The uterus was excised by cutting the mesometrium, and immediately placed on ice. The

embryos were released from the embryonic sac and placenta, and brain tissue was removed and submerged in TRIzol™ (Invitrogen #15596-026), for isolation of RNA and protein.

For collection of the postnatal P8 and P25 stages, pups were placed under anesthesia in the induction chamber, and sacrificed by decapitation. The brain tissue was removed, rinsed in 0.1M PBS (on ice), and immediately submerged in TRIzol™ for RNA and protein isolation.

2.4.1 Study 1

Three litters were obtained from each developmental stage from saline-administered dams at E16 ($n=16$: 5, 4 and 7 pups from each litter), E19 ($n=20$: 4, 7 and 9 pups from each litter) and P8 ($n=20$: 6, 6 and 8 pups from each litter), and PGE₂-administered dams at E16 ($n=21$: 6, 8 and 7 pups from each litter), E19 ($n=20$: 6, 8 and 6 pups from each litter), and P8 ($n=19$: 3, 9 and 7 pups from each litter).

2.4.2 Studies 2-4

For all COX knockout mouse model studies, three animals of each genotype (WT and *Ptgs-1*^{-/-} or *-2*^{-/-}), sex, and developmental stage required (E16, E19, P8, or P25) were obtained.

2.5 RNA/Protein Isolation

RNA and protein were isolated through the phase separation method using TRIzol™ reagent. Brain tissue (~100mg) was homogenized in 1ml of TRIzol™ using a tissue homogenizer by Polytron®, followed by centrifugation at 12,000 x g for 10 minutes at 4°C to separate the supernatant which contained RNA. The samples were centrifuged at 12,000 x g for 15 minutes at 4°C following addition of 20% chloroform to complete the separation of RNA, DNA, and Protein. For RNA isolation, the uppermost aqueous phase was mixed with 0.5ml of isopropyl alcohol, and centrifuged at 12,000 x g for 10 minutes at 4°C to precipitate the RNA into a pellet.

The RNA pellet was washed with 75% ethanol, dissolved in diethyl pyrocarbonate (DEPC) water, and treated with DNase (Bio-Rad #7326828) and 5mM EDTA (Sigma #E5134), to remove all DNases in the samples, and preserve RNA quality. The concentration of RNA in each sample was measured using the Nanodrop ND1000 Spectrophotometer.

Following phase separation, 1.5ml of isopropyl alcohol was added to the bottom phenol-ethanol layer and centrifuged at 12,000 x g for 10 minutes at 4°C to precipitate the protein pellet. The pellet was washed three times with 0.3M guanidine hydrochloride (Sigma #G4630) in 95% ethanol, followed by 100% ethanol, and dissolved in 1% SDS with proteinases in solution. The concentration of protein in each sample was measured using the bicinchoninic acid (BCA) assay (Thermoscientific #23225), and the Nanodrop ND1000 Spectrophotometer.

2.6 Microarray analysis

2.6.1 Study 2

RNA samples from WT males at E16 (n=3) and E19 (n=3), COX-1-KO males at E16 (n=3) and E19 (n=3), and COX-2-KO males at E16 (n=3) and E19 (n=3), were sent to Princess Margaret Genomics Center (Toronto, Canada; www.pmggenomics.ca) for microarray analysis. The samples were assessed with Mouse WG-6 V2 BeadChip (Illumina) microarray plates containing 45,281 target probes derived from the National Center for Biotechnology Information Reference Sequence (NCBI) *RefSeq* (Build 38 Release 22) (November 7, 2009), Mouse Exonic Evidence Based Oligonucleotide (MEEBO) and RIKEN FANTOM2 database. The facility ensured full quality control, and provided data analysis. Analysis and batch normalization were carried out using GENESPRING v12.6.1 and the statistical software, R, respectively.

2.6.2 Study 3

RNA samples from WT females at E16 (n=3) and E19 (n=3), and COX-2-KO females at E16 (n=3) and E19 (n=3), were sent to Princess Margaret Genomics Center (Toronto, Canada; www.pmgenomics.ca) for microarray analysis. The samples were assessed with Mouse Genome 2.0ST GeneChip® (Affymetrix) microarray plates containing 34,351 probes. Analysis and data normalization was carried out using GENESPRING v13.1.1 and RMA16, respectively.

2.6.3 Bioinformatics and identification of differentially expressed genes

Differential gene expression in COX-KO mice was normalized to WT data to obtain fold change (FC) values of all genes (WT = 1). One-way ANOVA *p*-values were corrected using Benjamini and Hochberg False Discovery Rate (FDR) method followed by post-hoc test (Tukey HSD). A cut-off of FDR corrected *p*-value < 0.05 and FC > absolute 1.5 was applied. For this study, we converted the gene names from Illumina gene IDs/Affymetrix to Official Gene Symbol (OGS) using DAVID Bioinformatics Resources (<http://david.abcc.ncifcrf.gov/>). These quality control and statistical procedures resulted in lists of significantly differentially expressed genes for COX-1-KO E16, COX-1-KO E19, COX-2-KO E16 and COX-2-KO E19 males (Study 2; genes and FC can be found in Appendix B1 Supplementary Table 1), and COX-2-KO E16 and COX-2-KO E19 females (Study 3; genes and FC can be found in Appendix B2 Supplementary Table 2). To determine genes in common within each knockout mouse model across developmental stages, Venn diagrams were created using Venny 2.0 tool (<http://bioinfogp.cnb.csic.es/tools/venny/>). List of common genes are in Appendix B (Males: Supplementary Table 3; Females: Supplementary Table 4).

2.6.4 Analysis of biological processes

Functional annotation lists of Gene Ontology: Biological Process (GO:BP) and Kyoto

Encyclopedia of Genes and Genomes (KEGG) categories were generated using DAVID Bioinformatics with the mouse BeadChip background list corresponding to the chip used in this study (MouseWG-6_V2_0R2_11278593_A) (Huang da *et al.*, 2009). Enrichment of genes belonging to particular annotations/pathways were determined using Benjamini FDR corrected EASE score (one-tail Fisher Exact Probability Value) P-value with < 0.1 cut off in DAVID. This P-value represents the probability of getting the same annotation cluster with random selection of genes. The lower the P-value, the more likely that the gene enrichment analysis is specific to the gene list, and not a random event. Only non-redundant annotations containing a gene count > 3 were reported. Some biological processes were identified by Eizaaz Ahmad (co-author of the publication).

2.6.5 Identification of ASD-risk genes and protein-interaction networks

Genes associated with ASD were identified using the Autism Database AutDB (Basu *et al.*, 2009) and cross referencing with additional genome wide studies of ASD individuals in the literature. ASD genes from COX-1^{-/-} or COX-2^{-/-} males were identified by Eizaaz Ahmad (co-author of the publication).

The ASD genes found in the COX-1^{-/-} and COX-2^{-/-} males, and in the COX-2^{-/-} females, were inputted individually into GeneMANIA (Warde-Farley *et al.*, 2010) to generate protein-interaction networks, using the Gene Ontology selection. The protein networks were manually organized according to biological processes and functions using Cytoscape (Merico *et al.*, 2010; RK *et al.*, 2017).

2.6.6 Comparison between Microarray Platforms

Genome-wide microarray analyses were conducted on male (Chapter 4) and female (Chapter 5) COX-2 gene KO mouse models at developmental stages E16 and E19. Due to a time gap

between the two studies, different microarray platforms were used: Males – Mouse WG-6 V2 BeadChip (Illumina) microarray; and Females – Mouse Genome 2.0ST GeneChip® (Affymetrix) microarray. There were 16,720 overlapping genes that were on both assays, and the majority of genes exclusive to each assay were predicted genes which are not essential for analyses as confirmed by the Princess Margaret Genomics Center (Toronto, Canada) (Figure 2-1). Therefore, it is feasible to present general comparisons and conclusions between male and female COX-2^{-/-} mouse models during prenatal brain development (Chapter 7).

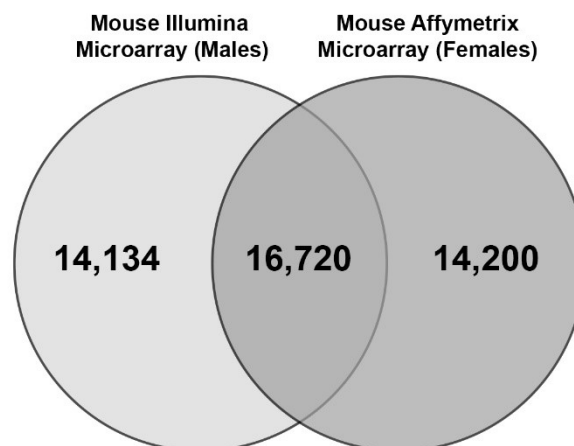


Figure 2-1. The number of genes that overlap between the two microarray platforms. The Mouse WG-6 V2 BeadChip (Illumina) microarray platform (left) was used to test the COX-1^{-/-} and COX-2^{-/-} male brain, and the Mouse Genome 2.0ST GeneChip® (Affymetrix) microarray platform (right) was used to test COX-2^{-/-} females. There were 16,720 overlapping genes that were on both platforms.

2.7 Reverse transcription

mRNA was converted to cDNA using Oligo-d(T)₁₈ mRNA primer (New England Biolabs #S1316), deoxynucleotide (dNTP) solutions (New England Biolab #N0446), and MMuLV reverse transcriptase reaction buffer and enzyme (New England Biolabs #0253). The mixture was heated at 42°C for 1 hour and at 90°C for 10 minutes in the Eppendorf® Mastercycler Gradient thermal cycler. The cDNA samples were used for quantitative real-time PCR (qRT-PCR). All primers for qRT-PCR were designed using Primer Express Software (Version 3.0; Applied Biosystems).

2.8 Quantitative real-time PCR

qRT-PCR was used to quantify the genes of interest using the 7500 Fast Real-Time PCR System (Applied Biosystems) with SYBR® Advantage qPCR Premix (Clontech CAT# 639676). Quantitative values were obtained from the threshold cycle (C_T) number. Two housekeeping genes were used: *Pgk1* (*phosphoglycerate kinase*) and *Hprt* (*hypoxanthine-guanine phosphoribosyltransferase*). The raw C_T values from experimental and WT mouse brain samples were normalized using the geometric mean of the housekeeping genes *Pgk1* and *Hprt* to obtain the ΔC_T values for all selected genes. The ΔC_T values were compared with a calibrator to generate the relative quantity (RQ) value which represents the FC expression of each target sample compared to the reference sample, known as the comparative C_T method.

2.8.1 Study 1

Custom Taqman plates (Applied Biosystems) were used as a preliminary screening tool to test 44 commercially available Wnt and Wnt-target developmental genes (*Apc*, *Axin1*, *Bmp2*, *Bmp3*, *Bmp4*, *Bmp7*, *Ccnd1*, *Cldn1*, *Creb1*, *Crebbp*, *Csnk1a1*, *Ctnnb1*, *Dvl1*, *Enc1*, *Fos11*, *Fzd1*, *Gli1*, *Gsk3b*, *Hnfla*, *Jun*, *Lef1*, *Lrp5*, *Lrp6*, *Mmp7*, *Myc*, *Nrcam*, *Ppard*, *Ptch2*, *Ptgs2*, *Pygo1*,

Smarca4, *Tcf3*, *Tcf4*, *Tcf7l2*, *Wnt1*, *Wnt2*, *Wnt3*, *Wnt3a*, *Wnt4*, *Wnt5a*, *Wnt5b*, *Wnt7a*, *Wnt8a*, *Wnt8b*). Each plate also contained 4 standard housekeeping control genes [*18S ribosomal RNA* (*18s*), *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*), *hypoxanthine phosphoribosyl transferase* (*Hprt*), and β -*glucuronidase* (*Gusb*)]. The screen test was performed on pooled samples from saline- and PGE₂-administered litters. Genes with FC of at least 1.5 – *Mmp7* and *Wnt2* – were chosen for further validation in each individual offspring.

For the EP-receptor genes, *EP1*, *EP2*, *EP3 α* , *EP3 β* , *EP3 γ* , and *EP4*, the ΔC_T values of the pooled brain samples (each developmental stage; E16, E19, and P8) were compared with a calibrator (adult stage) to generate the RQ value. For the genes *Mmp7* and *Wnt2*, the ΔC_T values of the brain samples from each individual PGE₂-administered animal, were compared with a calibrator (average of all saline-exposed pups from three different litters) to generate the RQ value.

2.8.2 Study 2

The ΔC_T values of each male COX-1^{-/-} or COX-2^{-/-} brain sample was compared with a calibrator (average of all WT pups) to generate the RQ value for the selected ASD-related genes: *myelin transcription factor 1 like* (*MYT1L*), *glyoxylase 1* (*GLO1*) and *dishevelled associated activator of morphogenesis 1* (*DAAMI*), to validate microarray analysis.

2.8.3 Study 4

The ΔC_T values of each male and female COX-1^{-/-} or COX-2^{-/-} brain sample was compared with an average of all WT pups for selected genes, *Spinophilin* (*Spn*) and *Actin beta* (*Actb*). Each experimental sample was run in triplicates on a 96-well plate which was considered as one RT-PCR experiment, and replicated three times. The primers are listed in Table 2.

Table 2-2. Forward and Reverse Primer Sequences for qRT-PCR

Gene	Primer Sequences (5'→ 3')	Amplicon size (base pairs)
<i>Hprt</i>	F: TCC ATT CCT ATG ACT GTA GAT TTT ATC AG R: AAC TTT TAT GTC CCC CGT TGA CT	75
<i>EP1</i>	F: GCA GCA CTG GCC CTC TTG R: TGT GCC ATT ATC GCC TGT TG	135
<i>EP2</i>	F: GCC TTT CAC AAT CTT TGC CTA CAT R: GAC CGG TGG CCT AAG TAT GG	135
<i>EP3α</i>	F: CGG AAG TTC TGC CAG ATC AGA R: TCC AGC TGG TCA CTC CAC ATC	101
<i>EP3β</i>	F: CGG AAG TTC TGC CAG ATG ATG R: CAG GGA AAC AGG TAC TGC AAT G	63
<i>EP3γ</i>	F: AGT TCT GCC AGG TAG CAA ACG R: GCC TGC CCT TTC TGT CCA T	61
<i>EP4</i>	F: CGC ACT GCG TGG GAA GA R: GAG CTC ACG GTT CGA TCT AGG A	67
<i>Mmp7</i>	F: TTGACAAGGATGAGTACTGGACTGAT R: AATTCATGGGTGGCAGCAAA	73
<i>Wnt2</i>	F: GCCCTGATGAACCTTCACAAC F: TGACACTTGCATTCTTGTTC	77
<i>MYTIL</i>	F - CTCTGGAAGAAAGACGGTATCC R - GGCAGCCAGACAGAGTTATTA	108
<i>GLO1</i>	F - CAAGACCCTGACGGCTACTG R - ATCCATTAATCCTCAAAGGCACA	97
<i>DAAMI</i>	F - CTAAGCGAAGAGTTGCGGGA R - ACTCCAGCTCCGTCTCTACA	115
<i>Spinophilin</i>	F - CAAGGACTACCAGCAAAAGGAGAT R - CCTGGCTAGCTCCGACTCTTC	85
<i>Actb</i>	F - GCTTCTTTGCAGCTCCTTCGT R - AGCGCAGCGATATCGTCAT	73

2.8.4 Selection of Optimal Housekeeping Genes

Previous studies have indicated that comparisons between different tissues, developmental ages or experimental conditions are challenging as even stable housekeeping genes can present different expression (Vandesompele *et al.*, 2002; Benn *et al.*, 2008; Boda *et al.*, 2009; Xu *et al.*, 2018). It is crucial for housekeeping genes to remain stable across all parameters, to ensure the validity of gene expression of all genes of interest. As per our knowledge, housekeeping genes across the developmental time points in the mouse brain have not previously been tested. The following genes were investigated: *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*), *hypoxanthine phosphoribosyl transferase* (*Hprt*), *phosphoglycerate 1* (*PGK1*), *transferrin receptor* (*TFRC*), and β -*glucuronidase* (*Gusb*), across development stages E16, E19, P8, and adult brain samples, using qRT-PCR. GeNorm software was used to analyze the resulting data. A geNorm M value of less than 0.5 for any given housekeeping gene is considered as stable as described in (Vandesompele *et al.*, 2002), and can be used for quantitative gene expression. Four out of the five housekeeping genes tested had stable expression across all developmental stages with geNorm values of less than 0.5 as described in (Vandesompele *et al.*, 2002) (Figure 2-2). Upon further investigation and experiments, we chose *PGK1* and *HPRT*, as they showed the most stable expression between developmental stages, treatments, and experiments.

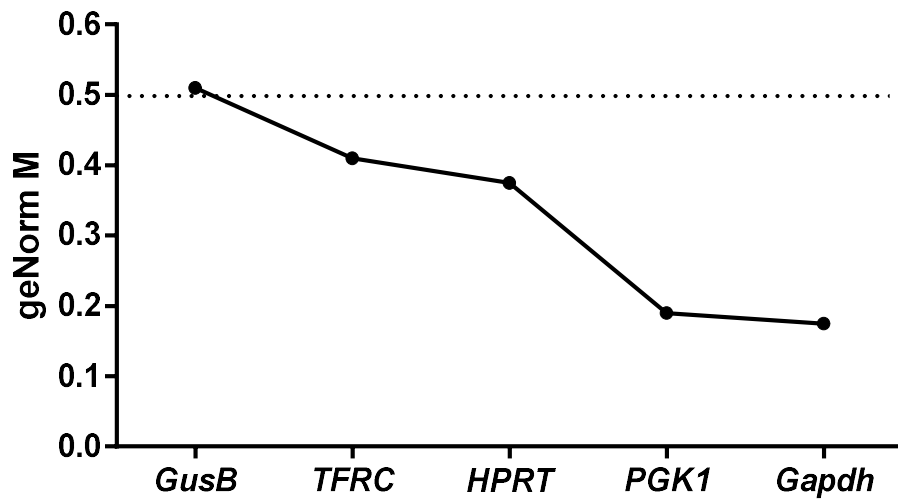


Figure 2-2. Optimal housekeeping genes in the mouse brain across developmental time points E16, E19, P8, and adult. Four out of the five housekeeping genes tested (*Gapdh*, *PGK1*, *HPRT*, and *TFRC*) had a geNorm value of less than 0.5, and are considered as stable and optimal housekeeping genes to use in the mouse brain across developmental stages E16, E19, P8, and adult.

2.9 Western Blot

Protein sample volume was calculated to a concentration of 30-40 μ g (dependent on protein of interest), volume adjusted with distilled water, and mixed with equal volume of laemmli buffer (with 5% β -mercaptoethanol). The samples were boiled for 5 minutes, and kept on ice. The entire sample was loaded onto a 12% polyacrylamide gel (30% acrylamide, 1.5M Tris-HCl pH 8.8, 20% SDS, 20% APS, TEMED, distilled water) and fractionated in 1X running buffer (25mM Tris, 192mM glycine, 0.1% SDS) using polyacrylamide gel electrophoresis (PAGE) (90V for 10 minutes and 100V for approximately 1.5 to 2.5 hours, depending on the molecular

weight of the protein). The gel was transferred onto a nitrocellulose membrane in 1X transfer buffer (25mM Tris, 192mM glycine, 20% methanol) with a spinner for 1 hour at 100V on ice. The membrane was washed 2X in TBS for 10 minutes and blocked in 5% solution (non-fat dry milk in TBS-T) for 1 hour at RT with agitation.

2.9.1 Study 1

Protein expression of EP receptors' was tested using the primary rabbit polyclonal antibodies anti-EP1 (1:200; Santa Cruz Biotechnology #sc-98388), anti-EP2 (1:200; Santa Cruz Biotechnology #sc-20675), anti-EP3 (1:200; Santa Cruz Biotechnology #sc-20676), and anti-EP4 (1:200; Santa Cruz Biotechnology #sc-20677). The anti-EP3 antibody recognizes all three isoforms (α , β , γ) of the EP3 protein.

Mmp7 protein was detected using monoclonal rabbit-anti-mmp7 (D4H5) (1:1000; Cell Signaling #3801) in 2.5% bovine serum albumin (BSA) and TBS-T, overnight at 4°C with agitation. Wnt2 protein was detected using goat-anti-Wnt2 (1:200; Santa Cruz Biotechnology #50361) in 2.5% BSA and TBS-T. β -actin was quantified using mouse-anti- β -actin (1:10000; Abcam #ab6276) in 2% non-fat dry milk and TBS-T, for 1 hour at RT with agitation.

The protein expression levels of two active forms of β -catenin were tested. One active form was β -catenin phosphorylated at Serine-552 by protein kinase A (PKA) using the polyclonal primary antibody against rabbit-phospho- β -catenin (Ser552) (1:1000; Cell Signaling #9566) in 5% BSA and TBS-T, overnight at 4°C with agitation. The second active form tested was non-phospho (active)- β -catenin (Ser33/37/Thr41), which is the stabilized form of β -catenin that is functionally active (Liu *et al.*, 2002), using a rabbit monoclonal anti-nonphospho- β -catenin (Ser33/37/Thr41) antibody (1:1000; Cell Signaling Technology #8814), in 5% BSA and TBS-T, overnight at 4°C with agitation.

2.9.2 Study 4

Spinophilin protein was detected by monoclonal rabbit- α -spinophilin (E1E7R) (1:500; Cell Signaling Technology #14136) in 5% non-fat dry milk and TBS-T, overnight at 4°C with agitation. β -actin was detected by mouse anti- β -actin (1:1000; Abcam #ab6276) in 2% non-fat dry milk in TBST, overnight at 4°C with agitation.

For comparative analysis, the same blots were used to probe with mouse anti-GAPDH (1:10000; Abcam #ab8245) in 2% non-fat dry milk in TBS-T, for 1 hour at RT with agitation. Horseradish peroxidase-conjugated secondary antibodies used were: goat α -rabbit (1:10000; Abcam #ab205718) in 2% non-fat dry milk in TBS-T for 2 hours at RT with agitation; donkey α -goat (1:2,000; Santa Cruz Biotechnology #sc-2020) in 2% non-fat dry milk in TBS-T for 1 hour at RT with agitation, or goat α -mouse (1:5,000; Abcam #ab6789). Clarity Western ECL Substrate reagent (Bio-rad, #1705060) was used for detection by Perkin Elmer Geliance 600 Imaging System.

2.1 Golgi staining

Brains from WT and COX-2^{-/-} males and females at the E19* and P25 developmental stages were extracted, dissected in half, rinsed in 0.1M PBS solution on ice, and immediately submerged in a combined fixative solution of 2% glutaraldehyde and 4% paraformaldehyde in 0.1M PB solution for one hour at room temperature in dark conditions (Koyama & Tohyama, 2012). The brain tissue was washed with distilled water, and submerged in golgi-cox solution (5% mercuric chloride, 5% dichromate solution and 5% potassium chromate), which was replaced with fresh solution after 24 hours, and kept incubated for 14 days at room temperature in dark conditions (Zaqout & Kaindl, 2016). The samples were then transferred to tissue

protectant solution (30% sucrose, 30% ethylene glycol, and 1% polyvinylpyrrolidone) for up to 7 days.

Sectioning of brain samples was achieved using a cryostat by the research histology lab at University Health Network (Toronto, ON). Sagittal sections of approximately 100µm were mounted onto gelatin-coated slides and allowed to dry. The stain was developed with 3:1 ammonia solution, followed by 5% sodium thiosulfate in water. The sections underwent a series of dehydration steps (70%, 95% and 100%), and were immersed in xylene until cover-slipped.

*Note: The E19 brain samples were mishandled and damaged by a different processing facility, and were no longer useable to obtain any data.

2.10 Microscopy

2.10.1 Cell Model (Results in Appendix A)

All cell images of neurospheres and neurite extensions were acquired using the Nikon Eclipse Ti-E microscope. The length of neurite extensions was measured from the point of attachment at the cell body to the tip of the growth cone, using line measurement tools in the NIS Elements software (Nikon).

2.10.2 Study 4

All images were acquired using light microscopy with the Zeiss Laser Scanning Confocal Microscope (LMS 700; Life Sciences Building at York University), in combination with the Carl Zeiss Zen software. The cerebellum was scanned using a 4X lens air objective, and areas of interest were zoomed in with 60X or 100X oil objectives. Dendrite images were captured every 0.15 µm, and compiled to create Z-stacks in the software. Incomprehensible images were

removed prior to compilation, to ensure visualization and ability to distinguish between all dendritic spines in the final image.

2.11 Spine classification and quantification

Dendritic spines from dendrites of 50-100 cells were classified and measured from each animal using the Imaris software 9.2.1. The z-stack files captured by the Zen software (as described above), were loaded in Imaris, and the image was processed using the invert and contrast change applications. The filament tracer tool was used to trace dendrites and spines using the most reliable semi-automatic method (Al-Absi *et al.*, 2018), in which dendrite and spine start and end points were manually chosen followed by automatic tracing in between the seed points by the software. A spine classification extension application was used to classify the spines according to the following parameters: Short Thin – spine head width $\geq$ spine neck width, $< 1\mu\text{m}$; Long Thin – spine head width $\geq$ spine neck width, $> 1\mu\text{m}$; Mushroom – Spine length $< 2\mu\text{m}$ and spine head width $>$ spine neck width*2; Stubby – spine length $< 0.5\mu\text{m}$, and Filopodia – $> 2\mu\text{m}$ (no head) (Table 3). These guidelines were chosen according to previous research, and ensured that no two spines could be classified under more than one category (Al-Absi *et al.*, 2018). Furthermore, each type of spine was overlaid with a distinguishing colour, allowing for unambiguous manual verification of accurate spine classification.

Table 2-3: Dendritic Spine Classification Criteria in IMARIS

Spine type	Classification Criteria in IMARIS
Short Thin	spine head width $\geq$ spine neck width, $< 1\mu\text{m}$
Long Thin	spine head width $\geq$ spine neck width, $> 1\mu\text{m}$
Mushroom	Spine length $< 2\mu\text{m}$ and spine head width $>$ spine neck width*2
Stubby	Stubby – spine length $< 0.5\mu\text{m}$
Filopodia	$> 2\mu\text{m}$ (no head)

Many types of measures were chosen for the quantification of spines, to determine whether the COX-2-KO genotype modifies their number or development in the male and female mouse cerebellum. Along with classifying the spines into their respective type categories, the following measurements were collected as per the literature: spine density (Smith *et al.*, 2016; Hernandez *et al.*, 2019); spine volume (Borczyk *et al.*, 2019); spine length (Dumanis *et al.*, 2011); spine neck length (Adrian *et al.*, 2017); spine head diameter (Vo *et al.*, 2019); and spine straightness (Gouder *et al.*, 2019). Various measures allowed for a greater amount of data regarding spine morphology changes in the COX-2-KO mouse and between sexes.

2.12 Statistics

2.12.1 Studies 1-4

qRT-PCR and Western blot data - The statistical analysis was conducted in the IBM SPSS Statistics program and organized in Graphpad Prism v6.1. The dataset of RQ values across three independently repeated experiments (qRT-PCR or Western blot), was analyzed using a

one-way ANOVA to compare the variance between groups, and generate an F-value. If the F-value deemed the data to be possibly significant, a post-hoc Tukey HSD test followed to confirm significance between independent groups. The Tukey HSD post-hoc test is considered as more conservative and reliable than other tests, and therefore more likely that the significance values are true. Groups were considered significant if the p value was less than 0.05 ($p < 0.05$). All numerical values were expressed as mean $\pm$ SEM.

2.12.2 Study 4

Spine morphology quantification measures – The experimenter was completely blind to animals' genotype and sex throughout the analysis. Dendritic spine measurements from approximately 50-100 cells from each animal were taken ($n=3$ animals per group). This entire data set was used to conduct a one-way ANOVA, followed by pairwise comparisons and post-hoc Tukey HSD test. Groups were considered significant if the p value was less than 0.05 ($p < 0.05$). All numerical values were expressed as mean $\pm$ SEM.

CHAPTER 3. RESULTS – STUDY 1

Study 1 –Maternal exposure to prostaglandin E₂ modifies expression of Wnt genes in mouse brain – An autism connection

Manuscript Citation:

Rai-Bhogal, R., Wong, C., Kissoondoyal, A., Davidson, J., Li, H., & Crawford, D. A. (2018). Maternal exposure to prostaglandin E₂ modifies expression of Wnt genes in mouse brain – An autism connection. *Biochemistry and Biophysics Reports* 14:43-53. (Appendix C1)

The dissertation was written in thesis format and the following is specific to the contributions in the chapter:

Contributions: Ravneet Rai-Bhogal designed and performed all experiments in the chapter, collected samples, acquired and analyzed all data, created figures and tables, and prepared the manuscript. Christine T. Wong redesigned some figures and assisted in editing the manuscript. Dr. Hongyan Li provided administrative and technical support throughout the study. Dr. Dorota A. Crawford supervised the design of the study and was involved with editing the manuscript.

3.1 Manuscript Introduction and Rationale

The brain contains high lipid content and its healthy development relies on the supply and function of these molecules (Calderon & Kim, 2004). Prostaglandin E₂ (PGE₂) is one of the major lipid metabolites released from phospholipid membranes through the action of phospholipase A₂ (PLA₂) and cyclooxygenase-1 or -2 (COX-1 or -2) (Funk, 2001). PGE₂ exerts its physiological function through four G-protein coupled E-prostanoid receptors (EP1-4) (Leclerc *et al.*, 2015a; Jones *et al.*, 2016; Sreeramkumar *et al.*, 2016). Recent research shows that PGE₂ can play an important role in brain development including dendritic spine formation (Amateau & McCarthy, 2002; Burks *et al.*, 2007) and neuronal plasticity (Chen *et al.*, 2002; Koch *et al.*, 2010). Increased levels of COX-2 during embryogenesis (Saint-Dizier *et al.*, 2011), and higher expression levels of EP receptors during neurogenesis (Tamiji & Crawford, 2010b), further strengthen the importance of PGE₂ in development.

We previously described that abnormalities in the PGE₂ signalling pathway due to genetic defects or exposure to environmental factors, during critical periods of brain development, have been associated with the etiology of neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) (Tamiji & Crawford, 2010b; Wong *et al.*, 2015b). The overwhelming evidence shows that various environmental risk factors, including deficient dietary supplementation, infections, exposure to drugs such as misoprostol (prostaglandin E analogue), air pollutants, chemicals found in food and personal care products like cosmetics, disrupt the level of PGE₂ and are independently linked to ASD (Tamiji & Crawford, 2010b; Wong *et al.*, 2015b). These exogenous chemicals can be shuttled through the BBB during a critical time in development and impact signaling of the PGE₂ pathway (Wong *et al.*, 2015b).

The molecular mechanisms by which changes in PGE₂ levels influence brain development are still not well known.

Thus far, *in vitro* research in our lab has shown that increased levels of PGE₂ can augment proliferation and migration of neuroectodermal stem cells (Wong et al. 2014), accelerate neuronal differentiation (Wong *et al.*, 2016), retract neuronal extensions (Tamiji & Crawford, 2010c) and elevate calcium level in the cytosol and growth cones (Tamiji & Crawford, 2010c; Davidson *et al.*, 2016). More interestingly, we showed for the first time in neuronal cell lines that PGE₂ signalling can interact with the major developmental pathway, *Wingless-Type MMTV Integration Site Family* (Wnt) canonical pathway (Wong *et al.*, 2014a). In neuroectodermal (NE-4C) stem cells PGE₂ can modulate the Wnt canonical pathway via protein kinase A (PKA), phosphoinositide 3-kinase (PI-3K) and β -catenin, the key regulator of the Wnt pathway (Wong *et al.*, 2014a; Wong *et al.*, 2016). In addition, higher level of PGE₂ modified the expression of many downstream Wnt-regulated genes important in development and previously implicated in ASD. We also found the cross-talk between the PGE₂ and Wnt signalling pathways for the first time in the brain of the COX-2^{-/-} knockout mouse (Rai-Bhagal *et al.*, 2018a). These findings are interesting because signalling of the Wnt signaling pathway is crucial during early brain development (Ciani & Salinas, 2005) and its abnormal regulation have also been associated with ASD (Kalkman, 2012; Zhang *et al.*, 2014b).

In this study, we use mouse as the experimental model system to determine the effects of maternal exposure to PGE₂ at the onset of neurogenesis (embryonic day 11) on expression of various developmental genes in the mouse brain. We identified differential expression of Wnt-regulated genes, including *Mmp7*, *Wnt2*, and *Wnt3a* (characterized by another student), in offspring from the same pregnancy. We found that the affected genes were previously linked to

neurodevelopmental disorders. PGE₂ greatly affected the normal expression level of these genes across three developmental stages tested: embryonic day 16 (E16), embryonic day 19 (E19) and early postnatal day 8 (P8). Moreover, we detected variations in expression level between offspring with the same genetic composition that persisted through E16, E19, and P8. We also show that PGE₂ increased the level of PKA activated p β -catenin, the major downstream regulator of gene transcription in the Wnt canonical signaling pathway. These findings provide a novel *in vivo* data that maternal exposure to exogenous PGE₂ during critical periods can affect the expression level of important developmental genes in the brain of offspring.

3.2 Objectives and Hypothesis

The primary aim of this study was to use mouse as an *in vivo* experimental model system to determine the effects of maternal exposure to PGE₂, at the onset of neurogenesis, on the brain throughout neurodevelopment. This was accomplished with the following major study objectives:

Objective 1 – To determine the effects of exogenous PGE₂ administration on the expression of critical genes and proteins during mouse brain development.

Objective 2 – To determine if a single prenatal administration of PGE₂ can lead to changes in gene and protein expression in the postnatal mouse brain at later developmental stages.

Objective 3 – To determine whether there are differences in expression of critical genes in individual pups that are genetically identical, and further assess sex differences.

Objective 4 – To confirm an interaction between PGE₂ and the Wnt pathway, *in vivo* in the mouse brain.

Hypothesis: Due to our previous findings in cell models and observed ASD-like behaviours in the COX-2-deficient and PGE₂-administered mouse models, we hypothesize that an exposure to PGE₂ during brain development, may lead to long-term changes in the brain, through an interaction with the critical early developmental Wnt pathway.

3.3 Experimental Model Systems

In the current study, maternal PGE₂ administration in a mouse model was used to represent increased levels of PGE₂ upon environmental exposures and misoprostol drug use, during pregnancy. PGE₂ administration was conducted at E11 – representative of a similar time-point of misoprostol use in clinical studies (Pastuszak *et al.*, 1998; Bandim *et al.*, 2003), at the onset of neurogenesis in the brain (Rodier, 1980; Clancy *et al.*, 2001; Clancy *et al.*, 2007). Three stages of neuronal development were scrutinized: E16 – peak of neurogenesis in many brain regions; E19 – when critical development in most brain regions has been completed (Rodier, 1980; Rice & Barone, 2000); and P8 – during which the prefrontal cortex, hippocampus, and the cerebellum are still undergoing postnatal development (Rodier, 1980; Semple *et al.*, 2013; Sengupta, 2013).

Results

3.4 Expression of *E-Prostanoid (EP)* Receptors in the Normally Developing and Adult Mouse Brain

In order to establish whether the wild type mouse in this study is suitable to for prenatal exposure to PGE₂, the expression profile of the EP receptors (EP1, EP2, EP3 α , EP3 β , EP3 γ , and EP4) in the brain at the E16, E19, and P8 stages, was examined using qRT-PCR assay. The expression level of all receptors for each sample was first normalized to the geometric mean of the

housekeeping genes *Pgkl* and *Hprt* (see methods) and presented as RQ values compared to the *EP4* receptor (RQ = 1), which was the same across all developmental stages tested (Figure 3-1, *left panels*). The expression pattern of all EP receptors at E16 shows that *EP2* was the most highly expressed (RQ = 355.8; $p < 0.0005$), followed by *EP3 γ* (RQ = 161.4; $p = 0.001$), *EP3 β* (RQ = 50.7; $p = 0.0013$), *EP3 α* (RQ = 0.9, $p = 0.41$), *EP4* (RQ = 1), and *EP1* (RQ = 0.01; $p < 0.0005$) (Figure 3-1A). Similarly, at E19, *EP2* was the most highly expressed (RQ = 316.3; $p < 0.0005$), followed by *EP3 γ* (RQ = 220.1; $p < 0.0005$), *EP3 β* (RQ = 56.2; $p < 0.0005$), *EP3 α* (RQ = 0.3; $p = 0.001$), and *EP1* (RQ = 0.002; $p < 0.0005$) (Figure 3-1B). At P8, the expression of *EP3 γ* was the highest (RQ = 392.0; $p = 0.0005$), followed by *EP2* (RQ = 221.7; $p = 0.002$), *EP3 β* (RQ = 155.0; $p = 0.001$), *EP3 α* (RQ = 3.1; $p = 0.02$), and *EP1* (RQ = 0.04; $p < 0.0005$) (Figure 3-1C). In the adult brain, *EP2* was again the most highly expressed (RQ = 133.0; $p = 0.007$), followed by *EP3 γ* (RQ = 111.4; $p = 0.0005$), *EP3 β* (RQ = 50.9; $p = 0.0005$), *EP3 α* (RQ = 3.6; $p = 0.01$), and *EP1* (RQ = 0.003; $p < 0.0005$) (Figure 3-1D). Overall, we can conclude that *EP2*, *EP3 γ* , and *EP3 β* are predominantly expressed in the mouse brain across all stages – prenatal to adult.

Western blot analysis confirmed the expression of all EP receptors in each developmental stage at the protein level (Figure 3-1, *right panels*). Although the protein expression between EP receptors cannot be compared, due to properties and binding affinities of individual antibodies, some interesting observations across developmental stages can be noted. The expression of *EP1* and *EP4* receptors remained consistent across all stages, whereas there was a decrease in expression of *EP2* and *EP3* receptors in the postnatal stages. This suggests a primary role of *EP2* and *EP3* receptors in prenatal brain development.

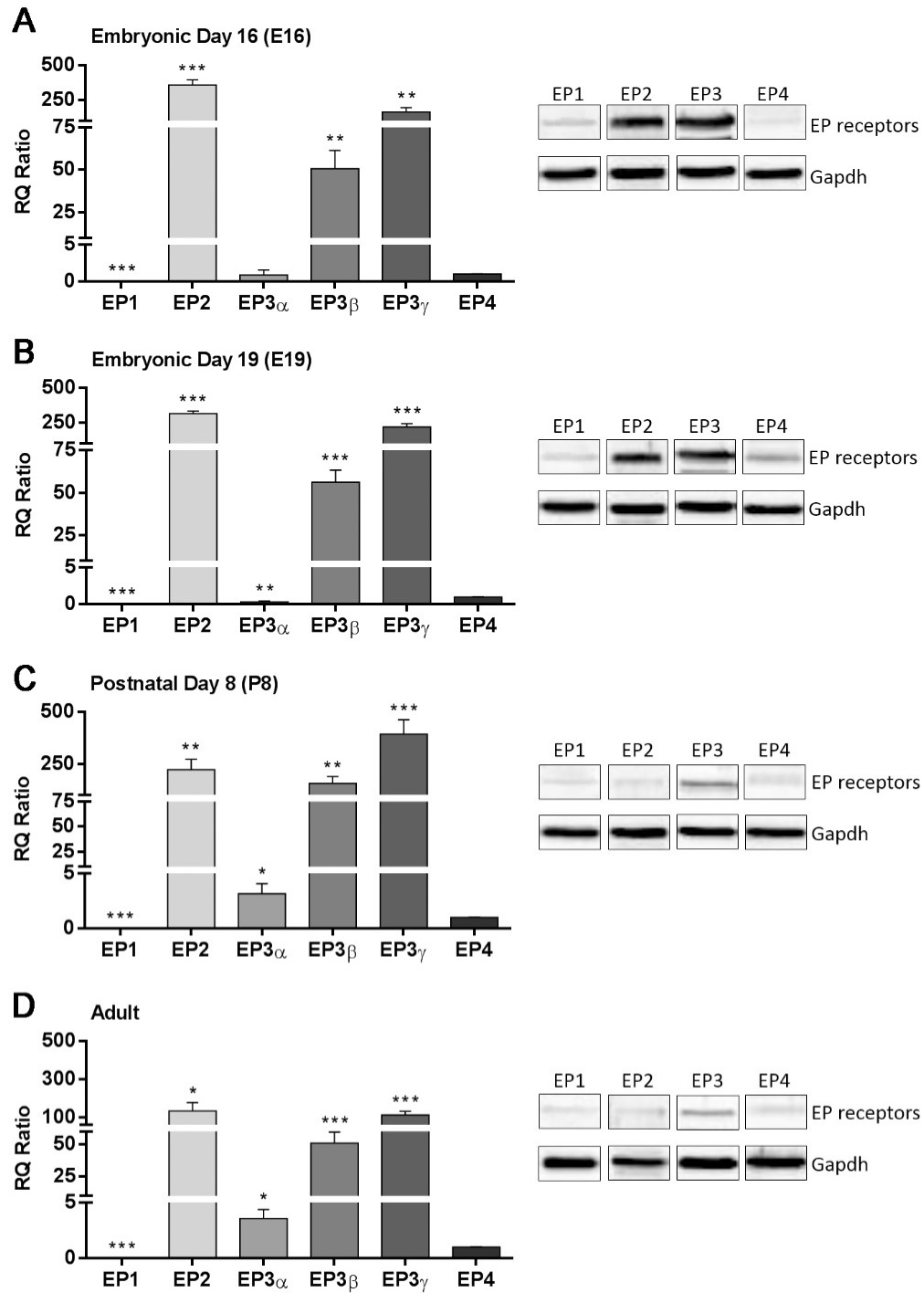


Figure 3-1. mRNA (left) and protein (right) expression of EP receptors during mouse brain development. (A) mRNA levels of the *EP2*, *EP3 β* , and *EP3 γ* receptors were the most predominantly expressed at Embryonic day 16 (E16). (B-D) Similar pattern was seen at Embryonic day 19 (E19), Postnatal day 8 (P8), and Adult stages. RQ values for *EP1*, *EP2*, *EP3 α* , *EP3 β* , and *EP3 γ* were normalized to RQ values of the *EP4* receptor. * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$. (A-D) Western blot confirmed protein expression of EP1, EP2, EP3 (all isoforms), and EP4 receptors in the developing and adult brain (right panel). Each value was averaged from three separate experiments ($n=3$).

3.5 Preliminary Screening of *Wnt* and *Wnt*-target Genes following Maternal Exposure to PGE₂

The next objective was to investigate whether exogenous PGE₂ administration interferes with Wnt signalling *in vivo* in the mouse brain during development, as was previously shown in neuronal cells (Wong *et al.*, 2014b; Wong *et al.*, 2016). To accomplish this, Custom Taqman plates were designed with 44 genes and used as a screening tool to test the mRNA expression of many *Wnt* and *Wnt*-target developmental genes, in brain tissue from PGE₂-exposed fetuses. Of the tested genes, three differentially expressed genes were detected: *Mmp7* (upregulated; RQ = 13.76), *Wnt2* (downregulated; RQ = 0.46), and *Fosl1* (downregulated; RQ = 0.45) (Figure 3-2). The expression of *Mmp7* and *Wnt2* will be further scrutinized in this dissertation, while *Fosl1* was characterized by another student.

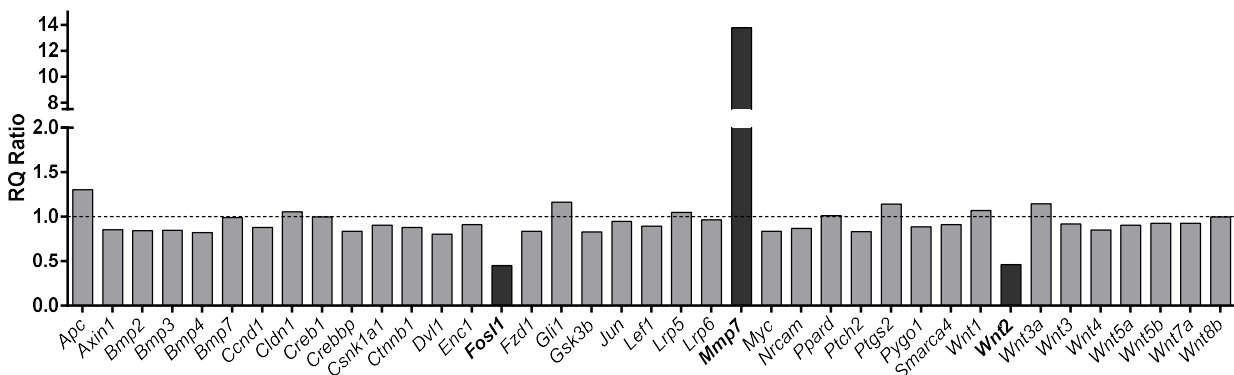


Figure 3-2. Screening of *Wnt* and *Wnt*-target Genes in the PGE₂-exposed embryo brain. Of the 44 *Wnt* and *Wnt*-target genes tested, mRNA of three genes was differentially expressed: *Mmp7* (upregulated), *Wnt2* (downregulated), and *Fosl1* (downregulated). The dotted line denotes an RQ value of 1.

3.6 Expression of *Mmp7* and *Wnt2* during Normal Mouse Brain Development

Normal mRNA and protein expression levels of *Mmp7* and *Wnt2* in the mouse brain at different developmental stages, were measured in WT animals. mRNA expression of *Mmp7* in E16, E19, and P8 was higher when compared to expression in the adult brain, with RQ values of 23.0 ($p < 0.0005$), 13.8 ($p = 0.01$), 10.9 ($p = 0.01$), and 1, respectively (Figure 3-3A). The protein expression of *Mmp7* in E16, E19, and P8 stages confirmed the mRNA results, following a similar pattern of expression compared to the adult brain, with fold-change values of 2.3, 1.9, and 1.5, respectively (Figure 3-3B). The highest expression of *Mmp7* was at E16 ($p = 0.0005$), followed by E19 ($p = 0.01$), and P8 ($p = 0.01$).

The expression of *Wnt2* in the brain at E16, E19, and P8 was lower than the adult brain, with RQ values of 0.1 ($p = 0.0008$), 0.3 ($p = 0.002$), 0.7 ($p = 0.03$), and 1, respectively (Figure 3-3C). The protein expression of *Wnt2* at E16 and E19 was significantly higher than the expression in the adult, with fold-change values of 7.0 and 4.9, respectively (Figure 3-3D). The highest expression was at E16 ($p = 0.006$), followed by E19 ($p = 0.03$), and no significant difference at P8, in comparison to the adult brain. Thus, the protein expression is high at the prenatal stages, but decreases in postnatal and adult brain, following a different trend than mRNA. Possible reasons for the discrepancy between mRNA and protein expression will be addressed in the discussion section.

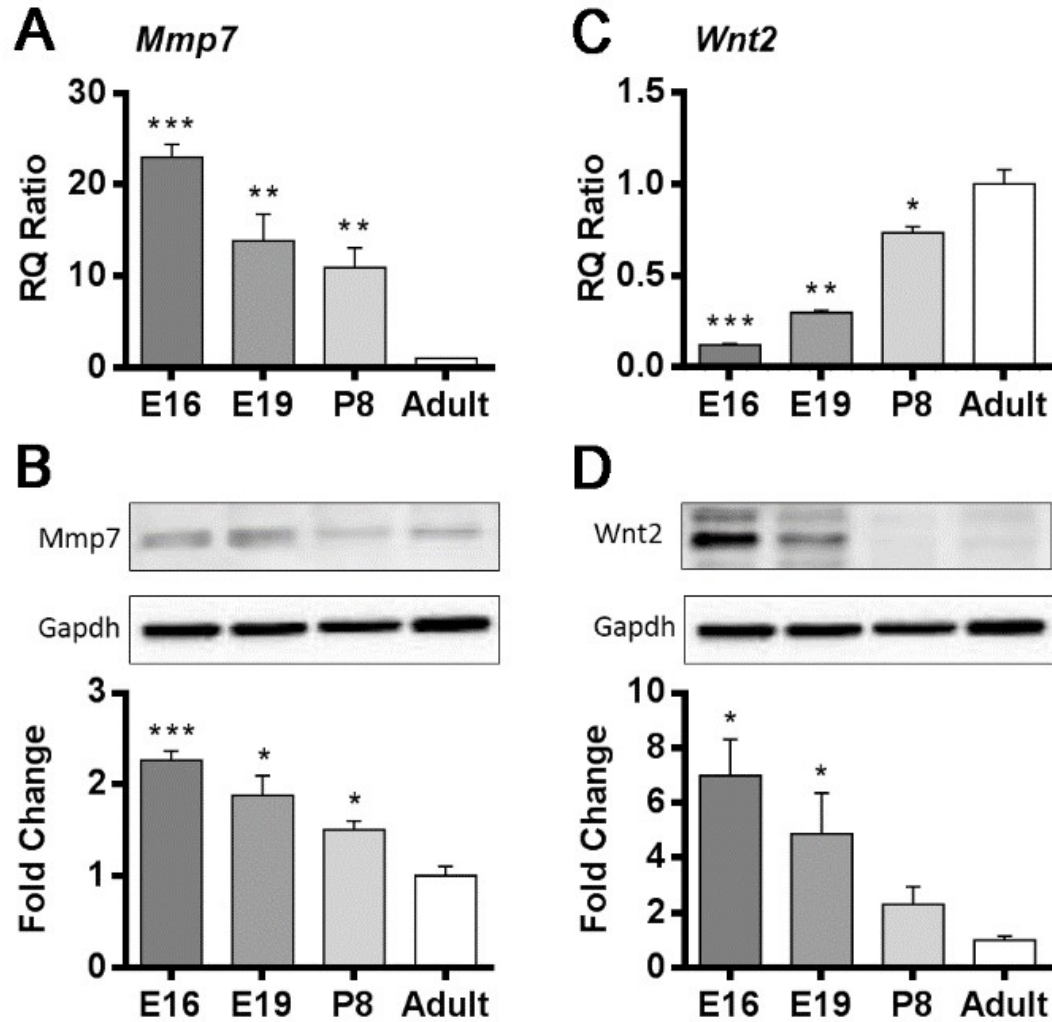


Figure 3-3. *Mmp7* and *Wnt2* mRNA and protein expression throughout normal mouse brain development. A. *Mmp7* mRNA expression in the brain is highest at E16, and decreases throughout development, compared to the adult brain. B. *Mmp7* protein expression is also highest at E16 and decreases throughout development. C. *Wnt2* mRNA expression is lowest at E16, and increases throughout development, compared to the adult brain. D. *Wnt2* protein expression is highest at the E16 stage, and decreases throughout development. Immunomembranes were re-probed with anti-Gapdh antibody as an equal loading control. Presented values were averaged from three independent experiments of pooled samples (n=3). * $p < 0.05$, ** $p < 0.005$, *** $p < .0005$.

3.7 mRNA Expression of *Mmp7* in the Brain of Individual Pups at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE₂

Next, the mRNA expression of *Mmp7* and *Wnt2* was measured in saline- and PGE₂-exposed pups during gestation. Values from individual pups of each litter (n=3 litters) were plotted, rather than mean values from each litter. Twin studies of ASD show a high concordance rate in individuals with ASD and verify the genetic component in its manifestation. However, the variability in behavioural symptoms even between monozygotic twins may be due to the environment. Therefore, the current study investigates whether maternal environmental exposure of PGE₂ can cause differential changes in gene expression in individual animals of the same pregnancy.

The mRNA expression levels of *Mmp7* and *Wnt2* genes was confirmed using qRT-PCR in each individual offspring from three independent litters following maternal exposure to PGE₂, and compared to control animals represented as RQ value of 1 across all stages (Figure 3-4 A-C). At E16, there was increased expression of *Mmp7* in all three litters (Figure 3-4A). Interestingly, the expression considerably varied between the pups of any given litter, suggesting that genetically identical pups of the same pregnancy are affected differently by the exogenous PGE₂ compound. Litter 1 (L1) had a total of six pups with RQ values of 95.2 ($p = 0.014$), 16.5 ($p = 0.017$), 7.2 ($p = 0.026$), 5.8 ($p = 0.0081$), 3.1 ($p = 0.27$) and 0.60 ($p = 0.12$). Eight pups in L2 had RQ values of 6.5 ($p = 0.0058$), 3.1 ($p = 0.017$), 2.0 ($p = 0.0072$), 1.0 ($p = 0.75$), 0.84 ($p = 0.24$), 0.53 ($p = 0.012$), and 0.38 ($p = 0.0048$), 0.10 ($p < 0.0005$). In L3, there were seven pups with RQ values of 13.8 ($p = 0.044$), 4.9 ($p = 0.015$), 4.1 ($p = 0.022$), 1.9 ($p = 0.16$), 1.8 ($p = 0.18$), 0.93 ($p = 0.84$), and 0.80 ($p = 0.057$). The average expression of *Mmp7* at E16 in PGE₂-exposed animals (L1, L2, and L3)

had RQ value of 8.2 ($p = 0.31$) above the level in control animals. This mean value did not reach significance because of the great variation between individual pups.

In contrast, at developmental stage of E19, the expression of *Mmp7* from the three litters was considerably decreased when compared to control animals (Figure 3-4B). In L4, there was a significant decrease of *Mmp7* expression in all six pups ($p < 0.0005$) with RQ values of 0.47, 0.34, 0.20, 0.048, 0.030, and 0.026. In L5, all eight pups showed a significant decrease of *Mmp7* expression ($p < 0.0005$) with RQ values of 0.12, 0.11, 0.10, 0.10, 0.08, 0.058 and 0.056. Significant reduction in *Mmp7* expression was also observed in all six pups of L6 ($p < 0.0005$) with RQ values of 0.11, 0.067, 0.050, 0.045, 0.033, and 0.022. The overall expression of *Mmp7* at E19 in PGE₂-exposed animals (L4, L5, and L6), decreased with an RQ value of 0.11 ($p < 0.0005$) or 9.1 times below the level from the controls.

Similar to E19, *Mmp7* expression in P8 remained decreased in three PGE₂-exposed pups of L7 (Figure 3-4C). The RQ values for each pup were 0.67 ($p = 0.040$), 0.65 ($p = 0.022$), and 0.40 ($p = 0.0012$). In L8, there were nine pups with RQ values of 1.8 ($p = 0.35$), 0.95 ($p = 0.56$), 0.90 ($p = 0.45$), 0.70 ($p = 0.24$), 0.68 ($p = 0.030$), 0.59 ($p < 0.0005$), 0.52 ($p = 0.0032$), 0.44 ($p < 0.0005$), and 0.43 ($p < 0.0005$). In L9, there were seven pups with RQ values of 1.09 ($p = 0.52$), 0.99 ($p = 0.89$), 0.75 ($p = 0.055$), 0.74 ($p = 0.23$), 0.63 ($p = 0.0039$), 0.60 ($p < 0.0005$), and 0.41 ($p < 0.0005$). The overall expression of *Mmp7* in all PGE₂-exposed animals (L7, L8, and L9) continued to be suppressed at P8 with an RQ value of 0.73 ($p = 0.0022$), which represents 1.4 times reduction from the normal level seen in controls.

The actual RQ values of normal expression level of *Mmp7* at E16, E19 and P8 in the control animals (represented as the RQ value of 1 in Figure 3-4 A-C) are plotted in Figure 5D. The expression level of *Mmp7* appears highest at E16, and continued to decline throughout

development (E19 and P8) with RQ values of 23.0 ($p < 0.0005$), 13.8 ($p = 0.012$), and 10.9 ($p = 0.010$), respectively. As indicated above, the expression of *Mmp7* was greatly influenced by exposure to PGE₂ in all developmental stages. Figure 3-4D shows that, at E16, the overall expression level of *Mmp7* in PGE₂-exposed animals was increased by 8.2 fold change. The expression level at E19 was overall decreased with PGE₂ by 9.1 and continued to be suppressed at P8 by 1.4.

In addition, sex differences in gene expression were examined between males and females following maternal exposure to PGE₂ (Figure 3-5). The values were normalized to control values, in which the control RQ value was set to 1. There was no significant difference in expression of *Mmp7* observed between males and females at E16 (males RQ = 10.1; females RQ = 1.8; $p = 0.69$), E19 (males RQ = 0.1; females RQ = 0.1; $p = 0.49$) or P8 (males RQ = 0.7; females RQ = 0.8; $p = 0.69$). However, there was a trend observed at the early E16 stage, during which, the mRNA expression of *Mmp7* in males seemed to be greater than in females.

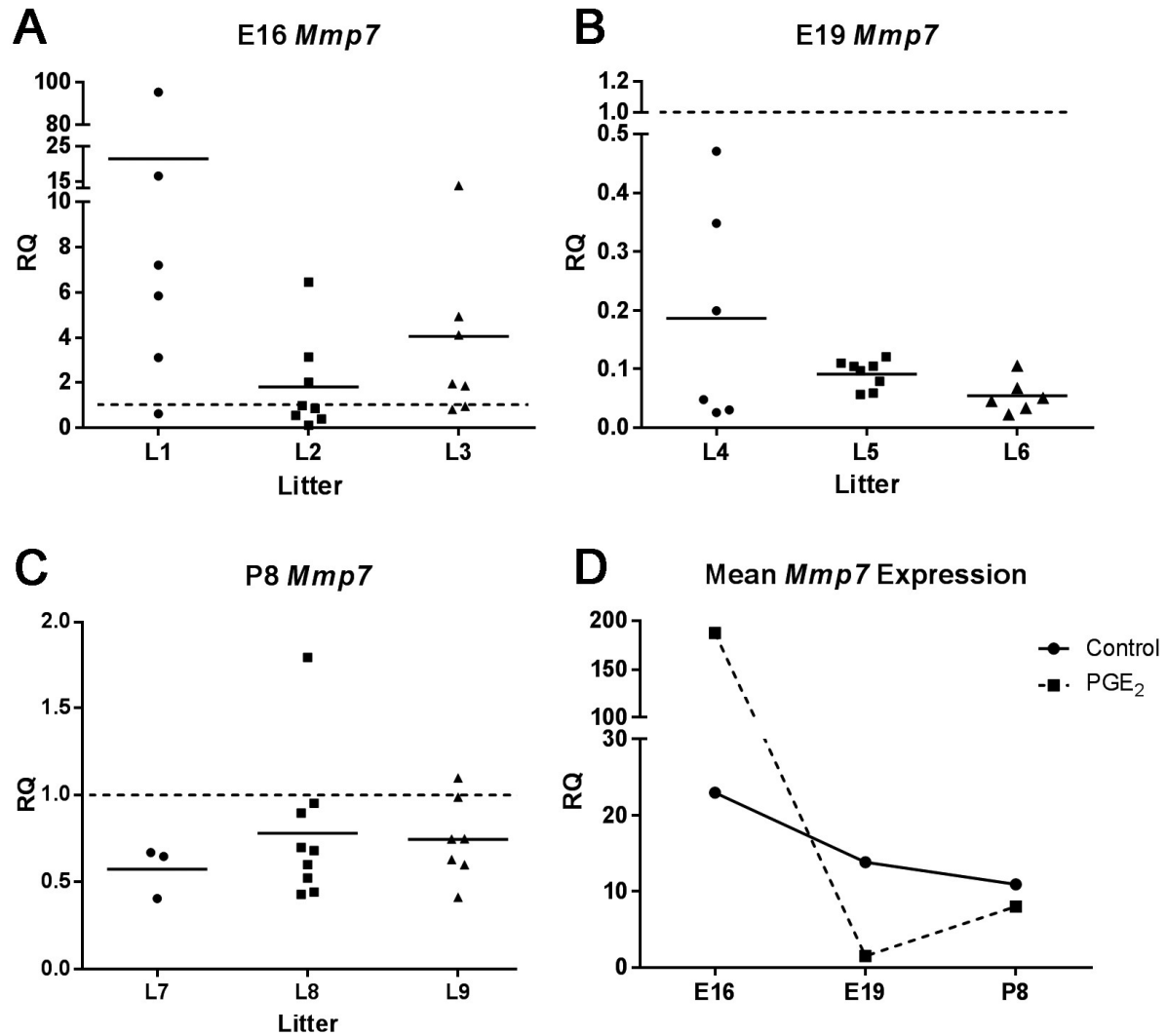


Figure 3-4. Expression of *Mmp7* mRNA in developing mouse brain following maternal exposure to PGE₂. (A) The expression of *Mmp7* increased at E16 in all three litters (L1-L3) following PGE₂ exposure. There were individual differences between pups. (B) At E19, the expression of *Mmp7* decreased in all three litters L4-L6. (C) There was continued decrease in expression of *Mmp7* at P8 in pups of all three litters L7-L9. Horizontal lines represent the mean value within each litter. (D) In the control mouse brain, *Mmp7* expression is highest at E16 ($p < 0.0005$), and decreases throughout development in E19 ($p = 0.012$) and P8 ($p = 0.010$). Following PGE₂ exposure, mean *Mmp7* expression from all pups is greater at E16 but not statistically significant ($p = 0.31$), and decreases at E19 ($p < 0.0005$) and P8 ($p = 0.0022$), from the normal expression in controls. Each value was averaged from animals from three litters and three independent experiments.

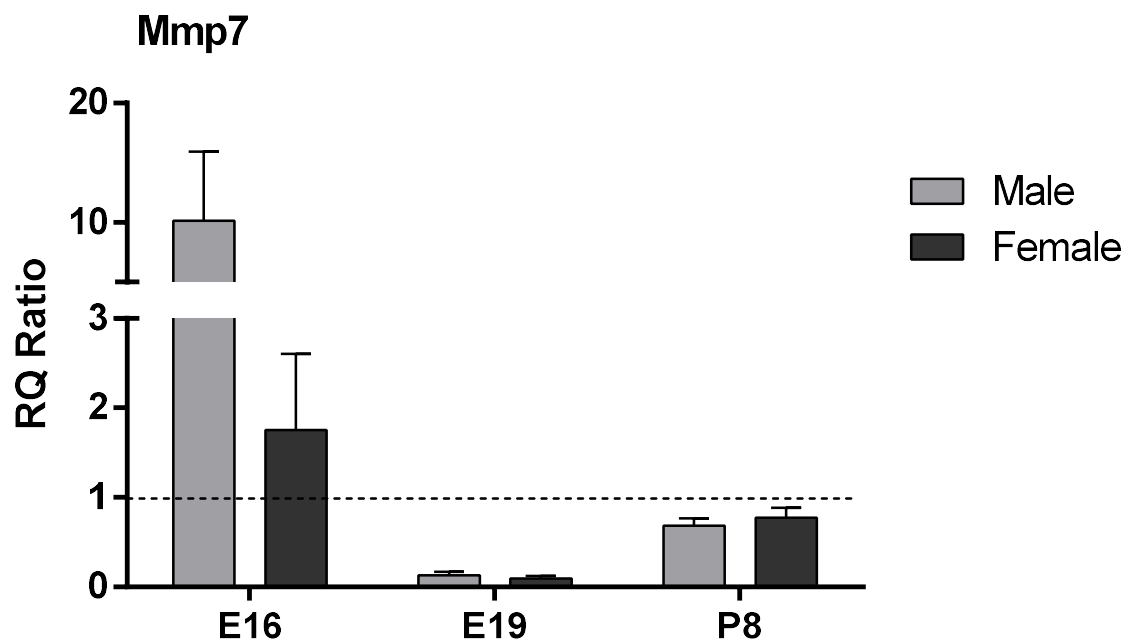


Figure 3-5. Sex differences in expression of *Mmp7* in brains of PGE₂-exposed animals throughout development. There were no significant differences in the expression of *Mmp7* between males and females at E16, E19, or P8. All values were normalized to controls (RQ = 1; dashed line).

3.8 mRNA Expression of *Wnt2* in the Brain of Individual Pups at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE₂

The mRNA expression of *Wnt2* in the brain was overall reduced at developmental stage of E16 and was elevated in E19 and P8 as compared to control animals (Figure 3-6). At E16, a significant decrease in expression of *Wnt2* was detected in all six pups in L1 with RQ values of 0.68 ($p < 0.0005$), 0.67 ($p = 0.041$), 0.66 ($p < 0.0005$), 0.64 ($p = 0.0022$), 0.59 ($p = 0.00052$), and 0.55 ($p < 0.0005$) (Figure 3-6A). In L2, there were eight pups with RQ values of 2.7 ($p < 0.0005$), 1.1 ($p = 0.37$), 0.87 ($p = 0.31$), 0.84 ($p = 0.16$), 0.80 ($p = 0.14$), 0.76 ($p = 0.028$), 0.74 ($p = 0.013$), and 0.60 ($p = 0.0046$). More variation was found in the expression level of *Wnt2* within the seven pups of L3 with RQ values of 2.4 ($p = 0.0059$), 1.6 ($p = 0.036$), 1.2 ($p = 0.54$), 0.87 ($p = 0.67$), 0.82 ($p = 0.45$), 0.69 ($p = 0.11$), and 0.66 ($p = 0.0023$). Although there are significant changes in *Wnt2* expression at E16 in individual PGE₂-exposed animals, the overall average across three litters was not significantly different from the controls with an average RQ value of 0.97 ($p = 0.82$).

Exposure to PGE₂ largely resulted in elevated *Wnt2* expression in the brain across the pups of three independent litters at developmental stage of E19 (Figure 3-6B). In L4, there were six pups with RQ values of 10.6 ($p = 0.00082$), 8.0 ($p = 0.0013$), 6.4 ($p < 0.0005$), 3.5 ($p = 0.0031$), 2.1 ($p = 0.034$), and 1.4 ($p = 0.33$). In L5, all eight pups show increased *Wnt2* expression with RQ values of 6.9 ($p < 0.0005$), and 5.2 ($p < 0.0005$), 3.4 ($p = 0.015$), 3.0 ($p = 0.0045$), 2.9 ($p = 0.014$), 2.7 ($p = 0.030$), 2.6 ($p = 0.023$), 2.5 ($p = 0.021$). Similarly, the expression of *Wnt2* was also increased in all six pups of L6 with RQ values of 6.3 ($p < 0.0005$), 5.1 ($p < 0.0005$), 3.8 ($p = 0.0026$), 2.6 ($p = 0.031$), 2.4 ($p = 0.049$), and 2.2 ($p = 0.045$). The overall expression of *Wnt2* in PGE₂-exposed animals at E19 (litter L4, L5, and L6), was 4.2 times greater than the control ($p < 0.0005$).

At developmental stage of P8, the expression of *Wnt2* continued to be increased (Figure 3-6C). In L7, there were three pups with RQ values of 3.0 ($p = 0.027$), 2.0 ($p = 0.032$), and 1.7 ($p = 0.20$). In L8, there were nine pups with RQ values of 2.9 ($p < 0.0005$), 2.9 ($p = 0.043$), 2.2 ($p = 0.0043$), 2.2 ($p < 0.0005$), 2.0 ($p = 0.0012$), 1.5 ($p = 0.16$), 1.1 ($p = 0.80$), 0.92 ($p = 0.82$), and 0.85 ($p = 0.62$). In L9, all seven pups had significantly increased expression of *Wnt2* with RQ values of 3.1 ($p < 0.0005$), 2.9 ($p = 0.00085$), 2.8 ($p < 0.0005$), 2.8 ($p = 0.00057$), 2.5 ($p = 0.0079$), 2.1 ($p = 0.0085$), 2.0 ($p = 0.035$). The overall expression of *Wnt2* in PGE₂-exposed animals at P8 (L7, L8, and L9) was 2.2 times greater than the controls ($p < 0.0005$).

In the control animals, the normal expression level of *Wnt2* in the brain (represented as the RQ value of 1 in Figure 3-6 A-C) was lowest at E16, and continued to increase throughout development (E19 and P8) with RQ values of 0.1 ($p = 0.00084$), 0.3 ($p = 0.0020$), and 0.7 ($p = 0.033$) respectively (Figure 3-6D). Maternal exposure to PGE₂ did not have a significant effect on the overall expression of *Wnt2* at developmental stage E16 with the average fold change decrease of 1.03. However, PGE₂ increased the overall *Wnt2* expression level at E19 by a fold change of 4.2, and remained elevated at P8 with a fold change of 2.2.

In addition, there was no significant difference in expression of *Wnt2* observed between males and females at E16 (males RQ = 1.0; females RQ = 1.0; $p = 0.69$), E19 (males RQ = 3.8; females RQ = 4.5; $p = 0.49$) or P8 (males RQ = 2.2; females RQ = 2.2; $p = 0.69$) (Figure 3-7).

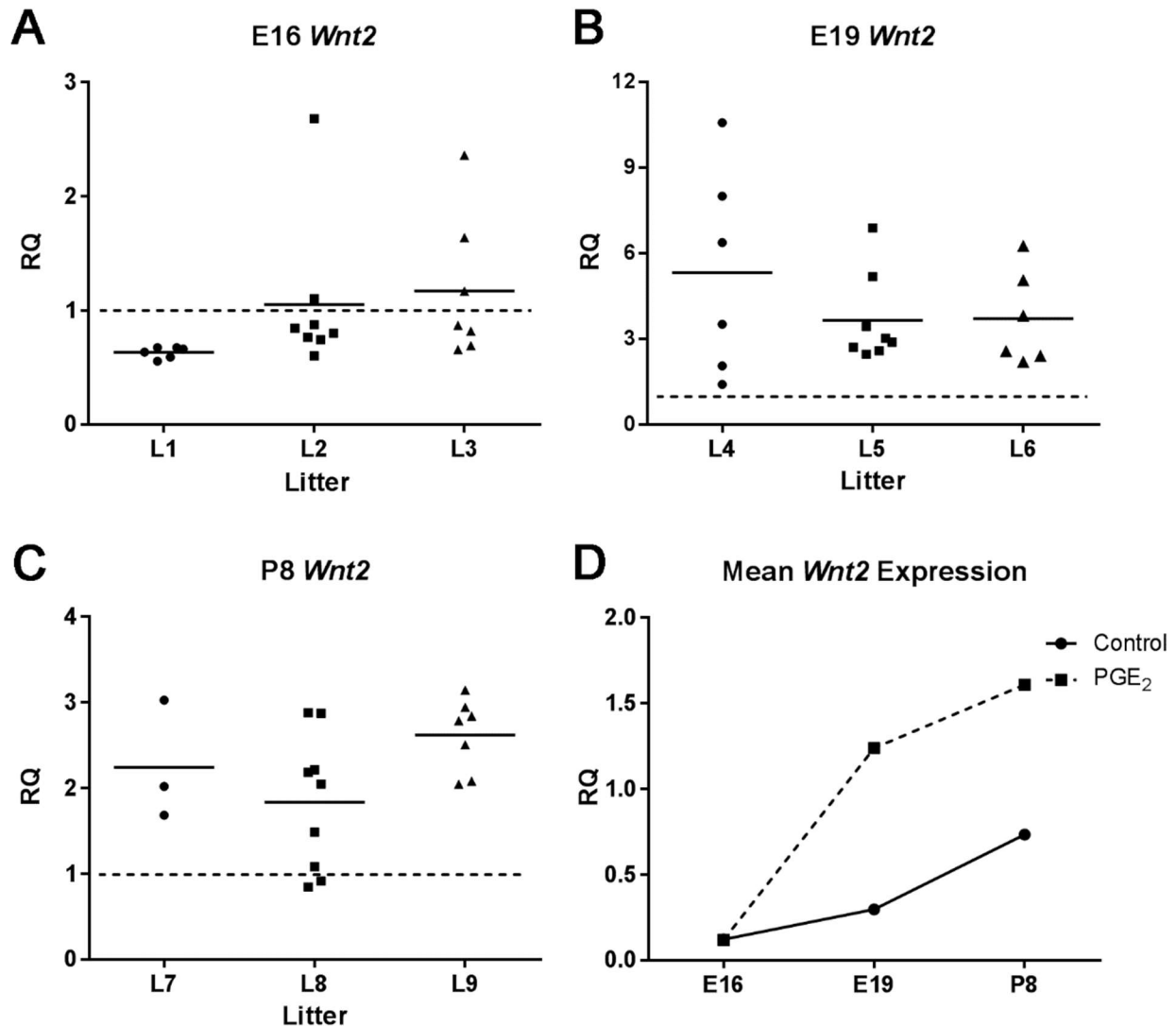


Figure 3-6. Expression of *Wnt2* mRNA in developing mouse brain following maternal exposure to PGE₂. (A) The expression of *Wnt2* decreased in most pups at E16 (L1-3), with the exception of a few pups in which levels were increased. (B) At E19, the expression of *Wnt2* increased in all three litters (L4-L6). (C) There was continued increase in expression of *Wnt2* at P8 in pups of all three litters (L7-L9). Horizontal lines represent the mean value within each litter. (D) In the control mouse brain, *Wnt2* expression is lowest at E16 ($p = 0.00084$), and increases throughout development in E19 ($p = 0.0020$) and P8 ($p = 0.033$). Following PGE₂ exposure, mean *Wnt2* expression from all pups is similar at E16 ($p = 0.82$), and significantly increases at E19 ($p < 0.0005$) and P8 ($p < 0.0005$), from the normal expression in controls. Each value was averaged from animals from three litters and three separate experiments.

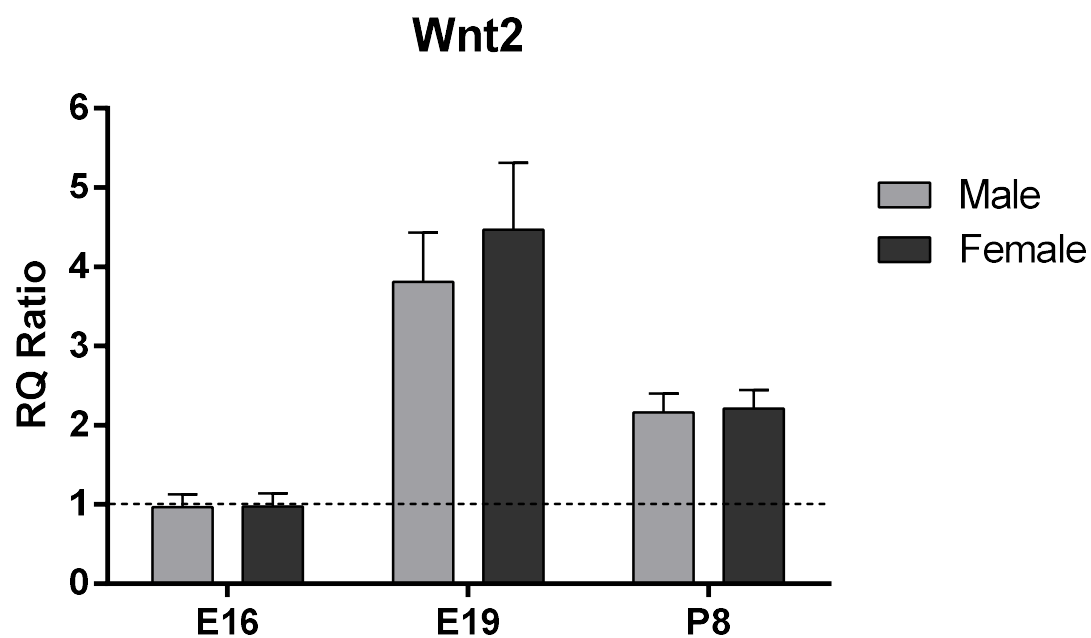


Figure 3-7. Sex differences in expression of *Wnt2* in brains of PGE₂-exposed animals throughout development. There were no significant differences in the expression of *Wnt2* between males and females at E16, E19, or P8. All values were normalized to controls (RQ = 1; dashed line).

3.9 Protein Expression of Mmp7 and Wnt2 in Mouse Brain at Developmental Stages E16 and E19 following Maternal Exposure to PGE₂

The next objective was to quantify the protein levels of Mmp7 and Wnt2 at developmental stages E16 and E19, following maternal exposure to PGE₂. The prenatal stages were chosen to quantify proteins as they showed contrasting gene expression (upregulation and downregulation or vice versa) from one stage to the other. It would be interesting to observe whether this switch occurs at the protein level as well. At E16, there was a significant 1.4-fold increase of Mmp7 protein expression in litter 1 ($p = 0.0009$) but no change was observed in litters 2 and 3 (Figure 3-8A). Increased expression of Mmp7 in litter 1 was consistent with the mRNA expression (Figure 3-4A). There was a significant 0.5-fold decrease in the expression of Wnt2 protein in litter 1 ($p = 0.007$), 0.6 in litter 2 ($p = 0.02$), and 0.5 in litter 3 ($p = 0.006$) (Figure 3-8B). Therefore, at the E16 developmental stage, the protein expression of Wnt2 reflects the mRNA expression following maternal PGE₂ exposure (Figure 3-6A).

At E19, there was a significant 0.5-fold decrease in Mmp7 protein expression in litter 2 ($p = 0.02$) and litter 3 ($p = 0.03$) (Figure 3-8C). In contrast, we observed an unexpected decrease in expression of Wnt2 protein in litter 3 with a fold-change of 0.7 ($p = 0.03$) compared to control animals (Figure 3-8D). At this stage, protein levels of Wnt2 do not reflect the observed mRNA expression (Figure 3-6B).

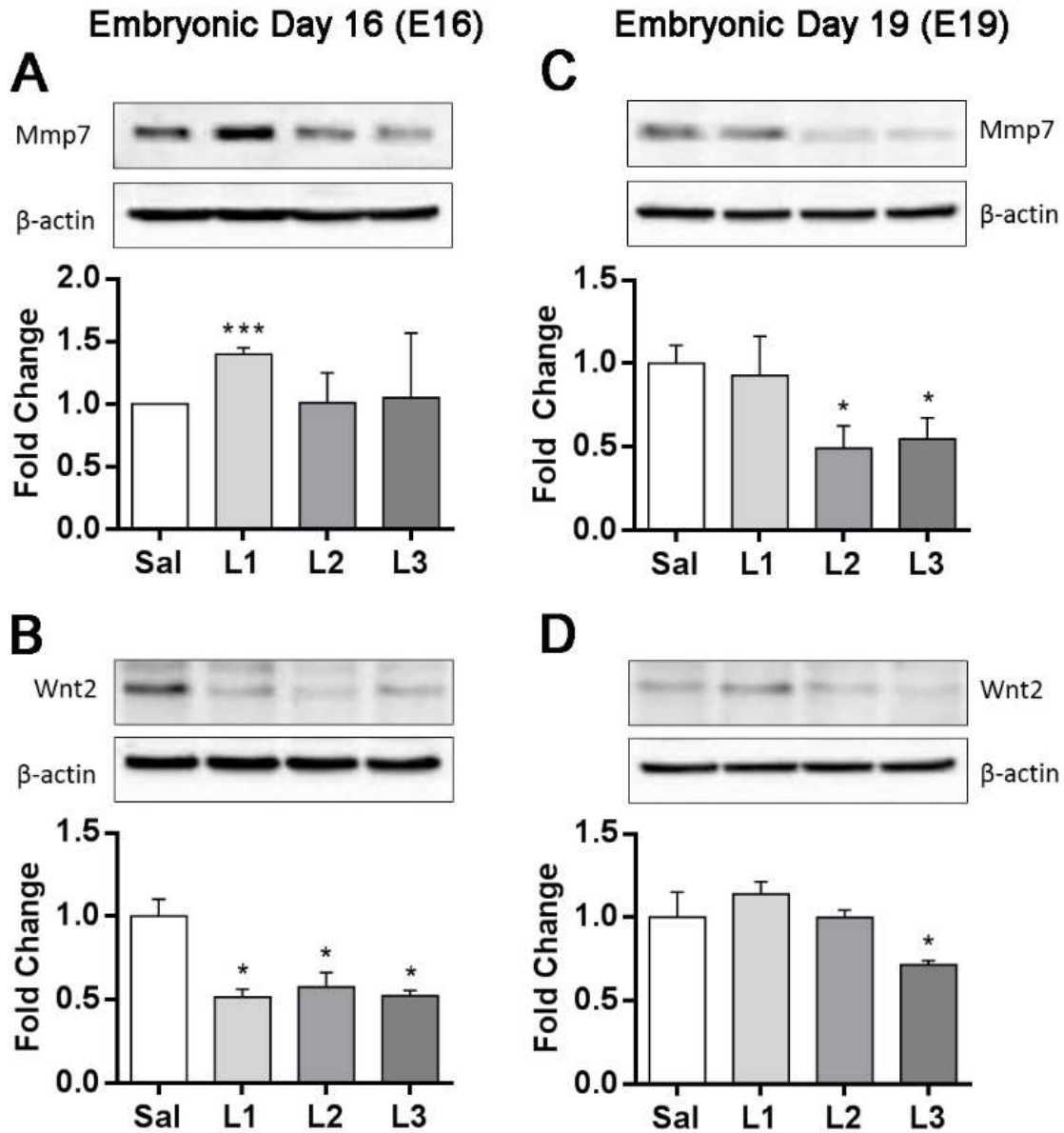


Figure 3-8. Protein expression of Mmp7 and Wnt2 at developmental stages E16 and E19 following maternal exposure to PGE₂. (A) At E16, Mmp7 protein expression was significantly increased in litter 1, and (B) Wnt2 was decreased in all three litters at E16. (C) At E19, Mmp7 protein level decreased in all three litters and (D) Wnt2 protein level was decreased in litter 3. Represented values were averaged from three independent experiments (n=3). * $p < 0.05$, *** $p < .0005$.

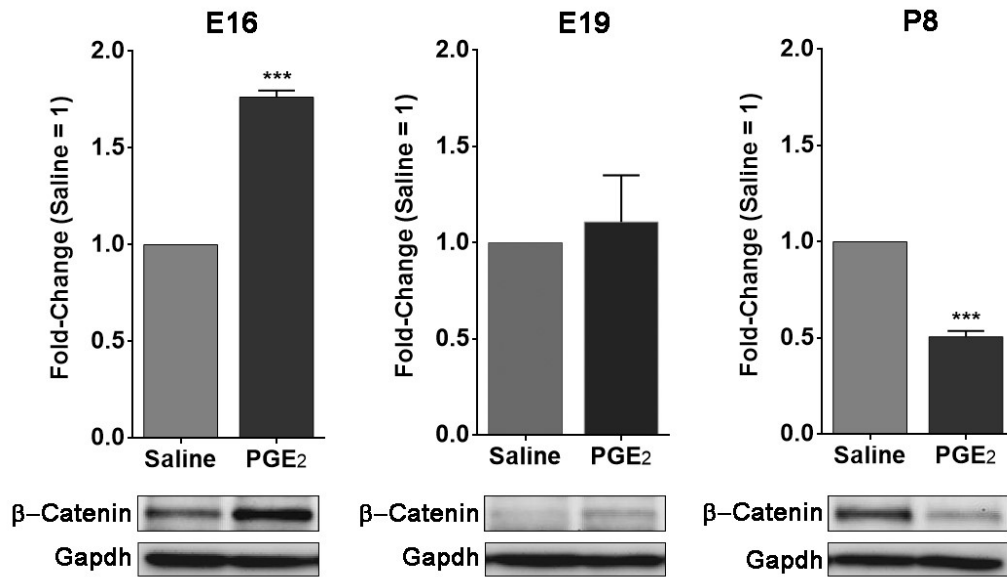
3.10 Protein Expression of Active β -catenin Forms in Mouse Brain at Developmental Stages E16, E19, and P8, following Maternal Exposure to PGE₂

Lastly, the convergence between PGE₂ and the Wnt canonical pathway in the brain during development was investigated. To accomplish this, the protein levels of two active forms of β -catenin, a key modulator of the Wnt canonical pathway were tested. Activation and degradation of β -catenin can be regulated by a complex multiple phosphorylation sites. Antibodies against β -catenin activated through phosphorylation of the Serine-552 site (phospho- β -catenin-Ser552) by PKA (Taurin *et al.*, 2006; Taurin *et al.*, 2008; Verheyen & Gottardi, 2010) were used. The levels of non-phospho (active) β -catenin (Ser33/37/Thr41), which is the most stable form of β -catenin (Gao *et al.*, 2014) were also tested. Phosphorylation at Ser33, Ser37, and Thr41 by GSK-3 β destabilizes β -catenin and marks it for degradation (Liu *et al.*, 2002; Kimelman & Xu, 2006; Wu & He, 2006). The western blot analysis showed that there was a significant 1.8 fold increase in the level of phospho- β -catenin (Ser552) at E16 in animals exposed to PGE₂ ($p < 0.0005$) (Figure 3-9A). At E19, there was no significant change detected ($p = 0.72$). However, at P8 there was a significant decrease in the level of phospho- β -catenin (Ser552) in PGE₂ exposed pups, with a fold change of 0.51 ($p < 0.0005$). Interestingly, the shift in the phosphorylation pattern of phospho- β -catenin (Ser552) and the change in expression levels of Wnt genes (Figures 3-4 and 3-6) both occur at E19. These results indicate that prenatal exposure to PGE₂ can interfere with the Wnt pathway via PKA and may potentially be specific to developmental stage.

The expression of non-phospho β -catenin (Ser33/37Thr41) was not affected at E16 and E19 with fold-change values of 1.1 ($p = 0.34$) and 0.91 ($p = 0.20$), respectively (Figure 3-9B). At P8, there was a significant decrease in PGE₂-exposed animals with a fold-change of 0.48 ($p < 0.0005$). These results indicate that the exposure to PGE₂ during prenatal stages result in

phosphorylation of the Wnt pathway mediator β -catenin by PKA at Ser-552 as well as the stabilization of non-phospho β -catenin at Ser33/37Thr41. Moreover, these effects might be developmentally regulated. A proposed diagram outlining these interactions between PGE₂ and Wnt are shown in Figure 3-10.

A Phospho- β -catenin (Ser552)



B Non-phospho (active) β -catenin (Ser33/37/Thr41)

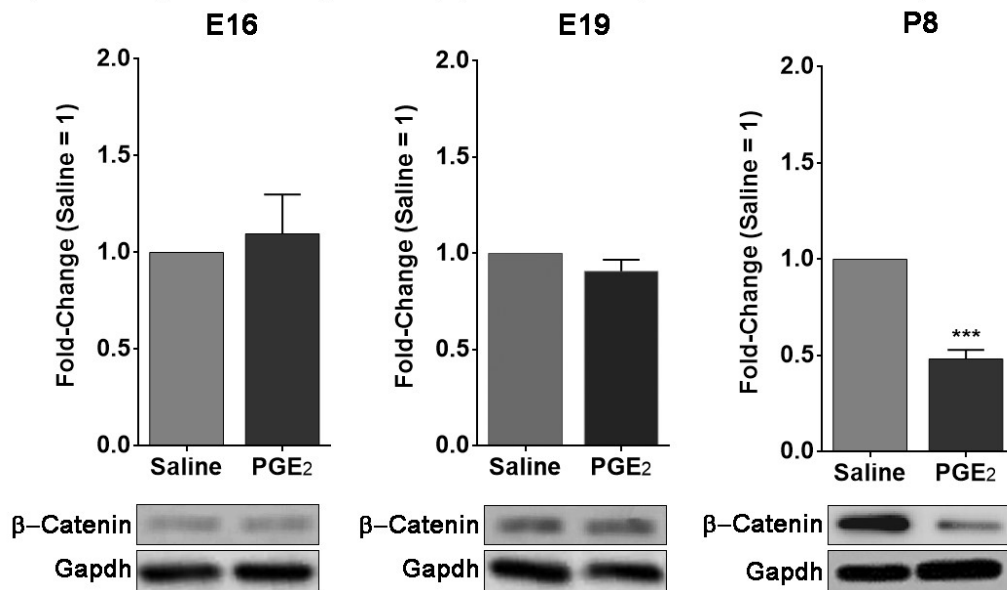


Figure 3-9. Protein expression of phosphorylated β -catenin (Ser-552) and non-phosphorylated β -catenin (Ser33/37/Thr41) in developing mouse brain following maternal exposure to PGE₂. The protein expression of two active forms of β catenin was confirmed by western blot from whole brain following maternal exposure to saline and PGE₂ at E16, E19 and P8. (A; left panel) There was a significant increase in phosphorylation of β -catenin (Ser-552) at E16, (A; middle panel) no change at E19, and (A; left panel) significant decrease at P8. (B; left and middle panels) There was no change in expression of non-phosphorylated β -catenin at Ser33/37/Thr41 sites at E16 and E19, (B; right panel) and significant decrease at P8. The results represent data from three litters and three independent experiments. *** $p < 0.0005$.

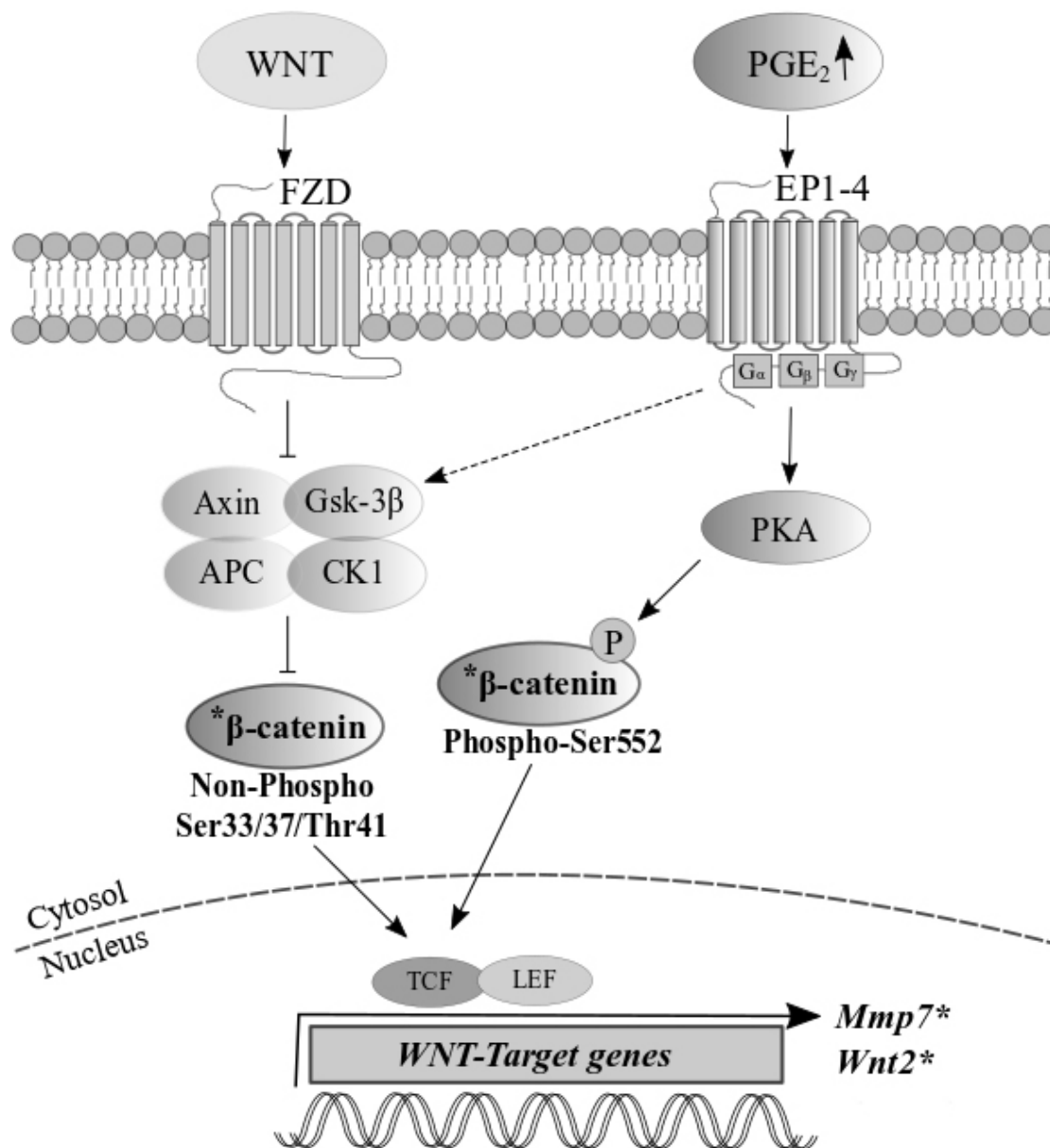


Figure 3-10. A proposed model of the interaction between PGE₂ and Wnt pathways in mouse brain. Elevated level of PGE₂ due to exposure to exogenous risk factors could affect phosphorylation of β-catenin by PKA at the Serine-552 site, and stabilization of active β-catenin (Non-phospho Ser33/37/Thr41) and its subsequent translocation to the nucleus to act on transcription factors to initiate transcription of Wnt-target genes. The Wnt genes and β-catenin affected by the exposure to PGE₂ in this study are shown in bold with an asterisk. The broken line represents proposed cross-talk.

CHAPTER 4. RESULTS – STUDY 2

Study 2 – Global microarray analysis in the cyclooxygenase-1 and -2 male knockout mice

Manuscript Citation:

Rai-Bhagal, R.*, Ahmad, E., Li*, H. & Crawford, D. A. (2018). Microarray analysis of gene expression in the cyclooxygenase knockout mice – a connection to autism spectrum disorder. *European Journal of Neuroscience* 47(6):750-766. *equal authorship (Appendix C2)

The dissertation was written in thesis format and the following is specific to the contributions in the chapter:

Contributions: Ravneet Rai-Bhagal designed and performed all experiments in the chapter, collected samples and acquired all data. Both Ravneet Rai-Bhagal and Eizaaz Ahmad analyzed the microarray data. Ravneet Rai-Bhagal created figures and tables, and prepared the manuscript. Dr. Hongyan Li provided administrative and technical support throughout the study. Dr. Dorota A. Crawford supervised the design of the study and was involved with editing the manuscript.

4.1 Manuscript Introduction and Rationale

During early brain development, there is abundant expression of COX-1, COX-2 (Gersting *et al.*, 2009), PGE₂ (Dumont *et al.*, 1998; Amateau & McCarthy, 2004; Alix *et al.*, 2008), and EP receptors (Rai-Bhogal *et al.*, 2018b) in the fetal or neonate brain. COX/PGE₂ signalling regulates gene expression involved in diverse physiological responses by activation of four G-protein coupled E-prostanoid (EP1-4) receptors (Furuyashiki & Narumiya, 2011), and plays an important role in events such as dendritic spine formation, learning and memory, synaptic transmission, and plasticity (Chen *et al.*, 2002; Chen & Bazan, 2005; Sang & Chen, 2006; Burks *et al.*, 2007) for healthy brain development.

Growing evidence indicates that altered COX/PGE₂ signalling contributes to the pathology of ASD, which affects approximately four times more males than females. There are various genetic and environmental risk factors that cause defects in the signalling of the COX-PGE₂ pathway and lead to neurodevelopmental disorders such as ASD, as outlined in the literature review (Tamiji & Crawford, 2010c; Wong *et al.*, 2014b; Wong *et al.*, 2015a) (Figure 1-6). Substantial evidence from human studies implicates abnormal expression or activity of multiple components of this pathway in ASD (Bell *et al.*, 2004; Bell *et al.*, 2010; El-Ansary *et al.*, 2011; Tostes *et al.*, 2013; Brigandi *et al.*, 2015; El-Ansary *et al.*, 2016; Jory, 2016). Of particular interest was that increased levels of COX-2 and PGE₂ correlated with abnormalities in sensory behaviours and processing in individuals with ASD (El-Ansary *et al.*, 2016; Qasem *et al.*, 2018). Previously, polymorphism of the *Ptgs2* gene encoding COX-2 was also found in ASD individuals with hyperactivity and atypical communication (Yoo *et al.*, 2008).

Animal studies, mostly in rodents, also provide convincing evidence to confirm that abnormal COX/PGE₂ signalling can result in ASD-like behaviours. Application of COX-2

inhibitors in a mouse model revealed atypical behaviours in complex social interactions, sensory integration, and attention (Dean *et al.*, 2012a). Recently, our lab provided the first evidence that COX-2 deficiency in a mouse model leads to ASD-like behaviours (Wong *et al.*, 2019). Juvenile and adults of both sexes were assessed for various behavioural tests, in which hyperactivity, anxiety, and repetitive behaviours were confirmed in all groups. Age- and sex-specific behaviours also included anxiety and social behaviours, and deficits in motor strength. All these studies combined, provide compelling evidence for the involvement of COX/PGE₂ pathway in the etiology of ASD.

Nevertheless, there is a gap in knowledge of the cellular and molecular mechanisms by which altered COX/PGE₂ signalling affects early brain development and neuronal function. Our lab has provided some *in vitro* evidence that PGE₂ can increase migration and proliferation of neuronal stem cells, accelerate neuronal differentiation (Wong *et al.*, 2014b), increase the length of neurite extensions of differentiated cells (unpublished results; Appendix A), alter intracellular calcium homeostasis in the cytosol and growth cones (Davidson *et al.*, 2016), and modulate the expression of critical risk genes for ASD (Wong *et al.*, 2014b; Davidson *et al.*, 2016). Furthermore, we have also provided *in vivo* evidence of dysregulation of gene and protein expression of critical developmental and ASD genes in the fetal mouse brain, possibly through an interaction with β -catenin (a modulator of the Wnt pathway) following a one-time environmental event of exogenous maternal PGE₂ administration in the mouse (Rai-Bhagal *et al.*, 2018b). Here, we would like to investigate the genetic impact of KO models (COX-1^{-/-} and -2^{-/-}) on expression of critical neurodevelopmental genes and pathways.

4.2 Objectives and Hypothesis

Objective 1 – To use the whole genome microarray analysis to examine gene expression profiling and affected biological pathways in the male COX-1^{-/-} and COX-2^{-/-} KO mice as our proposed experimental model systems for ASD.

Objective 2 – To investigate long-term differential gene expression, by examining the subset of genes that continue to be dysregulated from E16 to E19. This might provide some insight into the development of early diagnostic tools.

Objective 3 – To determine whether the differentially expressed genes from brains of COX-1^{-/-} and COX-2^{-/-} mice are implicated in other neurological disorders, as well as ASD.

Objective 4 – To reveal affected protein interaction networks involved in essential functions, during early brain development, and discuss how they may lead to molecular events that contribute to neurodevelopmental disorders such as ASD.

Hypothesis: Due to the previous findings from our lab where the COX-deficient mouse model exhibited ASD-like behaviours and altered morphology, branching, and density of microglia, we hypothesize that in the current study, various affected genes and biological processes will be uncovered that contribute to the pathological condition of these mice.

4.3 Experimental model systems

In the current study, genetic defects in the COX/PGE₂ signalling pathway were evaluated through male COX-1^{-/-} and COX-2^{-/-} mouse models, to understand the effect on expression of critical genes essential for prenatal brain development. Two prenatal stages were scrutinized: E16 — undergoing neurogenesis in many regions of the mouse brain, and E19 — a stage prior to

birth when neurogenesis is reaching completion in most regions (Rodier, 1980; Rice & Barone, 2000; Ohta *et al.*, 2005; Cina *et al.*, 2007; Xia *et al.*, 2015).

Results

4.4 Differential Gene Expression across E16 and E19 in COX-1^{-/-} and -2^{-/-} Male Mouse Brain

All differentially expressed genes identified in the brains of each COX gene knockout using the whole genome mRNA platform (Mouse WG-6 V2 BeadChip (Illumina) microarray) across developmental stages of E16 and E19, that had fold-change values ≥ 1.5 compared to the corresponding WT animals, were considered for further analysis. First, the number of up- and down-regulated genes in each COX-1^{-/-} mouse at E16 and E19 were identified (Figure 4-1). In the COX-1^{-/-} mouse, there were 229 (163 down- and 66 up-regulated) differentially regulated genes at E16, and 134 (74 down- and 60 up-regulated) at E19. In the COX-2^{-/-} mouse, there were 323 (208 down- and 115 up-regulated) differentially regulated genes at E16, and 148 (89 down- and 59 up-regulated) at E19 (Figure 4-1). Overall, there were a greater number of affected genes at the earlier E16 stage, in both the COX-1^{-/-} and COX-2^{-/-} models. There were also considerably more down-regulated genes in comparison to the number of up-regulated genes, in each model and developmental stage. Furthermore, the most number of affected genes were found in the COX-2^{-/-} mouse brain at E16. The global gene analysis indicates a potentially greater impact on development in the COX-2^{-/-} mice than COX-1^{-/-} with greater insult in the earlier stage of development during neurogenesis.

The next objective was to determine whether there are common genes altered in both COX-1^{-/-} and COX-2^{-/-} models, at each particular stage. Common defective genes between both developmental stages suggests continued dysregulation of certain genes across development. In

the COX-1-KO, there were 156 and 61 differentially expressed genes unique to each E16 and E19 stage, respectively, and 73 genes in common in both developmental stages (Figure 4-2A). In the COX-2-KO, there were 238 and 63 differentially expressed genes unique to each E16 and E19 stage, respectively, and 85 genes in common in both developmental stages (Figure 4-2B). Overall, the number of genes commonly expressed at both developmental stages was similar between both COX gene KOs, with slightly more genes in the COX-2^{-/-}.

In summary, the data showed that there were more differentially expressed genes at the earlier E16 stage in both COX gene KOs, than the later E19 stage. In addition, there was a greater number of down-regulated genes compared to up-regulated genes in both KOs across development. Investigation of genes that are differentially expressed across development revealed that there was a similar number of genes affected in both KOs, with slightly more genes in the COX-2^{-/-} model. The most substantial impact on gene regulation was in the COX-2^{-/-} model at the E16 developmental stage, during which period rapid neurogenesis is occurring in the brain. This subset of commonly affected genes is valuable as these genes are affected from mid- to end of neurogenesis, and may continue to be irregularly expressed and serve as early indicators of certain pathologies.

In order to validate the microarray findings, the expression of selected key developmental genes was quantified using qRT-PCR. ASD-risk genes *Myt1l* and *Glo1* were selected as they were the most down- or up-regulated genes, respectively (Appendix B; Supplementary Table 1). qRT-PCR showed a similar pattern of downregulated fold-change expression of the *Myt1l* gene in the COX-1^{-/-} (-1.64 ± 0.02 , $p=0.01$) and COX-2^{-/-} (-1.42 ± 0.04 , $p=0.02$) KOs, and confirmed upregulated expression of *Glo1* in both developmental stages of COX-2^{-/-} (2.26 ± 0.05 , $p=0.0006$; 1.95 ± 0.02 ,

$p=0.002$). Therefore, the similar pattern of regulation observed by qRT-PCR validated the microarray analysis.

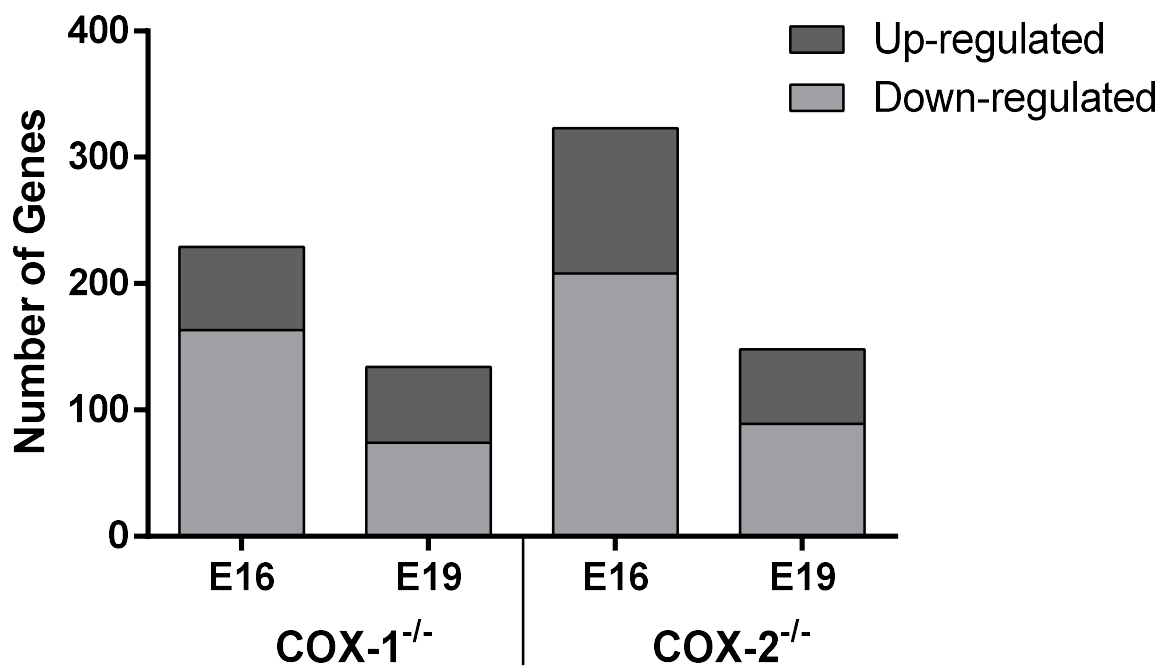


Figure 4-1. Number of differentially expressed genes in the brain of COX-1^{-/-} or COX-2^{-/-} males at E16 and E19. In the COX-1^{-/-} mouse brain, there were 163 down- and 66 up-regulated genes at E16, and 74 down- and 60 up-regulated at E19. In the COX-2^{-/-} mouse brain, there were 208 down- and 115 up-regulated genes at E16, and 89 down- and 59 up-regulated at E19.

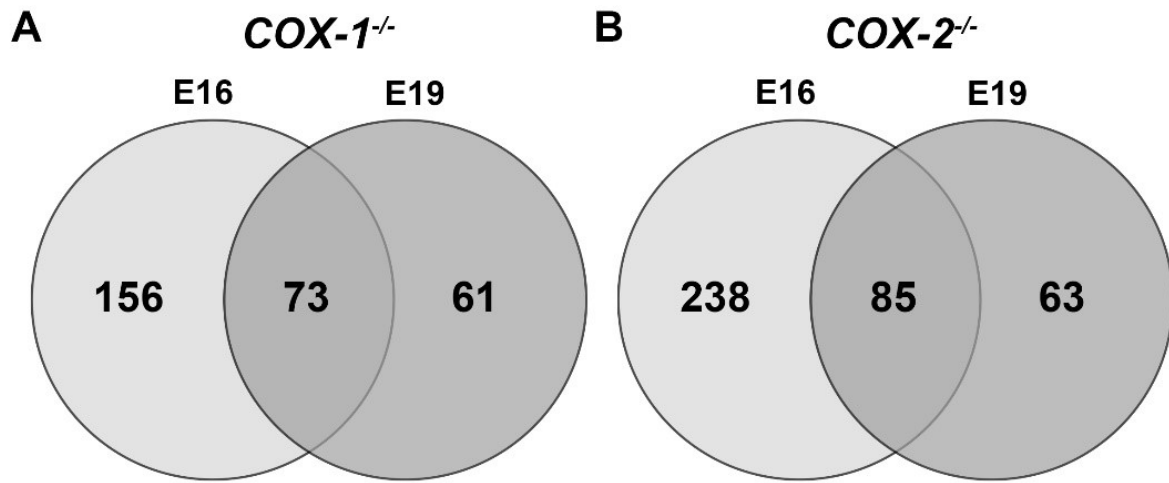


Figure 4-2. Commonly affected genes between the E16 and E19 developmental stages in each *COX-1^{-/-}* and *COX-2^{-/-}* male mouse. (A) In the *COX-1^{-/-}* knockout, there were 156 and 61 differentially expressed genes at E16 and E19, respectively, and 73 genes across both developmental stages. (B) In the *COX-2^{-/-}* knockout, there were 238 and 63 differentially expressed genes at E16 and E19, respectively, and 85 genes across both developmental stages.

4.5 Identification of Affected Biological Processes in the Male COX-1^{-/-} Mouse Brain

In order to gain some insight into the biological role of the affected genes found (Figure 4-1) and how they may contribute to ASD, the GO database was used for functional gene classification and KEGG pathway enrichment analysis (see methods for specific criteria) within the databases.

In the COX-1^{-/-} mouse, there were 13 biological pathways that were affected at E16, 8 of which encompassed ASD-risk genes (Table 4-1A). These pathways are found to be involved in regulation of neurological system process (GO:0031644), long-term potentiation (KEGG: mmu04720), adipocytokine signalling pathway (KEGG: mmu04920), secretion (GO:0046903), lipid biosynthetic process (GO:0008610), blood vessel development (GO:0001568), microtubule-based process (GO:0007017), and vesicle-mediated transport (GO:0016192). At E19, there was a total of 8 affected pathways, 3 of which included ASD-risk genes: transmission of nerve impulse (GO:0019226), secretion (GO:0046903), and induction of apoptosis (GO:0006917) (Table 4-1B).

Table 4-1. Biological Processes Affected in the COX-1^{-/-} E16 and E19 Male Mouse Brain using GO:BP and KEGG Analyses

(A) COX-1^{-/-} E16				
Database Category	Biological Process	Correct-ed p-value	Gene Count	Genes
GOTERM_BP_FAT	Regulation of neurological system process (GO:0031644)	0.002	7	<i>SYP</i> , <i>GRM5</i> , <i>GRIA2</i> , <i>NCDN</i> , <i>CSPG5</i> , <i>PPP3CA</i> , <i>CHRNA3</i>
GOTERM_BP_FAT	Regulation of cell morphogenesis (GO:0022604)	0.005	6	<i>EPB4</i> , <i>IL2</i> , <i>PALM</i> , <i>MAPT</i> , <i>CHRNA3</i> , <i>MYH10</i> , <i>THY1</i>
KEGG_PA_THWAY	Long-term potentiation (mmu04720)	0.008	5	<i>GRM5</i> , <i>GRIA2</i> , <i>CAMK4</i> , <i>CAMK2B</i> , <i>PPP3CA</i>
GOTERM_BP_FAT	Female gamete generation (GO:0007292)	0.026	4	<i>REC8</i> , <i>ZMIZ1</i> , <i>HEXB</i> , <i>ATM</i>
KEGG_PA_THWAY	Adipocytokine signaling pathway (mmu04920)	0.041	4	<i>IRS2</i> , <i>PRKAG2</i> , <i>ACSL6</i> , <i>CAMKK2</i>
GOTERM_BP_FAT	Secretion (GO:0046903)	0.047	7	<i>SYT4</i> , <i>HPS1</i> , <i>NRXN1</i> , <i>VPS33A</i> , <i>CHRNA3</i> , <i>EXOC6B</i> , <i>MYH10</i>
GOTERM_BP_FAT	Lipid biosynthetic process (GO:0008610)	0.047	8	<i>AGPAT5</i> , <i>INSIG2</i> , <i>HEXB</i> , <i>PRKAG2</i> , <i>MBOAT2</i> , <i>PBX1</i> , <i>ALG9</i> , <i>DHCR24</i>
KEGG_PA_THWAY	Viral myocarditis (mmu05416)	0.070	4	<i>ACTB</i> , <i>DAG1</i> , <i>CXADR</i> , <i>MYH10</i>
GOTERM_BP_FAT	Blood vessel development (GO:0001568)	0.071	7	<i>MEF2C</i> , <i>SEMA5A</i> , <i>RECK</i> , <i>HIF1A</i> , <i>IL18</i> , <i>ZMIZ1</i> , <i>THY1</i>
GOTERM_BP_FAT	Regulation of kinase activity (GO:0043549)	0.073	6	<i>PKIG</i> , <i>DGKH</i> , <i>PRDX2</i> , <i>ZEB2</i> , <i>CHRNA3</i> , <i>THY1</i>
GOTERM_BP_FAT	Response to oxidative stress (GO:0006979)	0.085	4	<i>HIF1A</i> , <i>GPX8</i> , <i>PRDX2</i> , <i>DHCR24</i>
GOTERM_BP_FAT	Microtubule-based process (GO:0007017)	0.093	6	<i>SPC25</i> , <i>KIF1B</i> , <i>MTAP2</i> , <i>MAPT</i> , <i>TUBB6</i> , <i>HOOK3</i>
GOTERM_BP_FAT	Vesicle-mediated transport (GO:0016192)	0.095	10	<i>SYP</i> , <i>GRIA2</i> , <i>SYT4</i> , <i>CAP1</i> , <i>NAPB</i> , <i>VPS33A</i> , <i>HOOK3</i> , <i>EXOC6B</i> , <i>MYH10</i> , <i>TRAPPC2</i>
(B) COX-1^{-/-} E19				
GOTERM_BP_FAT	Regulation of transcription from RNA polymerase II promoter (GO:0006357)	0.005	11	<i>TXNIP</i> , <i>EGR1</i> , <i>FOS</i> , <i>CEBPB</i> , <i>SOX11</i> , <i>NKX6-2</i> , <i>PKIG</i> , <i>NKX2-1</i> , <i>ST18</i> , <i>KLF4</i> , <i>PITX2</i>
GOTERM_BP_FAT	Glucose metabolic process (GO:0006006)	0.010	5	<i>PPP1CA</i> , <i>UEVLD</i> , <i>PHKB</i> , <i>PKM2</i> , <i>PYGB</i>
GOTERM_BP_FAT	Transmission of nerve impulse (GO:0019226)	0.014	6	<i>NRXN2</i> , <i>CAMK4</i> , <i>NRXN3</i> , <i>NKX6-2</i> , <i>HEXB</i> , <i>CHRNA3</i>
KEGG_PA_THWAY	Focal adhesion (mmu04510)	0.029	5	<i>ACTB</i> , <i>PPP1CA</i> , <i>GRLF1</i> , <i>CAPN2</i> , <i>COL5A2</i>
GOTERM_BP_FAT	Lipid biosynthetic process (GO:0008610)	0.031	6	<i>AGPAT5</i> , <i>LASS4</i> , <i>INSIG2</i> , <i>HEXB</i> , <i>MBOAT2</i> , <i>ALG9</i>
GOTERM_BP_FAT	Secretion (GO:0046903)	0.051	5	<i>NRXN2</i> , <i>NRXN3</i> , <i>STXBP2</i> , <i>VPS33A</i> , <i>CHRNA3</i>

GOTERM_ BP_FAT	Forebrain development (GO:0030900)	0.084	4	<i>FEZF1, GRLFI, NKX2-1, PITX2</i>
GOTERM_ BP_FAT	Induction of apoptosis (GO:0006917)	0.086	4	<i>SERINC3, CEBPB, APOE, PHLDA3</i>

For each term, an EASE score cut-off of P-value <0.1 was applied along with gene count >3. Along with database category for GO and KEGG using DAVID, Benjamini false discovery rate (FDR)-corrected P-value, gene count and gene lists are presented. Genes in bold are autism-risk candidates found in AutDB.

There was one common biological process that was impacted at both E16 and E19 developmental stages: lipid biosynthetic process – which encompasses the chemical reactions and pathways involved in the formation of lipids. This is an expected result as the COX-1 enzyme is responsible for breaking down arachidonic acid into various prostaglandin molecules. Across the two developmental stages, defects in processes of long-term potentiation (E16) and transmission of nerve impulse (E19) suggest abnormal synaptic functions. Within both these processes there is dysregulation of *CAMK4*, an ASD-associated gene. Furthermore, the biological process of secretion is also inclusive in the synaptic function group, as the genes affected in this process are well known for their involvement in neurotransmitter secretion.

Next, predicted protein networks that may be affected from E16 to E19 in the male COX-1^{-/-} mouse model were identified to determine which developmental processes have sustained dysregulation for a long period of time close to birth. The subset of common genes between the two stages (Figure 4-2A; Supplementary Table S2) was inputted using Cytoscape and the plug-in geneMANIA, to reveal affected protein interactions. In the COX-1^{-/-} mouse, the

HOPS complex was found to be defective over the developmental period of E16 to E19, at least (Figure 4-3). The HOPS complex is responsible for autophagy roles, and its potential involvement in neuropathology will be explored in the discussion.

COX-1^{-/-} Model (Common genes)

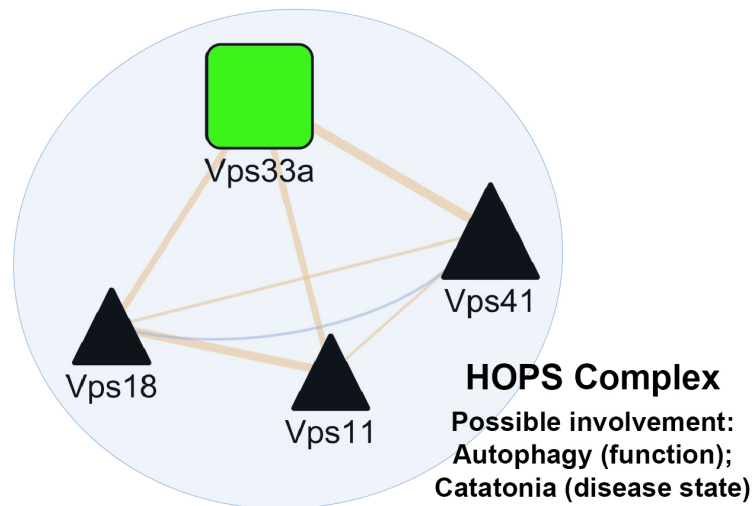


Figure 4-3. Protein interaction network affected in the male COX-1^{-/-} model. The common genes found to be dysregulated in both E16 and E19 stages of development in the COX-1^{-/-} model, were used to create protein interaction networks, using Cytoscape with the plug-in geneMANIA. In this model, the HOPS complex pathway was found to be affected, which may function in an autophagy role, and in the disease state, catatonia. Rounded square represents gene found in this study (*green = upregulated*); Black triangles represent genes also important in this biological process found in other studies. Pink and blue interaction lines denote co-expression and co-localization, respectively

4.6 Identification of Affected Biological Processes in the Male COX-2^{-/-} Mouse Brain

In the COX-2^{-/-} mouse, at the E16 developmental stage, 10 of the 14 affected pathways [Wnt signalling (KEGG: mmu04310), DNA metabolic process (GO:0006259), chordate embryonic development (GO:0043009), skeletal system development (GO:0001501), intracellular transport (GO:0046907), axon guidance (KEGG: mmu04360), regulation of translation (GO:0006417), long-term depression (KEGG: mmu04730), and negative regulation of apoptosis (GO:0043066)] include ASD-risk genes (Table 4-2A). At E19, 4 of the 6 affected pathways [sensory perception of sound (GO:0007605), lipid transport (GO:0006869), tissue morphogenesis (GO:0048729), and intracellular protein transport (GO:0006886)] include ASD-risk genes (Table 4-2B).

The list of common genes (Figure 4-2B; Supplementary table S2) between E16 and E19, was used to detect potential protein networks that were affected in the brain of COX-2^{-/-} male mice, as described for the COX-1^{-/-} model above. This dataset revealed possible dysregulation in functions of wound healing, cell-substrate adhesion, extracellular matrix, and neural tube formation (Figure 4-4). Each protein interaction network shown encompassed at least one ASD gene – dysregulated in the current study or previous studies. These networks will be addressed in the discussion.

Table 4-2. Biological Processes Affected in the COX-2^{-/-} E16 and E19 Male Mouse Brain using GO:BP and KEGG Analyses

(A) COX-2^{-/-} E16				
Database Category	Biological Process	Correct-ed p-value	Gene Count	Genes
GOTERM_BP_FAT	Sulfur metabolic process (GO:0006790)	0.015	6	<i>ADII, AHCY, SEPP1, DCN, TPMT, MGST1</i>
KEGG_PA_THWAY	Wnt signalling pathway (mmu04310)	0.023	7	<i>SFRP1, CCND2, CSNK1E, SFRP2, GSK3B, PPP3CA, DAAMI</i>
GOTERM_BP_FAT	Somitogenesis (GO:0001756)	0.028	4	<i>SFRP1, SFRP2, FOXC2, ZEB2</i>
GOTERM_BP_FAT	Regulation of protein complex disassembly (GO:0043244)	0.031	4	<i>MTAP2, MAPT, TMOD3, CLASP1</i>
GOTERM_BP_FAT	DNA metabolic process (GO:0006259)	0.031	13	<i>RAD23B, UBE2A, MELA, LOC100046025, ESCO2, MCM6, RPA3, GIN1, UBE2N, BCL11B, RRM1, MLL1, NFIB</i>
GOTERM_BP_FAT	Chordate embryonic development (GO:0043009)	0.035	13	<i>NRP, UBE2A, HIF1A, SFRP1, SFRP2, ZMIZ1, TGFB2, TSC2, FOXC2, ZEB2, KEAP1, DLK1, MYH10</i>
GOTERM_BP_FAT	Skeletal system development (GO:0001501)	0.036	10	<i>MEF2C, RAI1, HIF1A, HEXB, TGFB2, SOX5, FOXC2, DLK1, SOX6, COL5A2</i>
GOTERM_BP_FAT	Intracellular transport (GO:0046907)	0.043	13	<i>APIB1, NAPB, HOOK3, KIF1B, SRPR, GSK3B, ARCN1, TSC2, PPP3CA, KPN2, SRP9, TRAPPC2, MYH10</i>
KEGG_PA_THWAY	Axon guidance (mmu04360)	0.044	6	<i>NGEF, GNAI1, PLXNA2, CFL2, GSK3B, PPP3CA</i>
KEGG_PA_THWAY	Tight junction (mmu04530)	0.046	6	<i>ACTB, EPB4.1L2, EPB4.1L1, GNAI1, JAM3, MYH10</i>
GOTERM_BP_FAT	Regulation of translation (GO:0006417)	0.075	5	<i>TNRC6C, RBM8A, PAIP1, TIA1, SRP9</i>
KEGG_PA_THWAY	P53 signaling pathway (mmu04115)	0.077	4	<i>CCND2, TSC2, CCNG1, CCNG2</i>
KEGG_PA_THWAY	Long-term depression (mmu04730)	0.089	4	<i>GRM5, GRIA2, GNAI1, GUCY1A3</i>
GOTERM_BP_FAT	Negative regulation of apoptosis (GO:0043066)	0.094	8	<i>BCL11B, GSK3B, FCER1G, FOXC2, PRDX2, GLO1, DNAJC5, DHCR24</i>
(B) COX-2^{-/-} E19				
GOTERM_BP_FAT	Translation (GO:0006412)	0.003	8	<i>RPL30, RPS3A, MRPL55, WARS2, LARS2, IARS2, RPS2, RPL29</i>
GOTERM_BP_FAT	Sensory perception of sound (GO:0007605)	0.014	4	<i>MYO6, CDKN1B, HEXB, GJB2</i>
GOTERM_BP_FAT	Sulfur metabolic process (GO:0006790)	0.021	4	<i>ADII, MTHFD1, AHCY, MGST1</i>
GOTERM_BP_FAT	Lipid transport (GO:0006869)	0.040	4	<i>SORL1, ATP10D, GLTPD1, ATP8A1</i>
GOTERM_BP_FAT	Tissue morphogenesis (GO:0048729)	0.066	5	<i>SFRP1, TSC2, GRLF1, PBX1, PITX2</i>

GOTERM_ BP_FAT	Intracellular protein transport (GO:0006886)	0.099	5	<i>MYO6</i> , <i>SRPR</i> , <i>ARCNI</i> , <i>TSC2</i> , <i>IPO9</i>
-------------------	---	-------	---	--

For each term, an EASE score cut-off of P-value <0.1 was applied along with gene count >3. Along with database category for GO and KEGG using DAVID, Benjamini false discovery rate (FDR)-corrected P-value, gene count and gene lists are presented. Genes in bold are autism-risk candidates found in AutDB.

4.7 COX-2/PGE₂ Interaction with Wnt Signalling Pathway

Previous research in our lab has shown a cross-talk between PGE₂ signalling and the Wnt canonical pathway in neuronal cell line (Wong *et al.*, 2014b; Davidson *et al.*, 2016), and maternal PGE₂ exposure in a mouse model [Chapter 3; (Rai-Bhogal *et al.*, 2018b)]. In the current study, the Wnt signalling pathway was dysregulated in the COX-2^{-/-} mouse, which included 8 differentially expressed genes – *SFRP1*, *SFRP2*, *DAAMI*, *CCND2*, *CSNK1E*, *GSK3β*, *PPP3CA*, and *ACTβ* (Table 4-2A). Of these, *DAAMI* is an ASD-risk gene, which plays a significant role in regulating Wnt activity to the actin cytoskeleton – important in dendritic spine formation. It is also interesting to note that this interaction was present only at the E16 developmental stage, during a period of rapid neurogenesis and synaptogenesis. This provides evidence for an interaction between COX-2/PGE₂ and Wnt signalling pathways at a critical time-point during neurodevelopment in the mouse.

COX-2^{-/-} Model (Common genes)

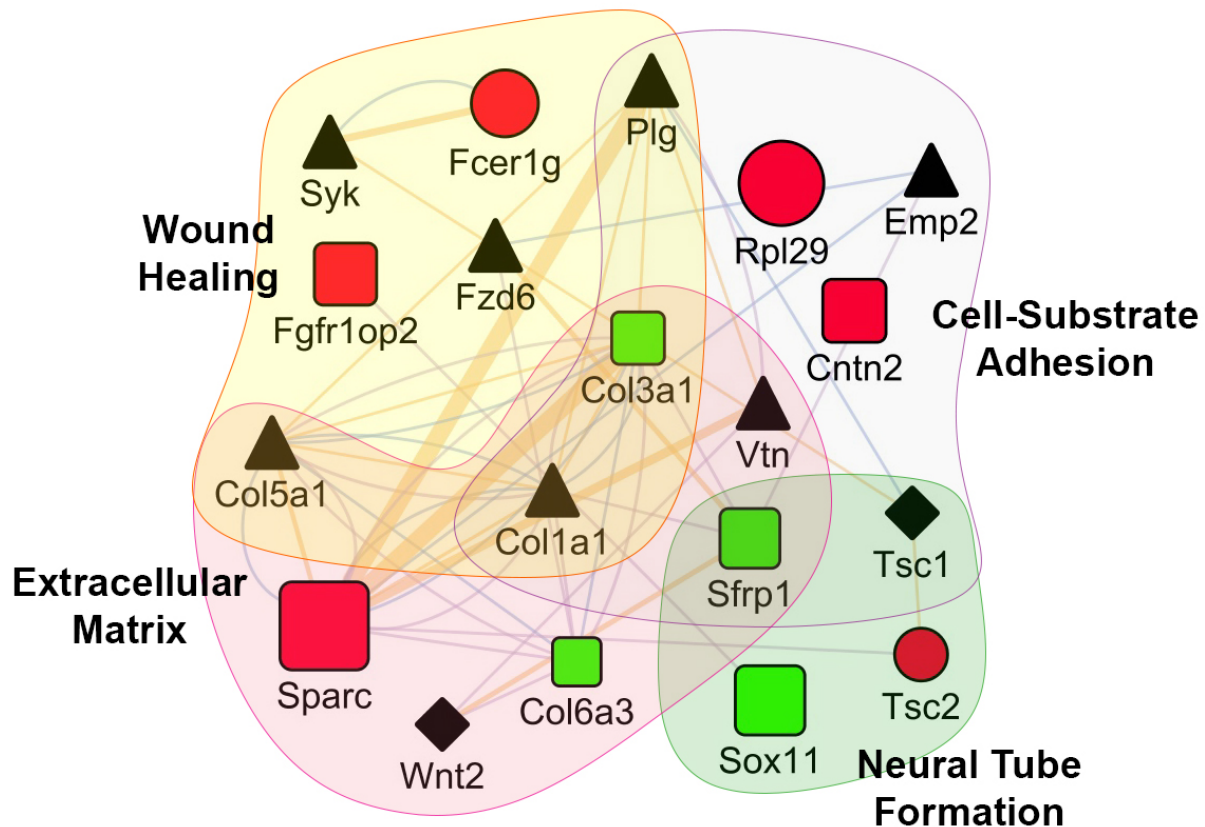
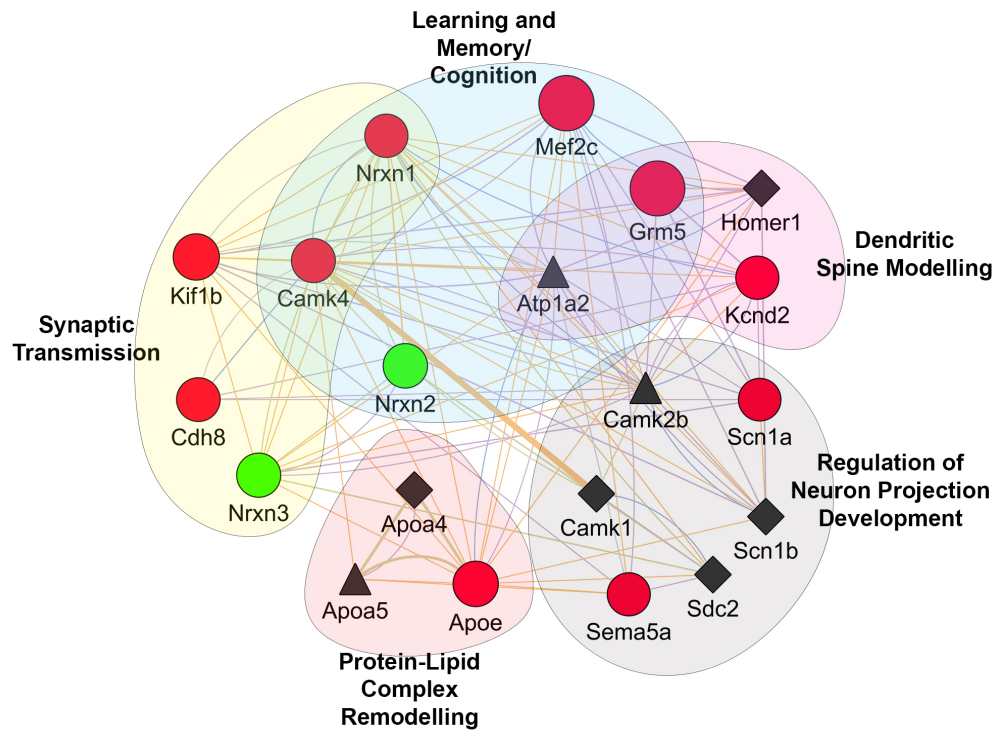


Figure 4-4. Protein interaction networks affected in the COX-2^{-/-} male mouse model. The common genes found to be dysregulated in both E16 and E19 stages of development in the COX-2^{-/-} model, were used to create protein interaction networks, using Cytoscape with the plug-in geneMANIA. In this model, critical functions of wound healing, cell-substrate adhesion, extracellular matrix, and neural tube formation were found to be affected. Rounded square represents gene found in this study (*red* = downregulated; *green* = upregulated); Circles represent ASD-risk genes found in this study; Black diamonds represent ASD-risk genes found in previous studies; Black triangles represent non-ASD-risk genes also important in these biological processes found in other studies. Purple, blue and orange interaction lines denote co-expression, co-localization and predicted interactions, respectively.

4.8 Protein Interaction Networks of ASD genes in the COX-1^{-/-} and -2^{-/-} Male Mouse Brain

The ASD-risk genes (Table 4-1; Table 4-2) found in each COX gene KO were characterized by constructing protein network analyses using Cytoscape and GeneMania (Rai-Bhagal *et al.*, 2018a), to reveal deficits in a number of important developmental processes. In the COX-1^{-/-} model, the genes were enriched in 5 key biological processes, including learning or memory and cognition, regulation of neuron projection development, dendritic spine modelling, synaptic transmission, and protein-lipid complex remodelling (Figure 4-5A). In the COX-2^{-/-} model, ASD-risk genes were enriched in regulation of adaptive immune response, dendritic spine modelling and dendrite morphogenesis, and synaptic transmission (Figure 4-5B). Interestingly, both COX-1^{-/-} and -2^{-/-} models revealed networks involved in dendrite and/or synaptic transmission processes.

A) COX-1^{-/-} Model - ASD genes



B) COX-2^{-/-} Model - ASD genes

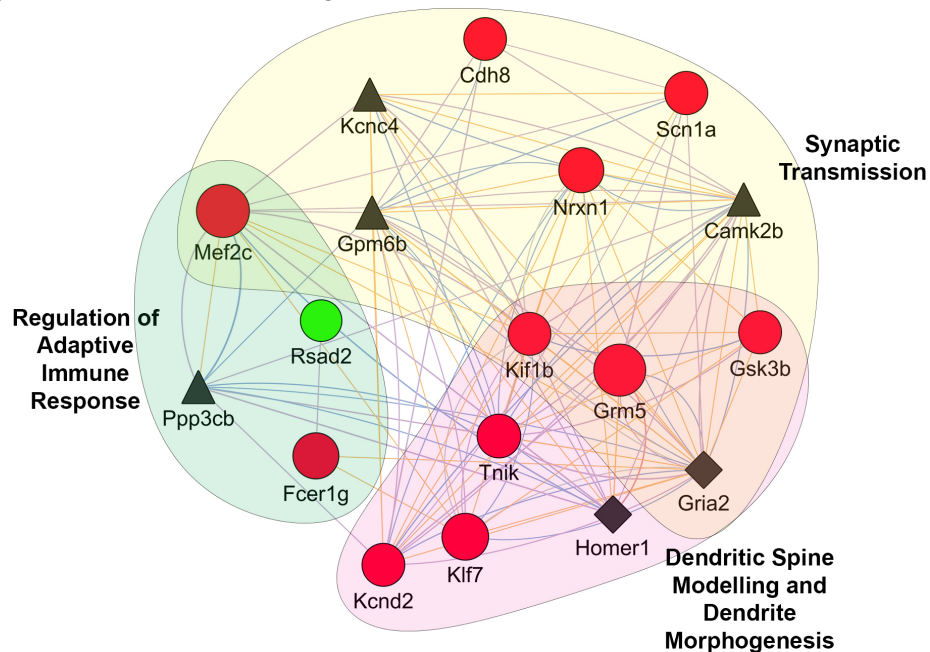


Figure 4-5. Protein interaction networks of ASD-risk genes in the COX-1^{-/-} and COX-2^{-/-} male mouse model. The ASD-risk genes found in the COX-1^{-/-} and COX-2^{-/-} models were used to create interaction networks using GeneMANIA and organized in Cytoscape. (A) The affected networks found in the COX-1^{-/-} regulate biological processes of synaptic transmission, learning and memory/cognition,

dendritic spine modelling, regulation of neuron projection development, and protein-lipid complex remodelling. (B) In the COX-2^{-/-} model the affected networks regulate synaptic transmission, dendritic spine modelling and dendrite morphogenesis, and adaptive immune response processes. Circles represent ASD-risk genes found in this study (*red* = *downregulated*; *green* = *upregulated*); Black diamonds represent ASD-risk genes found in previous studies; Black triangles represent non-ASD-risk genes also important in these biological processes found in other studies. Purple, blue and orange interaction lines denote co-expression, co-localization and predicted interactions, respectively.

4.9 Identification of Differentially Expressed Genes from COX-1^{-/-} and -2^{-/-} Male Mouse Brain, Involved in Co-morbid Neurological Disorders.

The current study has strengthened the involvement of the COX/PGE₂ pathway during early neurodevelopment and in ASD pathology. Previous studies have also indicated that dysfunction of this pathway may be involved in the etiology of other diseases and disorders. It is plausible that timing of the COX/PGE₂ pathway dysfunction is key in understanding the neurological outcome. There is also a substantial amount of emerging evidence which confirms that ASD displays co-morbidity with other key neurological disorders. Thus, we put forth a hypothesis that irregular COX/PGE₂ signalling may potentially be involved in co-morbid conditions to ASD. Analysis was conducted through the Gene-Disease Associations tool, DisGeNET, and research studies. Other than ASD, a substantial number of differentially expressed genes have previously been implicated in Intellectual disability (ID), Alzheimer's disease (AD) and Schizophrenia (SZ). The number of genes categorized under each disorder is displayed in Figure 4-6. It is evident that the greater number of dysregulated genes related to

neurological disorders was at the E16 developmental stage in the COX-2^{-/-} mouse. It is also interesting to note that a majority of differentially expressed genes belong to the same four disorders (ID, AD, SZ, and ASD) in both COX-1^{-/-} and COX-2^{-/-} mice at both E16 and E19 developmental stages. The affected genes encompassed within these four disorders were further scrutinized with a VENN diagram to detect the genes that overlap and which are exclusive to each respective disorder.

In the COX-1^{-/-} mouse, a total of 42 genes were identified as being involved in pathology of multiple disorders, and 27/42 genes showed an overlap with ASD (Figure 4-7A). In addition, there were 27 genes implicated exclusively in ID, 20 genes in AD, 8 genes in SZ, and 4 genes in ASD (Figure 4-7A). In the COX-2^{-/-} mouse, a total of 51 genes were identified as being involved in pathology of multiple disorders, and 26/51 genes showed an overlap with ASD (Figure 4-7B). Additionally, there were 34 genes implicated exclusively in ID, 19 in AD, 9 in ASD, and 8 in SZ (Figure 4-7B). Overlapping genes may be of importance to understand the heterogeneity and co-morbid nature of ASD. Furthermore, there is a high rate of co-morbidity between ASD and ID, and ASD was once characterized as childhood SZ due to clinical similarities. Therefore, we can make some hypotheses that defects in COX signalling may wreak havoc on the developing brain, and cause dysregulation in genes of childhood and adult-onset disorders with similar presentation. These topics will be described in further detail in the discussion section.

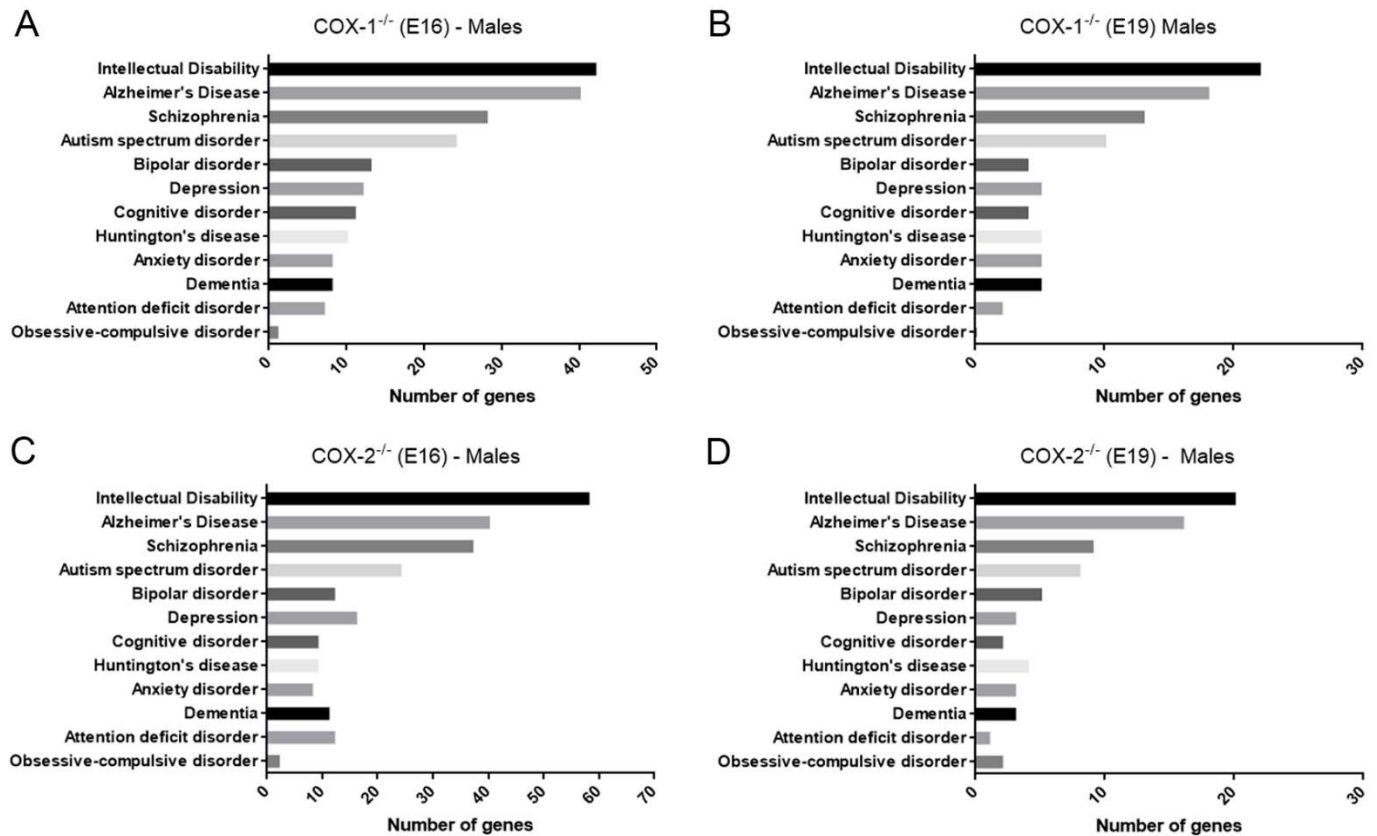
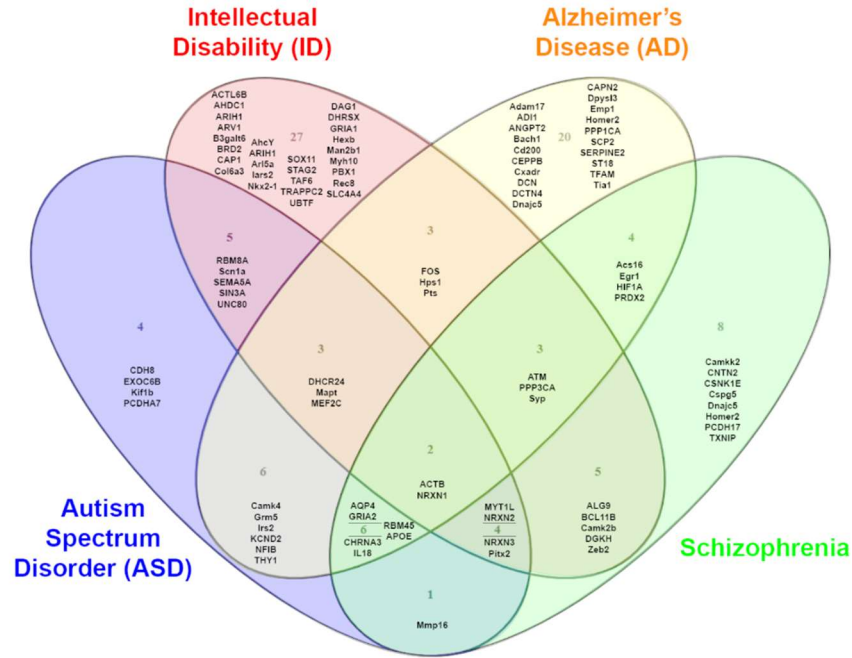


Figure 4-6. Number of genes differentially expressed in COX-1^{-/-} and COX-2^{-/-} male mouse at E16 and E19 involved in various neurological disorders. (A-D) In both COX^{-/-} gene knockouts at each developmental stage of E16 and E19, a substantial number of differentially expressed genes have been implicated previously in the pathology of the following neurological disorders: Intellectual disability; Alzheimer's disease; Schizophrenia; and Autism spectrum disorder. In each COX^{-/-} gene knockouts, a greater number of genes are involved at the (A,C) E16 stage, compared to (B,D) the later E19 stage.

A) COX-1^{-/-} Model



B) COX-2^{-/-} Model

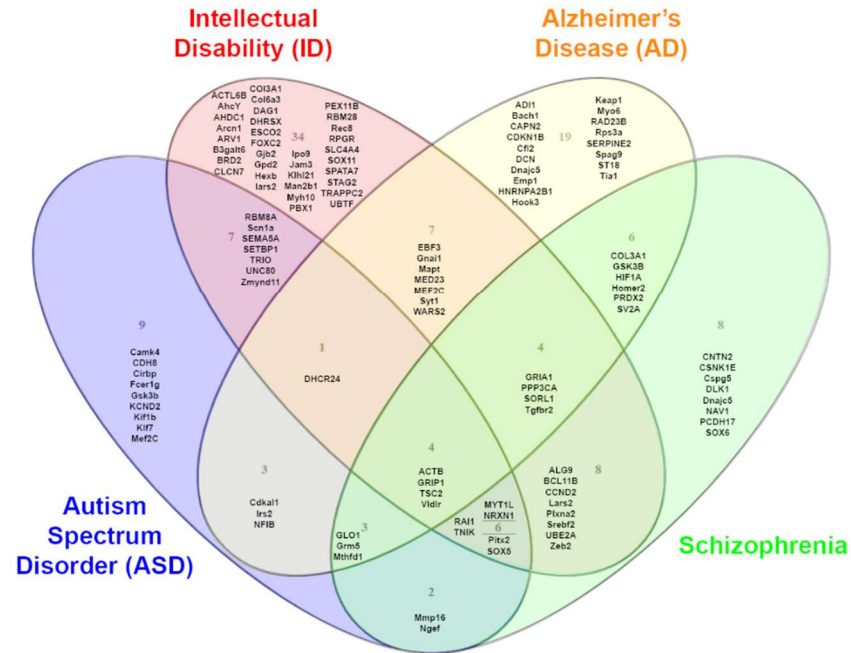


Fig 4-7. Differentially expressed genes from the COX-1^{-/-} and COX-2^{-/-} male mice have implications in multiple disorders. This VENN diagram illustrates which genes are involved in the pathology of Autism spectrum disorder (ASD); co-morbid disorder – Intellectual disability (ID); Schizophrenia (SZ), and Alzheimer's Disease (AD).

4.10 Summary of Results

In summary, the current global gene analysis revealed that both COX-1^{-/-} and -2^{-/-} mouse models exhibited a greater impact on gene regulation at the E16 stage, during which neurogenesis occurs in many brain regions, compared to the later E19 stage during which neurogenesis is mostly complete but other events such as apoptosis and pruning are occurring. Of the two KO models, the COX-2 deficiency had a greater impact on the expression of critical developmental genes, involved in ASD and various pathologies. These relationships will be examined further in the discussion (Chapter 7).

This study also uncovered a relationship between COX-2 and Wnt signalling pathway, through the differential expression of multiple Wnt pathway genes. Therefore, it is also of great importance that these pathways are likely to interact around developmental day E16 in the mouse brain.

CHAPTER 5. RESULTS – STUDY 3

Study 3 – Global microarray analysis in the cyclooxygenase-2 (COX-2) knockout female mouse. Manuscript in preparation.

Contributions: Ravneet Rai-Bhagal designed and performed all sample collections, acquired and analyzed all data, and created all figures and tables. Dr. Dorota A. Crawford supervised the design of the study and will be involved in editing the manuscript.

5.1 Introduction and Rationale

Autism spectrum disorder (ASD) is a heterogeneous disorder that affects four times more boys than girls. It also seems to present itself differently in females, where they appear to exhibit less of the typical rigid and repetitive symptoms of their male counterparts (Frazier *et al.*, 2014; Ros-Demarize *et al.*, 2020). The molecular mechanisms behind why ASD is a male-dominant disorder, and how it manifests in females, is currently unknown.

Hormones and the molecules that they act on are major contributors of sex differences between males and females, impacting processes and pathways which play a pivotal role in development of the brain. Estradiol and its receptors, for example, are very highly expressed in the developing brain – involved in growth of dendrites (Lorenzo *et al.*, 1992) and axons in the male and female brain in early development (Blanco *et al.*, 1990; Diaz *et al.*, 1992; Cambiasso *et al.*, 1995). They are also responsible for dendritic spine formation, and an increase in glutamatergic activity, specifically through action of the COX-2/PGE₂ pathway. Moreover, estradiol-induced PGE₂ is a key regulator of sex differences through its actions in the preoptic area, which is a highly dimorphic region in the brain (Amateau & McCarthy, 2004; Burks *et al.*, 2007; Wright & McCarthy, 2009).

Recently, our lab successfully confirmed ASD-like behaviours in the female COX-2^{-/-} mouse (Wong *et al.*, 2019). COX-2^{-/-} females displayed an increase in hyper ambulatory activity, anxiety-driven marble-burying and decreased sociability behaviours, compared to the WT females. In the previous chapter, we revealed affected genes and pathways through global microarray analysis in two prenatal stages (E16 and E19) in the male brain of mice deficient for COX-1 or COX-2. The COX-2^{-/-} mutation resulted in a more significant number of genes and pathways affected, compared to COX-1^{-/-}. In addition, we found an interaction between COX-2

and the Wnt signalling pathway in the males. In this study, our aim was to also conduct whole genome analysis of gene expression and affected interaction networks/pathways in the female COX-2-KO mouse brain.

5.2 Objectives and Hypothesis

Objectives: The aims of the current study are to provide a comprehensive and detailed gene expression analysis in the female COX-2-deficient mouse brain, as completed for males (Study 2; Chapter 4). This study will also examine the same prenatal developmental time-points of E16 and E19. The main objectives of this study are:

Objective 1 – To use whole genome microarray analysis to examine gene expression profiling and affected biological pathways in the female COX-2^{-/-} mouse as our proposed experimental model system for ASD.

Objective 2 – To investigate long-term differential gene expression, by examining the subset of genes that continue to be expressed from E16 to E19.

Objective 3 – To examine the affected genes/pathways that may have pathologic consequences for the brain, and

Objective 4 - To make comparisons of the affected processes and biological mechanisms between males and females, to determine possible sex differences in the brain.

Hypothesis: Based on the results obtained from the gene analysis in COX-2^{-/-} male mice, we hypothesize that COX-2 deficiency in the female brain will also result in dysregulated genes and biological pathways, essential for normal development. However, as previous research has shown that COX/PGE₂ can act to produce sex differences in the brain, we expect to find some sex-specific changes.

5.3 Experimental Model System

In the current study, genetic defect in COX-2 female mouse was used as a model at stages E16 and E19, to determine the effects on expression of critical genes essential for prenatal brain development.

Results

5.4 Differential Gene Expression across E16 and E19 in COX-2^{-/-} Female Mouse Brain

To determine the effects of the COX-2/PGE₂ pathway on prenatal gene expression in females, whole genome mRNA platform (Mouse Genome 2.0ST GeneChip® (Affymetrix) microarray) was conducted on brain tissue from COX-2^{-/-} female mice at prenatal stages of E16 and E19. All genes identified from the microarray analysis were analyzed as described in chapter 4. Differentially expressed genes with fold change values ≥ 1.5 were scrutinized for further data analysis. At E16, there was a total of 261 differentially expressed genes, with a greater number of genes that were downregulated (160 down- and 101 up-regulated). At E19, there was a total of 276 differentially expressed genes, with a greater number of genes that were upregulated (67 down- and 209 up-regulated) (Figure 5-1). The total number of differentially affected genes was similar across both developmental stages.

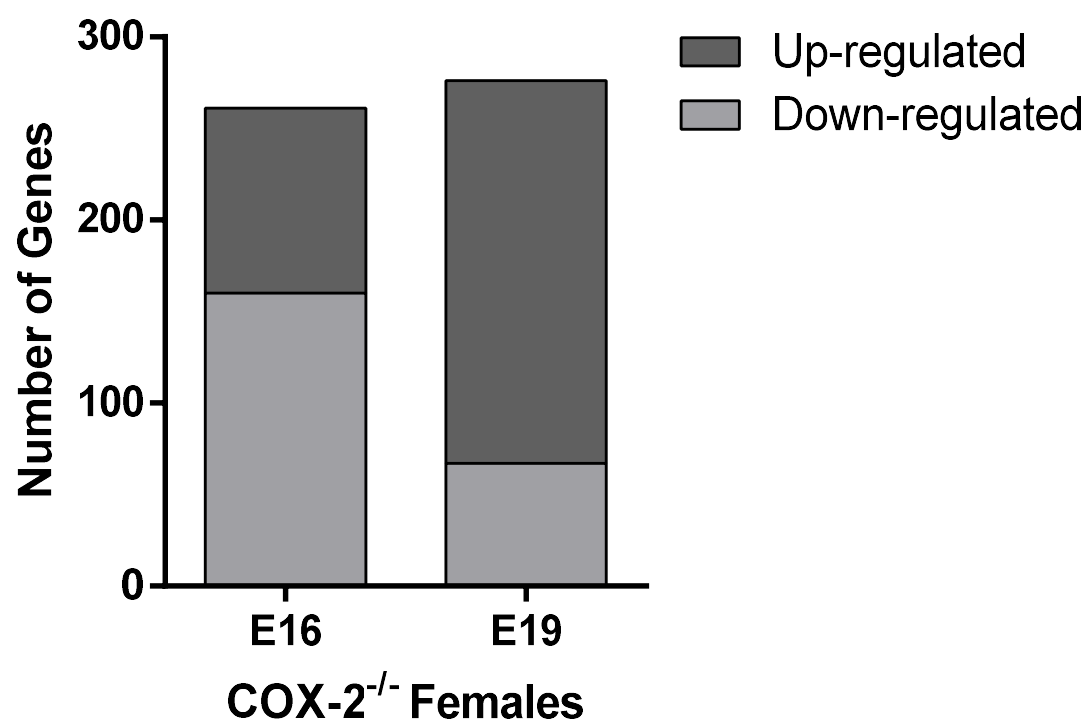


Figure 5-1. Number of genes differentially expressed in the brain of COX-2^{-/-} females at embryonic stages E16 and E19. There were 160 down- and 101 up-regulated genes at E16, and 67 down- and 209 up-regulated at E19, in the COX-2^{-/-} female mouse brain.

The next objective was to determine whether there are common genes altered in the female COX-2^{-/-} mouse, between the E16 and E19 developmental stages. Common defective genes between both stages suggests continued dysregulation of certain genes across neurodevelopment. There were 94 and 104 affected genes expressed exclusively at the E16 and E19 stages, respectively, and 139 common genes differentially expressed at both stages of

development (Figure 5-2). Of the 139 genes, there were 28 upregulated and 111 downregulated genes at the E16 stage. The expression of all genes that were upregulated at the E16 stage, remained upregulated at the E19 stage. However, of the 111 downregulated genes at E16, 97 of them switched the expression to being upregulated at the E19 stage (Appendix B; Supplementary Table 2).

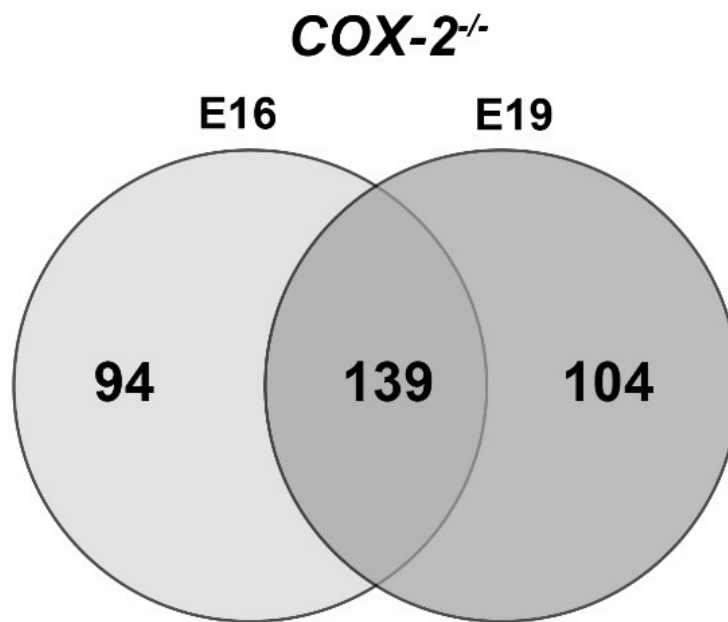


Figure 5-2. Commonly affected genes between the E16 and E19 developmental stages in the *COX-2*^{-/-} female mouse. There were 94 and 104 differentially expressed genes at E16 and E19, respectively, and 139 genes across both developmental stages.

Overall, the data indicates that there is a similar number of differentially regulated genes at both stages in brain tissue of the COX-2^{-/-} female mouse. At the E16 stage, there was a higher number of down-regulated genes expressed, whereas there was a substantially larger number of up-regulated genes at E19. Furthermore, there was a significant number of common genes that were dysregulated between both stages, many of which switched their expression from downregulated at E16 to upregulation by the E19 stage.

5.5 Identification of Affected Biological Processes in the Female COX-2^{-/-} Mouse Brain

The affected genes from each developmental stage were classified according to the biological processes and functions they are involved in, using the DAVID bioinformatics functional annotation tool. A minimum of three genes must be dysregulated to be grouped and considered an affection process/function. At E16, there was an enrichment of genes in many pathways and biological functions such as acetylation, cytokinesis (GO:0000910), lipid biosynthesis, transport, translation (GO:0006412), chromatin silencing at rDNA (GO:0000183), postsynaptic cell membrane, ribosome (mmu03010), phospholipid biosynthetic process (GO:0008654), phosphoprotein, protein transport, nucleosome core, lipid metabolism, arachidonic acid metabolism (mmu00590), cytoplasm, and metabolic pathways (mmu01100) (Table 5-1A). Of the 16 biological pathways found, 3 included ASD-risk genes: Transport; Postsynaptic cell membrane; and Phosphoprotein. It is interesting to note that processes in lipid synthesis and metabolism, as well as arachidonic acid metabolism were affected at the E16 stage. Therefore, a deficit in COX-2, can potentially affect the metabolism of arachidonic acid, which is an upstream component in the pathway (Figure 1-6), and lead to further hindrances in the lipid signalling mechanism.

At the later E19 stage, an enrichment of genes was found in pathways involved in processes such as acetylation, ribonucleoprotein, ribosome (mmu03010), metal-binding, translation (GO:0006412), and isopeptide bond (Table 5-1B). Of the 6 affected biological processes, 2 included ASD-risk genes: acetylation; and metal-binding.

5.6 ASD-Risk Genes and Biological Pathways in the COX-2^{-/-} Female Mouse Brain

In chapter 4, a study of the COX-2 gene deficiency on the developing male brain in mice revealed an association to ASD by the abnormal expression of many ASD-risk genes and pathways. In the current study, similar analysis tools were used to indicate dysregulation of ASD-risk genes in the COX-2^{-/-} female mouse brain. There was a total of 6 ASD-risk genes found to be dysregulated during development – three at E16, four at E19, inclusive of one that is common for both stages (Table 5-2). At the E16 stage, there was increased expression of *Cdh10* (*Cadherin 10*) and *Gabrb1* (*Gamma-aminobutyric acid (GABA) A receptor, beta 1*). At E19, there was decreased expression of *Glo1* (*Glyoxylase I*), *Slc6a8* (*Solute carrier family 6 (neurotransmitter transporter, creatine), member 8*), and *Camk2a* (*Calcium/calmodulin dependent protein kinase II alpha*). Most of these genes are involved in critical biological processes of synaptic functions that are associated with the pathology of ASD (Table 5-2). At both E16 and E19 stages, there was decreased expression of *Gabra2* (*Gamma-aminobutyric acid type A receptor subunit alpha2*), which indicates continued dysregulation of this gene, and may be investigated as a potential ASD marker. Interestingly, the expression of *Glo1* was also dysregulated in males in both COX-1 and 2 KO models (Chapter 4).

Table 5-1. Biological Processes Affected in the COX-2^{-/-} E16 and E19 Female Mouse Brain using GO:BP, KEGG, and UniProtKB analyses. Genes depicted in bold are linked to ASD.

(A) COX-2^{-/-} E16				
Database Category	Biological Process	Correct-ed p-value	Gene Count	Genes
UP_KEYWOR DS	Acetylation	0.001119	21	<i>CYB5R3, HIST1H2BC, HIST4H4, NDUFA3, EXOSC9, CAPZA1, LIN7C, RPS8, DSTN, CBRI, RPL30, C1QBP, PTCD3, EIF3E, RANBP6, DAD1, RPL5, MAP9, CHORDC1, HIST1H4I, DYNLRB1</i>
GOTERM_ BP_DIRECT	Cytokinesis (GO:0000910)	0.008285	3	<i>MAP9, DSTN, ARL3</i>
UP_KEYWOR DS	Lipid biosynthesis	0.013877	4	<i>CYB5R3, CYP51, ISYNA1, PTGDS</i>
UP_KEYWOR DS	Transport	0.014388	13	GABRA2 , <i>NDUFA3, SEC24A, GABRB1, LIN7C, FABP3-PS1, ARL3, PTGDS, SDHC, RANBP6, IFT27, DYNLRB1, VPS39</i>
GOTERM_ BP_DIRECT	Translation (GO:0006412)	0.016432	6	<i>RPL30, RPL21, EIF4A2, EIF3E, RPL5, RPS8</i>
GOTERM_ BP_DIRECT	Chromatin silencing at rDNA (GO:0000183)	0.017702	3	<i>HIST4H4, TAF1D, HIST1H4I</i>
UP_KEYWOR DS	Postsynaptic cell membrane	0.01786	4	GABRA2 , GABRB1 , <i>LRRTM2, LIN7C</i>
KEGG_PATH WAY	Ribosome (mmu03010)	0.018188	4	<i>RPL30, RPL21, RPL5, RPS8</i>
GOTERM_ BP_DIRECT	Phospholipid biosynthetic process (GO:0008654)	0.020204	3	<i>ISYNA1, PISD-PS2, PISD-PS3</i>
UP_KEYWOR DS	Phosphoprotein	0.028886	33	<i>CYB5R3, HIST4H4, SRSF10, TAF1D, CAPZA1, PDIA6, DSTN, PAK6, RPL30, SFR1, CBRI, GOT1, EIF3E, ZFP131, RANBP6, RPL5, HIST1H4I, ESCO1, WDFY1, HIST1H2BC, EXOSC9, APOA1BP, SNAPC3, STMN4, RPS8, ARL3, C1QBP, RGS4, EIF4A2, MAP9, CHORDC1, CDH10, PLA2G4E</i>
UP_KEYWOR DS	Protein transport	0.034569	6	<i>SEC24A, RANBP6, LIN7C, IFT27, VPS39, ARL3</i>
UP_KEYWOR DS	Nucleosome core	0.039285	3	<i>HIST4H4, HIST1H2BC, HIST1H4I</i>
UP_KEYWOR DS	Lipid metabolism	0.041794	5	<i>CYB5R3, CYP51, ISYNA1, PTGDS, PLA2G4E</i>
KEGG_PATH WAY	Arachidonic acid metabolism (mmu00590)	0.046401	3	<i>CBRI, PTGDS, PLA2G4E</i>
UP_KEYWOR DS	Cytoplasm	0.047555	21	<i>CYB5R3, SEC24A, EXOSC9, AKR1E1, CAPZA1, IDE, RPS8, ARL3, PAK6, CBRI, ISYNA1, GOT1, C1QBP, PTGDS, EIF3E, RANBP6, RPL5, MAP9, DYNLRB1, VPS39, PLA2G4E</i>
KEGG_PATH WAY	Metabolic pathways (mmu01100)	0.048382	10	<i>CYP51, CBRI, ISYNA1, NDUFA3, GOT1, PTGDS, B3GALT2, SDHC, DAD1, PLA2G4E</i>
(B) COX-2^{-/-} E19				
UP_KEYWOR DS	Acetylation	0.007195	17	<i>ATP5E, SORD, S100A10, HDDC3, RPS8, STK3, TPM3, I810037117RIK, RPL30, PTCD3, TCEB2, H2AFZ, ALDH2, GLO1, RPL5, NOP56, HIST1H2AN</i>
UP_KEYWOR DS	Ribonucleoprotein	0.009487	5	<i>RPL30, RPL21, RPL5, NOP56, RPS8</i>
KEGG_PATH WAY	Ribosome (mmu03010)	0.009839	4	<i>RPL30, RPL21, RPL5, RPS8</i>
UP_KEYWOR DS	Metal-binding	0.032702	16	<i>CYP51, ESCO1, 2610044015RIK8, WDFY1, SORD, ZFP40, APOA1BP, IDE, HDDC3, ZFP458, STK3, GM14412, GLO1, CAMK2A, ZFP938, PLA2G4E</i>
GOTERM_ BP_DIRECT	Translation (GO:0006412)	0.041752	5	<i>RPL30, RPL21, EIF4A2, RPL5, RPS8</i>
UP_KEYWOR DS	Isopeptide bond	0.042455	7	<i>ESCO1, TFAP2B, H2AFZ, RPL5, NOP56, HIST1H2AN, TPM3</i>

Table 5-2. ASD-associated Genes Differentially expressed in the COX-2^{-/-} E16 and E19 Female Mouse Brain

COX-2^{-/-} E16 Female KO mouse					
Gene	Gene name	Fold change	Human cytoband	Number of studies listed in AutDB	DAVID biological process
<i>Cdh10</i>	<i>Cadherin 10</i>	1.55	5p14.2-p14.1	9	Cell adhesion (GO:0007155)
<i>Gabrb1</i>	<i>Gamma-aminobutyric acid (GABA) A receptor, beta 1</i>	1.55	4p12	6	Central nervous system development (GO:0021954) Ion transport (GO:0006811) Cellular response to histamine (GO:0071420)
<i>Gabra2</i>	<i>Gamma-aminobutyric acid type A receptor subunit alpha2</i>	-1.69	4p12		Synaptic transmission, GABAergic (GO:0051932) Regulation of neurotransmitter levels (GO:0001505)
COX-2^{-/-} E19 Female KO mouse					
<i>Gabra2</i>	<i>Gamma-aminobutyric acid type A receptor subunit alpha2</i>	-2.09	4p12		Synaptic transmission, GABAergic (GO:0051932) Regulation of neurotransmitter levels (GO:0001505)
<i>Glo1</i>	<i>Glyoxylase I</i>	-2.09	6p21.2	9	Carbohydrate metabolic process (GO:0005975) Negative regulation of apoptotic process (GO:0043066)
<i>Slc6a8</i>	<i>Solute carrier family 6 (neurotransmitter transporter, creatine), member 8)</i>	-1.60	Xq28	11	Neurotransmitter transport (GO:0006836) Embryonic brain development (GO:1990403) Ion transport (GO:0006811)
<i>Camk2a</i>	<i>Calcium/calmodulin dependent protein kinase II alpha</i>	-1.58	5q32	7	Regulation of neurotransmitter secretion (GO:0046928) Regulation of neuronal synaptic plasticity (GO:0048168) Dendrite morphogenesis (GO:0048813) Ionotropic glutamate receptor signaling pathway (GO:0035235)

AutDB is the autism database.

5.6 Protein interaction networks of ASD genes in the COX-2^{-/-} female mouse brain.

Next, protein network analysis was conducted on the subset of 6 ASD-risk genes (Table 5-2) found in the current study using Cytoscape with GeneMANIA and the David Functional Annotation Tool (see methods; Chapter 2), to uncover important interactions that may potentially be defective during neurodevelopment. Two clusters of genes involved in biological processes of Dendrite/Synapse (*Gabrb1*, *Camk2a*) and Phosphoprotein (*Slc6a8*, *Cdh10*) were found to be affected in the female COX-2^{-/-} mouse (Figure 5-3). Similarly, in the male COX-2^{-/-} mouse, synaptic processes such as dendritic spine morphology and synaptic transmission were affected as well (Chapter 4; Figure 4-5B)

5.7 Summary of Results

In summary, the current global gene analysis revealed many differentially expressed genes in the female COX-2^{-/-} mouse at both E16 and E19 developmental stages. An interesting observation that was unique to the females was the switch from downregulation expression at E16 to upregulation at E19 in many genes. In contrast, in the COX-2^{-/-} males, there was a greater number of downregulated genes at both E16 and E19 stages. There may be two possible reasons for this difference between the two sexes: 1) The gene expression needs during normal brain development is different between males and females; or 2) The female brain tries to correct for the abnormal downregulation of genes, by increasing their expression by the later developmental stage. Lastly, there were 6 ASD risk genes that were identified in this study – most of them responsible for dendrite or synaptic functions.

COX-2^{-/-} Model

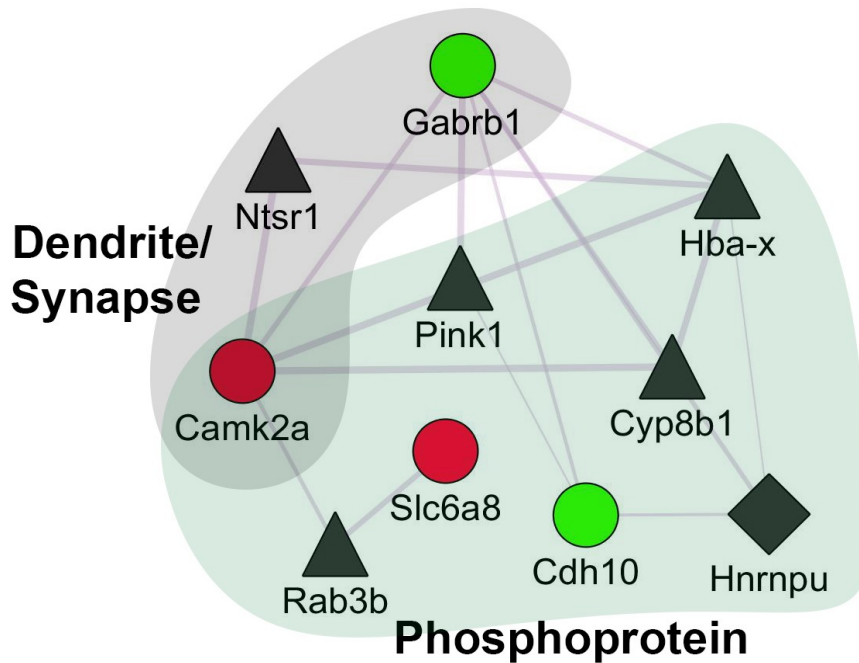


Figure 5-3. Protein interaction networks of ASD-risk genes in the female COX-2^{-/-} model. The ASD-risk genes found in the female COX-2^{-/-} model were used to create interaction networks using David Functional Annotation Tool and organized in Cytoscape with GeneMANIA. The affected networks found in the COX-2^{-/-} model include Synapse/Dendrite and Phosphoprotein. Circles represent ASD-risk genes found in this study (*red* = down-regulated; *green* = up-regulated); Black diamonds represent ASD-risk genes found in previous studies; Black triangles represent non-ASD-risk genes also important in these biological processes found in other studies. Purple, blue and orange interaction lines denote co-expression, co-localization and predicted interactions, respectively.

CHAPTER 6. RESULTS – STUDY 4

Study 4 – Sex differences in dendritic spine morphologies in cerebellar neurons of the COX-2^{-/-} male and female mouse brain – implications in ASD

Contributions: Ravneet Rai-Bhogal designed and performed all sample collections, acquired and analyzed all data, and created all figures and tables. Dr. Dorota A. Crawford supervised the design of the study and will be involved in editing the manuscript.

6.1 Introduction and Rationale

Abnormal synaptic transmission plays a role in the manifestation of many neurological disorders, including ASD (Guang *et al.*, 2018; Bagni & Zukin, 2019). Dendrites are responsible for receiving and integrating signals, and those of principal neuron types have small projections called dendritic spines, which are postsynaptic sites for excitatory glutamatergic synapses in the brain (Gray, 1959; Harris & Kater, 1994; Shepherd, 1996). Dendritic spines can be classified into different types, each with their own respective structure and function: thin spines have thin necks and small heads; mushroom spines have a thin neck and large head; stubby spines are without a distinguishable neck; and filopodia are long, thin protrusions known as precursors to the formation of dendritic spines (Hering & Sheng, 2001; Arellano *et al.*, 2007; Risher *et al.*, 2014). The morphology of dendritic spines can be affected by various factors, such as dysregulation of genes and proteins involved in spine development and structure, synaptic transmission, synaptic plasticity (Phillips & Pozzo-Miller, 2015; Martinez-Cerdeno, 2017), and environmental factors (Hatanaka *et al.*, 2017; Pinzon-Parra *et al.*, 2019).

An important protein enriched in dendritic spines is Spinophilin (SPN) (Allen *et al.*, 1997), which plays a significant role in spine development (Hu *et al.*, 2010; Evans *et al.*, 2015; Mi *et al.*, 2017), and thus synaptic transmission (Feng *et al.*, 2000). SPN is able to manipulate dendritic spine shape by binding to actin (Satoh *et al.*, 1998), which is also highly concentrated in dendritic spines (Fifkova & Delay, 1982; Matus *et al.*, 1982; Kaech *et al.*, 1997; Fischer *et al.*, 1998), and its dynamic and highly motile properties are essential for dendritic spine morphology (Fischer *et al.*, 1998). Therefore, changes in SPN expression or activity may lead to abnormalities in formation and maintenance of synapses.

The COX-2/PGE₂ pathway has also been implicated in dendritic spine dysgenesis. First, previous studies have established increased expression of COX-2/PGE₂ in the cerebellum upon inflammation in the body (Yan *et al.*, 2004; Schmidt *et al.*, 2016; Wright *et al.*, 2019), possibly through increased activity of aromatase (Wright *et al.*, 2019). This led to changes in the expression of SPN, abnormalities in dendritic spine development in the cerebellum (Dean *et al.*, 2012a; Dean *et al.*, 2012b), and presentation of ASD-like symptoms in animal models (Dean *et al.*, 2012a; Hoffman *et al.*, 2016). In these studies, the increase or decrease of COX-2 or PGE₂ levels was a result of an inflammatory response, or direct exposures through postnatal local injections or inhibitors, at specific time-points. The cerebellum is highly implicated in ASD pathology (D'Mello & Stoodley, 2015), with injury to the cerebellum during a critical window being one of the most prevalent factors in its manifestation (Limperopoulos *et al.*, 2007).

In the previous chapters, whole genome analyses were conducted on the COX-2^{-/-} male (Chapter 4) (Rai-Bhagal *et al.*, 2018a) and female (Chapter 5) mouse KOs, revealing many affected genes (developmental and disease-related), protein networks, and biological functional groupings that affect the dendrite and synapse, synaptic transmission, dendritic spine modelling, and dendrite morphogenesis at prenatal stages of embryonic days 16 (E16) and 19 (E19). Interestingly, we have also found that the mRNA expression of β -actin is downregulated in male mice with either COX-1 or -2 gene-deficit. This study aims to further explore the effects of COX-2^{-/-} gene mutation by investigating its role in dendritic spine morphology in the mouse cerebellum.

6.2 Objective and Hypotheses

Previous studies have examined the environmental impact of disruptions to the COX-2/PGE₂ pathway, through the use of inhibitors of PGE₂ production and local injections at specific

time-points. This allowed for determination of sensitive time windows during which the exposure may modify the development of dendritic spines in the cerebellum, leading to pathological conditions of the brain and ASD-like symptoms. The current study aims to investigate the genetic impact of the COX-2^{-/-} mutation on characteristics of dendritic spine morphology in cerebellar neurons at postnatal day (P25). The main objectives of this study are:

Objective 1 – To quantify the gene and protein expression of SPN and Actin (essential proteins of dendritic spine structure), in the brain of WT and COX-2^{-/-} males and females, at developmental stages of E19 and P25.

Objective 2 – To characterize and compare the density and type of dendritic spines expressed on dendrites of cerebellar neurons in the brain of WT and COX-2^{-/-} males and females at P25.

Objective 3 – To measure various morphological parameters of dendritic spines such as area, volume, length, neck length, head diameter, and straightness (as investigated in previous studies), in dendrites of cerebellar neurons in the brain of WT and COX-2^{-/-} males and females at P25.

Hypothesis: Based on previous studies and the current investigations of this dissertation, the involvement of COX-2 in spinogenesis and synaptogenesis is becoming very evident. Thus, we hypothesize that deficit of COX-2 expression in the brain following sensitive cerebellar development periods, will affect the expression of critical proteins, such as SPN and Actin, and alter dendritic spine morphologies in males and females.

6.3 Experimental Model System

In the current study, male and female COX-2^{-/-} gene knockout mouse models were used to investigate effects of the gene mutation on cerebellar development between sexes. Two stages were scrutinized: E19 – early period of cerebellar development, and P25 – following cerebellar development.

RESULTS

6.4 mRNA Expression of *SPN* and *β-actin* in the Normally Developing Male and Female Mouse Brain

First, the mRNA expression of *SPN* and *β-actin* in the brain was investigated in male and female mice during normal brain development. The developmental stages of E19 and P25 were carefully selected to parallel development of the cerebellum. CT Values for all tested conditions were compared to the gene expression measurement from WT male mouse at E19 for each respective gene (Figure 6-1).

The mRNA expression of *SPN* in brain tissue between WT males and females, was not significantly different at E19 (RQ: males = 0.70, females = 0.74; $p = 0.92$), but it was increased in females (RQ = 1.52; $p = 0.029$) compared to males (RQ = 1.39) at P25. However, between time-points, the expression of *SPN* significantly increased from E19 to P25 in both males ($p = 0.046$) and females ($p = 0.029$) (Figure 6-1A). This indicates a potential increase in the need for *SPN* at P25, during when the brain is rapidly developing its synaptic structure and formations.

Next, the mRNA expression of *β-actin* was measured in male and female mice at E19 and P25 during normal brain development. There were no significant differences found between males and females at E19 (RQ: males = 0.75, females = 0.86; $p = 0.64$) or P25 (RQ: males = 0.19, females = 0.21; $p = 0.20$). However, there was a significant decrease in the mRNA

expression of β -actin from E19 to P25 in the brains of both male ($p = 0.021$) and female mice ($p = 0.013$) (Figure 6-1B). This may be indicative of the process of postnatal maturation and pruning of synapses that typically occurs between these time-points. In conclusion, there are significant changes that occur in the expression level of critical genes *SPN* (increase) and β -actin (decrease) from E19 to P25 in both male and female mice, during normal brain development.

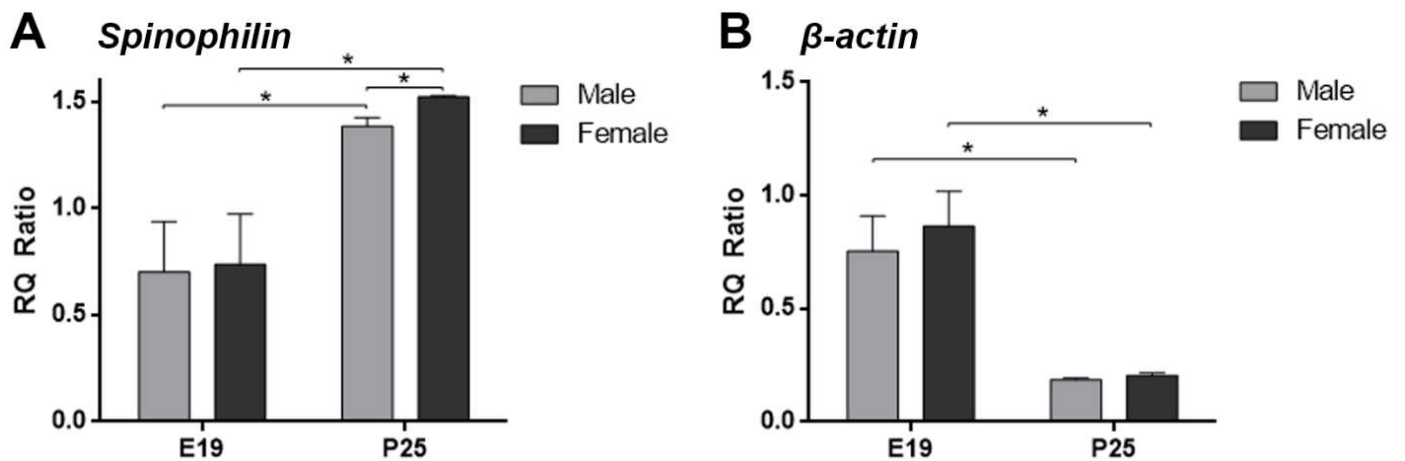


Figure 6-1. mRNA Expression of *SPN* and β -actin in the brains of normally developing males and females at E19 and P25. The mRNA expression levels of (A) *SPN* increased from E19 to P25 and (B) β -actin decreased from E19 to P25 in normally developing brain of WT male and female mice. (n=3). * $p < 0.05$

6.5 mRNA Expression of *SPN* and β -*actin* in COX-2^{-/-} Males and Females at E19 and P25.

In order to study the effect of COX-2^{-/-} genotype on the mRNA expression of *SPN* and β -*actin* in the brains of COX-2^{-/-} males and females, qRT-PCR was used to test these genes at developmental time-points of E19 and P25 (Figure 6-2). At each stage, CT values from the COX-2^{-/-} males and females were normalized to their WT counterparts, which were designated as RQ=1. At E19, there was a significant decrease in the expression of *SPN* in both males (RQ = 0.80; $p = 0.003$) and females (RQ = 0.71; $p = 0.008$) (Figure 6-2A). At the P25 stage, *SPN* in was significantly decreased only in females (RQ = 0.75; $p = 0.004$), with no change of expression in the male (RQ = 1.05; $p = 0.71$) COX-2^{-/-} brain (Figure 6-2B).

The same pattern was seen in the expression of β -*actin* between both sexes and time points. At E19, there was a significant decrease in the expression of β -*actin* in both male (RQ = 0.75; $p = 0.009$) and female (RQ = 0.69; $p = 0.001$) COX-2^{-/-} mouse brains (Figure 6-2C). At P25, there was a significant decrease of β -*actin* in the female COX-2^{-/-} mouse (RQ = 0.75; $p = 0.007$), and no change in the males (RQ = 1.06; $p = 0.137$) (Figure 6-2D). These results suggest that there is an impact of the COX-2^{-/-} mutation on the mRNA expression levels of these critical genes in both sexes at the earlier E19 stage, but the expression continues at abnormal levels in the females only at P25. The mRNA expression of both *SPN* and β -*actin* returns back to normal levels in the males by P25.

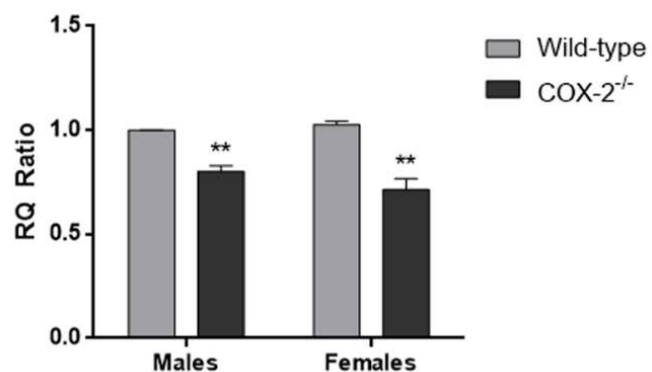
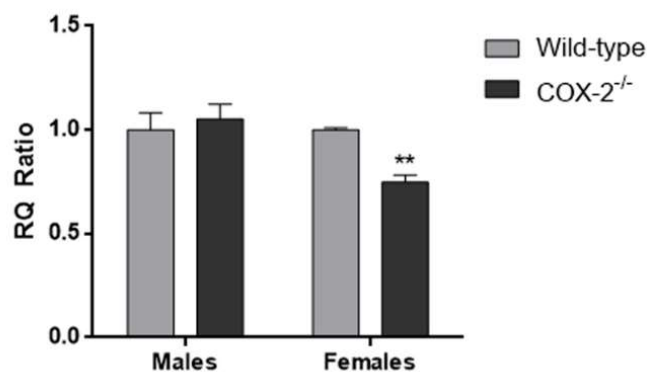
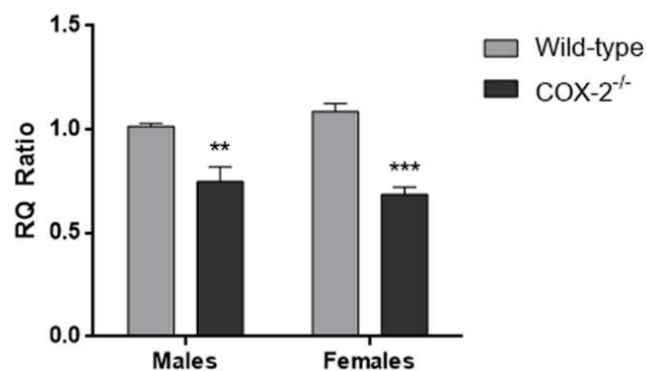
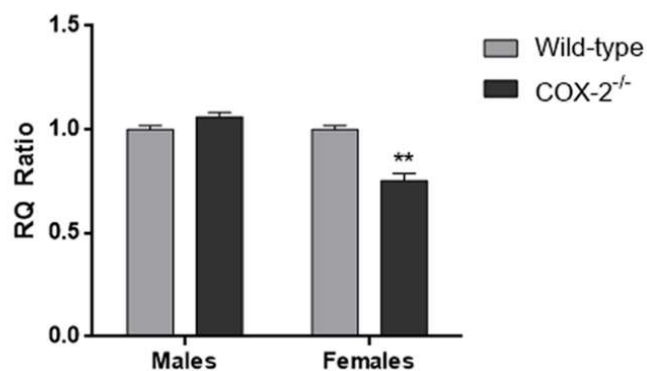
A *Spinophilin* (E19)**B** *Spinophilin* (P25)**C** β -actin (E19)**D** β -actin (P25)

Figure 6-2. mRNA Expression of *SPN* and β -actin in the *COX-2^{-/-}* male and female mouse brains at E19 and P25. There was a decrease in expression levels of *SPN* in the brain of, A) *COX-2^{-/-}* male and females at E19, B) and only in the females at P25. C) There was a decrease in expression levels of β -actin in the brain of *COX-2^{-/-}* male and females at E19, D) and only in the females at P25. (n=3). ** $p < 0.005$, *** $p < 0.0005$.

6.6 Protein Expression of SPN and β -actin in COX-2^{-/-} Males and Females at E19 and P25.

Due to the changes observed in the mRNA expression of *SPN* and *β -actin*, the protein products of both genes were measured in COX-2^{-/-} male and female brain to verify their expression (Figure 6-3). There was no change in expression of SPN in the COX-2^{-/-} mice at the E19 stage (fold-change: males = 0.63, $p = 0.22$; females = 1.17; $p = 0.65$) (Figure 6-3A). In contrast, there were significant changes in the expression of SPN at the later P25 stage in both sexes: 0.61-fold decrease in males ($p = 0.033$) and 7.2-fold increase in females ($p = 0.003$) (Figure 6-3B). This was interesting as the regulation of protein expression is the opposite in COX-2^{-/-} males and females.

The expression of β -actin was unaltered in COX-2^{-/-} males at E19 (Fold-change = 1.03; $p = 0.785$), but decreased at 0.73-fold in COX-2^{-/-} females ($p = 0.003$) (Figure 6-3C). At the P25 stage, there was a 0.30-fold decrease ($p = 0.008$) in the expression levels of β -actin in the COX-2^{-/-} male brain, and a 3.98-fold increase in the COX-2^{-/-} females ($p = 0.029$) (Figure 6-3D). Therefore, there were significant changes in protein expression of SPN and β -actin at the P25 stage, compared to the E19 stage, in brains of the COX-2^{-/-} mouse. It is important to note that the expression pattern of both proteins in males and females was similar at the P25 stage in the COX-2^{-/-} mouse. This data also indicates sex-specific expression of both SPN and β -actin in COX-2^{-/-} mouse. Due to this expression pattern specifically at P25, this stage was suitable to further investigate the effect on dendritic spine development.

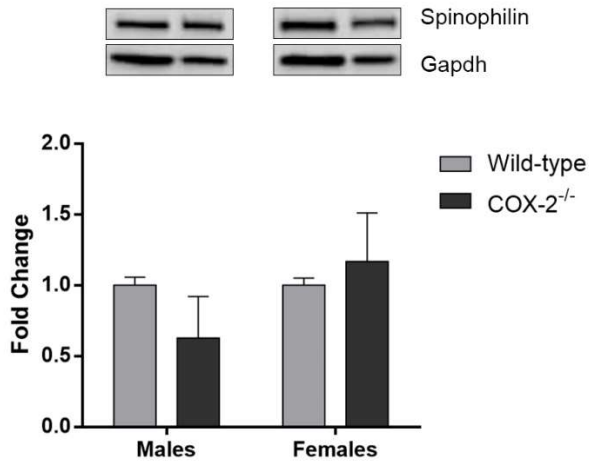
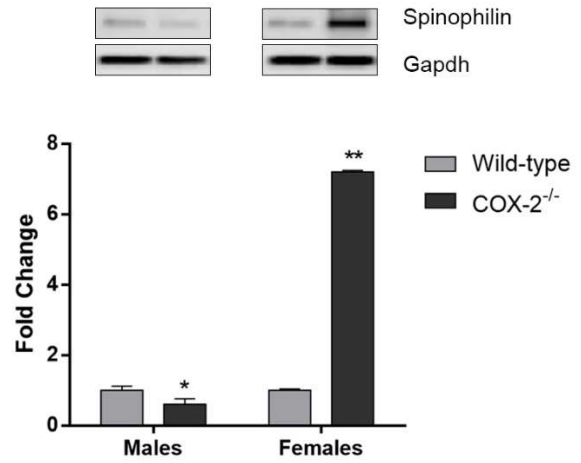
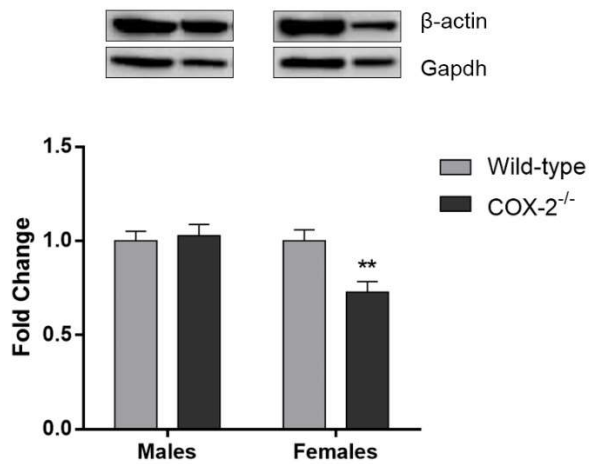
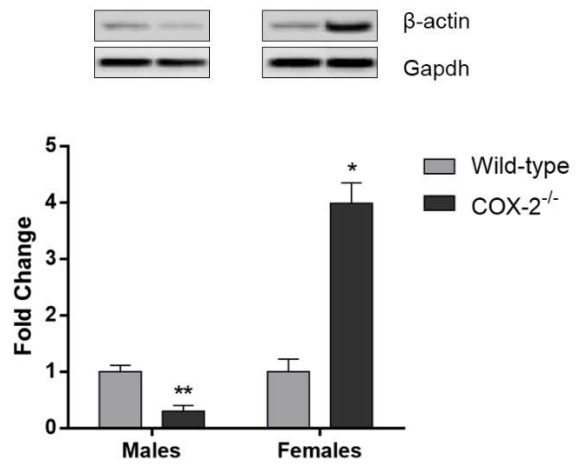
A Spinophilin (E19)**B Spinophilin (P25)****C β-actin (E19)****D β-actin (P25)**

Figure 6-3. Protein expression of SPN and β-actin in the COX-2^{-/-} male and female mouse brains at E19 and P25. (A) There was no change in the expression of SPN protein in the COX-2^{-/-} males or females at E19, (B) but significant changes in expression (decrease in males and increase in females) at P25 in the mouse brain. (C) There was a decrease in protein expression levels of β-actin in the brain of COX-2^{-/-} females only at E19, and (D) significant changes in expression (decrease in males and increase in females) at P25 in the mouse brain. (n=3). * $p < 0.05$, ** $p < .005$.

6.7 Dendritic Spine Characteristics of Cerebellar Neurons in WT and COX-2^{-/-} Male and Female Mice at P25

Due to previous research in the cerebellum, and the sex-specific changes in SPN and β -actin protein expression in the COX-2^{-/-} mice from the current study, dendritic spine morphology was scrutinized in the cerebellum between COX-2^{-/-} males and females.

6.7.1 Spine Density

Spine density was measured as the number of spines per 10 μ m length of dendrite, in cerebellar neurons of WT and COX-2^{-/-} male and female mice at P25 (Figure 6-4). There was a total of 4.4 spines per 10 μ m of dendrite in WT males, 4.0 spines in COX-2^{-/-} males, 4.6 spines in WT females, and 4.2 spines in COX-2^{-/-} females ($p = 0.90$) (Figure 6-4A). Figure 6-4B shows a representative dendrite exhibiting the average spine density seen across samples of all subjects. Although there is a range in dendritic spine density values dependent on region and developmental timing, the spine density measurement values found in our study are in accordance with the literature (Murphy & Segal, 1996; Smith *et al.*, 2016; Hernandez *et al.*, 2019). In conclusion, there were no significant changes in spine density in cerebellar neurons of the COX-2^{-/-} mouse in either males or females.

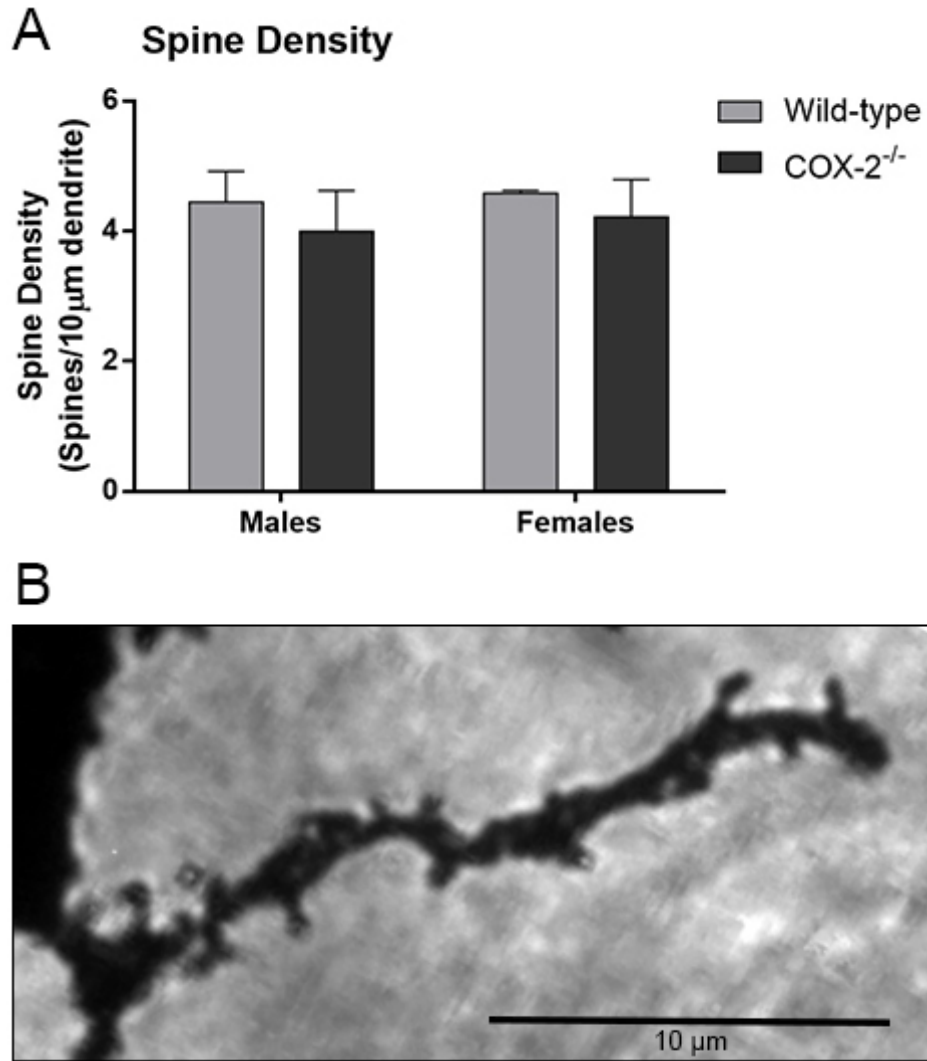


Figure 6-4. Dendritic spine density in the cerebellum of WT and COX-2^{-/-} males and females at P25. (A) There were no significant differences in the density of dendritic spines in cerebellar neurons between WT and COX-2^{-/-} mice in either sex. (B) A representative image of a dendrite with dendritic spines (captured from WT female) to exhibit the average density observed across all experimental groups, as there was no difference observed. n=3 samples per group.

6.7.2 Dendritic Spine Types

Next, dendritic spines were classified according to their type (thin, mushroom, stubby and filopodia) and quantified to determine the ratio of each type relative to the total number of spines on a dendrite (Figure 6-5). The spines were characterized using a reliable semi-automatic method using Imaris software with the filament tracer tool as described in methods (Chapter 2). The percent of each spine type was calculated as: (number of each spine type/total number of spines)*100.

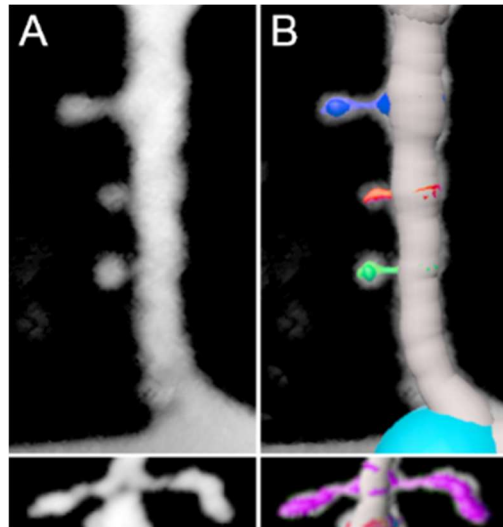


Figure 6-5. Types of dendritic spines. A) Four types of dendritic spines are identified, and B) classified using a semi-automatic method in Imaris software with filament tracer tool. *Blue* = Thin spines; *red* = Stubby spines; *green* = Mushroom spines; *purple* = Filopodia.

Overall, the cerebellar neurons encompassed the greatest number of thin spines followed by stubby, mushroom and filopodia, in all mice. In the thin spine class, there were $54.5\% \pm 1.2$ spines in WT male brains, $48.0\% \pm 1.5$ in COX-2^{-/-} males, $48.8\% \pm 1.5$ in WT females, and $51.4\% \pm 3.2$ in COX-2^{-/-} females ($p = 0.294$) (Figure 6-6). For the mushroom spine type, there were $6.7\% \pm 1.2$ spines in WT male brains, $4.7\% \pm 0.3$ in COX-2^{-/-} males, $5.8\% \pm 1.0$ in WT females, and $6.3\% \pm 1.2$ in COX-2^{-/-} females ($p = 0.437$). There were $36.0\% \pm 5.8$ stubby spines in WT male brains, 44.7 ± 2.6 in COX-2^{-/-} males, $41.8\% \pm 1.8$ in WT females, and $39.3\% \pm 5.5$ in COX-2^{-/-} females ($p = 0.078$). Lastly, of the filopodia designation, there were $5.2\% \pm 1.5$ spines in WT male brains, $3.4\% \pm 0.3$ in COX-2^{-/-} males, 4.0 ± 0.3 in WT females, and 3.0 ± 0.6 in COX-2^{-/-} females ($p = 0.497$). In conclusion, there were no significant changes observed in the percentage of any of the spine types in the cerebellum between WT and COX-2^{-/-} mice, or males and females.

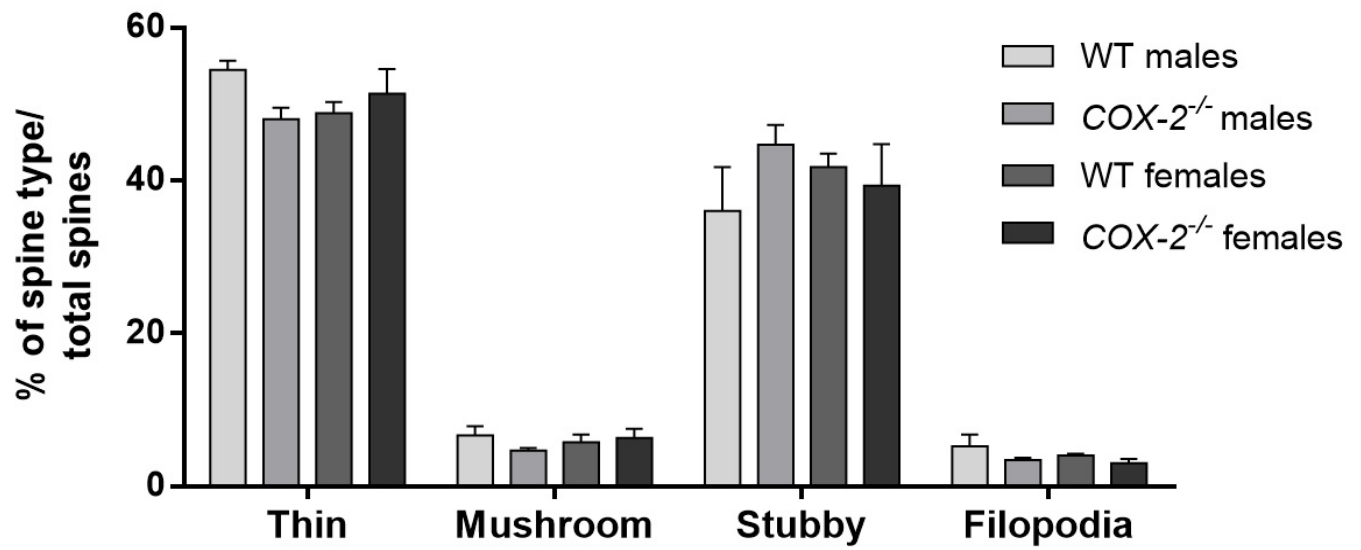


Figure 6-6. Dendritic spine types in the cerebellum of WT and COX-2^{-/-} males and females at P25. There was an abundance of thin spines expressed by cerebellar neurons, followed by stubby, mushroom and filopodia spines. There were no significant differences found between WT and COX-2^{-/-} males and females. n=3 samples per group.

6.7.3 Spine Area

Dendritic spines function in most of the excitatory synapses in the brain. Thus, changes in their morphology or structure may affect synaptic function leading to deficits in learning and memory. Various parameters of spine morphology (chosen according to the literature) were investigated to determine whether the COX-2-deficiency causes modifications to these structures. Spine area of each dendritic spine was determined, and averages from each spine type were calculated for each WT and COX-2^{-/-} males and females. For all further analyses, thin spines were subcategorized into long thin spines (< 2µm) and short thin spines (< 1µm), as presented in previous studies (Risher *et al.*, 2014; Ohta *et al.*, 2017), and described in methods (Chapter 2). **Thin spines:** The average area of short and long thin spines was 0.79 ± 0.02 and $1.63 \pm 0.05 \mu\text{m}^2$ in WT males, 0.79 ± 0.02 and $1.63 \pm 0.05 \mu\text{m}^2$ in COX-2^{-/-} males, 0.76 ± 0.02 and $1.63 \pm 0.05 \mu\text{m}^2$ in WT females, and 0.81 ± 0.02 and $1.69 \pm 0.05 \mu\text{m}^2$ in COX-2^{-/-} females (short: $p = 0.579$, long: $p = 0.701$), respectively (Figure 6-7A). **Mushroom:** The average area of mushroom spines was $1.53 \pm 0.08 \mu\text{m}^2$ in WT males, $1.45 \pm 0.09 \mu\text{m}^2$ in COX-2^{-/-} males, $1.65 \pm 0.08 \mu\text{m}^2$ in WT females, and $1.62 \pm 0.08 \mu\text{m}^2$ in COX-2^{-/-} females ($p = 0.529$) (Figure 6-7B). **Stubby:** The average area of stubby spines was $0.32 \pm 0.01 \mu\text{m}^2$ in WT males, $0.31 \pm 0.01 \mu\text{m}^2$ in COX-2^{-/-} males, $0.30 \pm 0.01 \mu\text{m}^2$ in WT females, and $0.30 \pm 0.01 \mu\text{m}^2$ in COX-2^{-/-} females ($p = 0.753$) (Figure 6-7C). **Filopodia:** Lastly, the average area of filopodia spines was $3.99 \pm 0.20 \mu\text{m}^2$ in WT males, $4.00 \pm 0.23 \mu\text{m}^2$ in COX-2^{-/-} males, $3.89 \pm 0.19 \mu\text{m}^2$ in WT females, and $3.87 \pm 0.23 \mu\text{m}^2$ in COX-2^{-/-} females ($p = 0.965$) (Figure 6-7D). In conclusion, the COX-2^{-/-} mutation did not have an affect on dendritic spine area in cerebellar neurons of males or females.

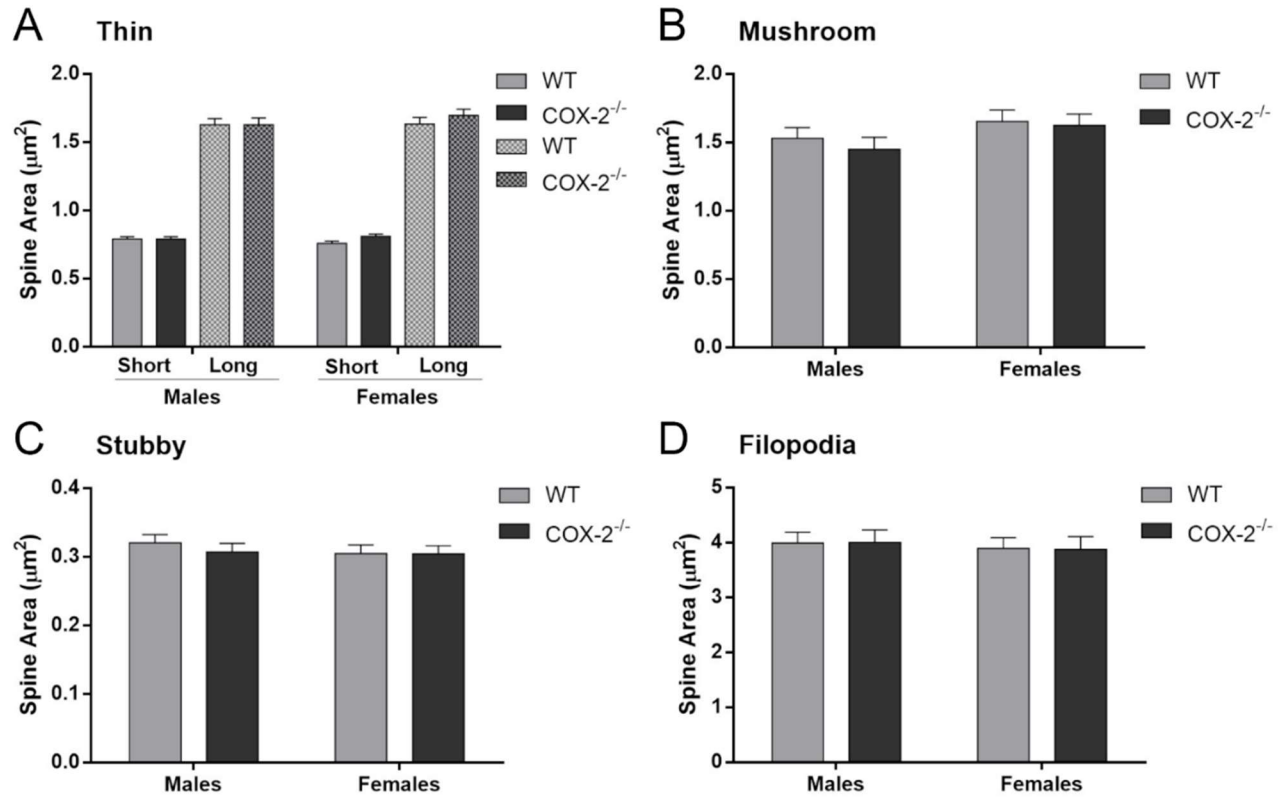


Figure 6-7. Spine area measurement of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. There were no changes in spine area of (A) Thin (short and long); (B) Mushroom; (C) Stubby; and (D) Filopodia spines between WT and COX-2^{-/-} males and females at P25. n=3 subjects per group.

6.7.4 Spine Volume

Dendritic spine volume was the next parameter that was measured in cerebellar neurons of WT and COX-2^{-/-} males and females. Spine volume may directly influence maintenance of synaptic connections in the brain (Kasai *et al.*, 2010). **Thin:** We found that the spine volume of short and long thin spines was $0.08 \pm 0.003 \mu\text{m}^3$ and $0.18 \pm 0.007 \mu\text{m}^3$ in WT males, $0.08 \pm 0.003 \mu\text{m}^3$ and $0.20 \pm 0.025 \mu\text{m}^3$ in COX-2^{-/-} males, $0.07 \pm 0.003 \mu\text{m}^3$ and $0.19 \pm 0.009 \mu\text{m}^3$ in WT females, and $0.08 \pm 0.003 \mu\text{m}^3$ and $0.19 \pm 0.008 \mu\text{m}^3$ in COX-2^{-/-} females (short: $p = 0.352$, long: $p = 0.860$), respectively (Figure 6-8A). **Mushroom:** The spine volume of mushroom spines was $0.14 \pm 0.01 \mu\text{m}^3$ in WT males, $0.12 \pm 0.01 \mu\text{m}^3$ in COX-2^{-/-} males, $0.16 \pm 0.01 \mu\text{m}^3$ in WT females, and $0.16 \pm 0.02 \mu\text{m}^3$ in COX-2^{-/-} females ($p = 0.860$) (Figure 6-8B). **Stubby:** The spine volume of stubby spines was $0.024 \pm 0.002 \mu\text{m}^3$ in WT males, $0.022 \pm 0.002 \mu\text{m}^3$ in COX-2^{-/-} males, $0.023 \pm 0.001 \mu\text{m}^3$ in WT females, and $0.0225 \pm 0.0009 \mu\text{m}^3$ in COX-2^{-/-} females ($p = 0.786$) (Figure 6-8C). **Filopodia:** Lastly, the spine volume of filopodia spines was $0.54 \pm 0.04 \mu\text{m}^3$ in WT males, $0.53 \pm 0.04 \mu\text{m}^3$ in COX-2^{-/-} males, $0.49 \pm 0.03 \mu\text{m}^3$ in WT females, and $0.50 \pm 0.03 \mu\text{m}^3$ in COX-2^{-/-} females ($p = 0.707$) (Figure 6-8D). There were no significant differences found in spine volume of any spine type in COX-2^{-/-} males or females at P25.

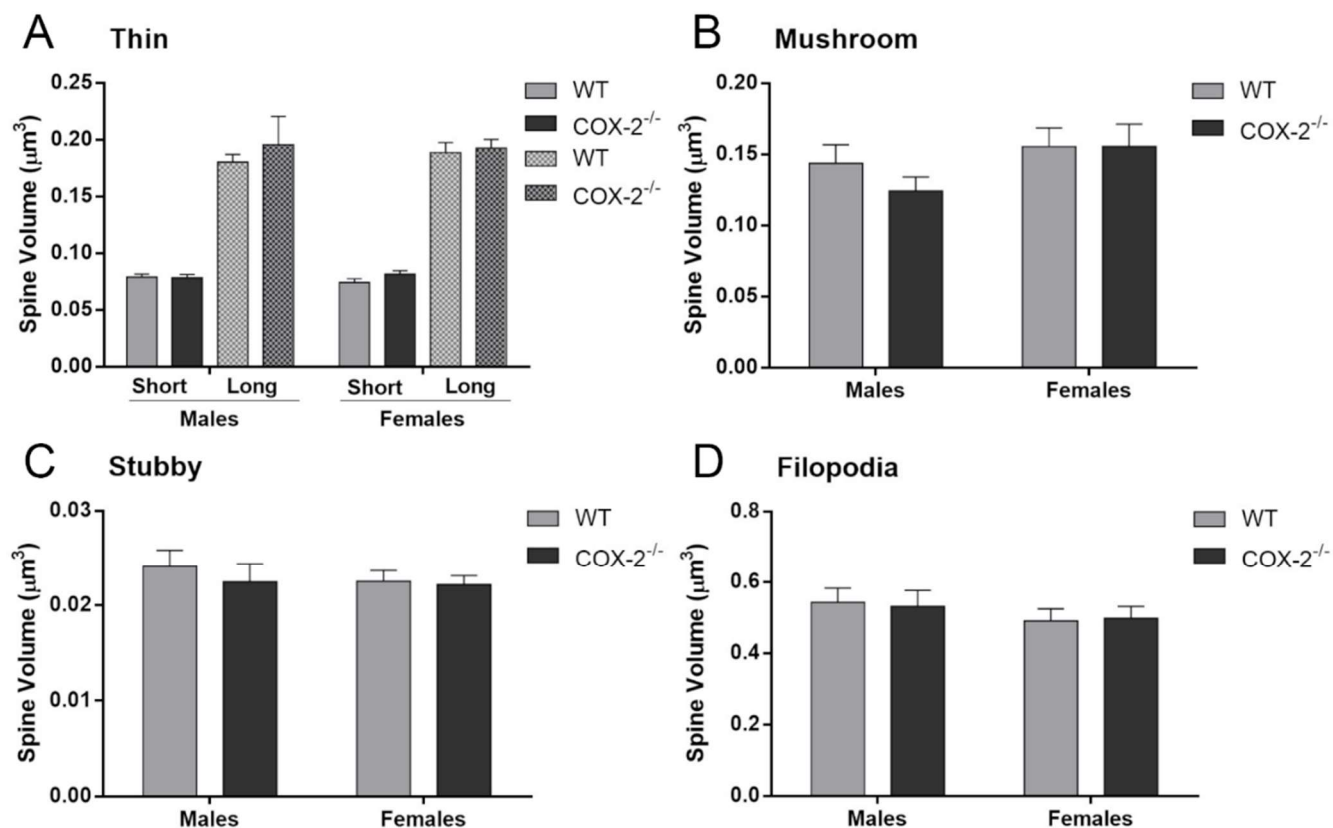


Figure 6-8. Spine volume measurement of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. There were no changes in spine volume of (A) Thin (short and long); (B) Mushroom; (C) Stubby; and (D) Filopodia spines between WT and COX-2^{-/-} males and females at P25. n=3 subjects per group.

6.7.5 Spine Length

Dendritic spine length is a measurement from the tip of the spine to where it connects it to the dendrite. Spine length may have an inverse relationship with synaptic potential, and result in modifications to synaptic plasticity (Araya *et al.*, 2006). **Thin:** The average spine length for short and long thin spines was 0.72 ± 0.005 and 1.35 ± 0.01 μm in WT males, 0.71 ± 0.006 and 1.34 ± 0.01 μm in COX-2^{-/-} males, 0.73 ± 0.006 and 1.32 ± 0.01 μm in WT females, and 0.72 ± 0.005 and 1.35 ± 0.01 μm in COX-2^{-/-} KO females (short: $p = 0.771$, long: $p = 0.242$), respectively (Figure 6-9A). **Mushroom:** The length of mushroom spines was 1.54 ± 0.04 μm in WT males, 1.52 ± 0.05 μm in COX-2^{-/-} males, 1.59 ± 0.04 μm in WT females, and 1.54 ± 0.05 μm in COX-2^{-/-} females ($p = 0.732$) (Figure 6-9B). **Stubby:** The average spine length for stubby spines was 0.35 ± 0.01 μm in WT males, 0.35 ± 0.01 μm in COX-2^{-/-} males, 0.34 ± 0.01 μm in WT females, and 0.326 ± 0.005 μm in COX-2^{-/-} females ($p = 0.337$) (Figure 6-9C). **Filopodia:** Lastly, the length of filopodia spines was 2.65 ± 0.06 μm in WT males, 2.73 ± 0.07 μm in COX-2^{-/-} males, 2.59 ± 0.06 μm in WT females, and 2.57 ± 0.07 μm in COX-2^{-/-} females ($p = 0.667$) (Figure 6-9D). There were no significant differences found in spine length in any spine type in COX-2^{-/-} males or females at P25.

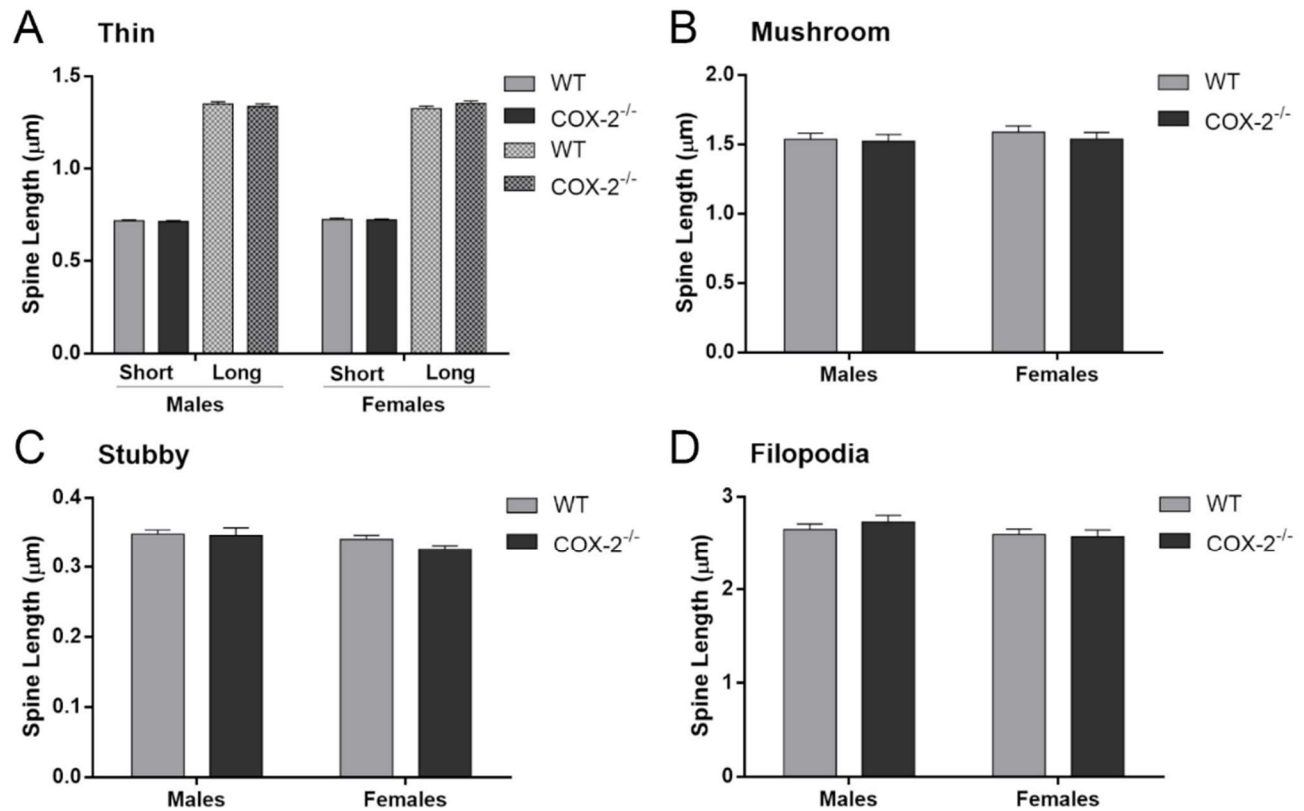


Figure 6-9. Spine length measurement of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. There were no changes in spine length of (A) Thin (short and long); (B) Mushroom; (C) Stubby; and (D) Filopodia spines between WT and COX-2^{-/-} males and females at P25. n=3 subjects per group.

6.7.6 Spine Neck Length

Thin and mushroom spines have a neck that connects the dendrite shaft to the bulb of the spine, and is known for the compartmentalization of signals, and may change in size depending on potentiation. Changes in spine length can alter the strength of signals. **Thin:** The average

spine neck length for short and long thin spines was 0.42 ± 0.007 and 1.02 ± 0.01 μm in WT males, 0.43 ± 0.007 and 1.01 ± 0.02 μm in COX-2^{-/-} KO males, 0.44 ± 0.008 and 0.98 ± 0.01 μm in WT females, and 0.43 ± 0.007 and 1.01 ± 0.01 μm in COX-2^{-/-} KO females (short: $p = 0.181$, long: $p = 0.278$), respectively (Figure 6-10A). **Mushroom:** The average spine neck length of mushroom spines was 1.18 ± 0.04 μm in WT males, 1.19 ± 0.05 μm in COX-2^{-/-} males, 1.22 ± 0.04 μm in WT females, and 1.18 ± 0.04 μm in COX-2^{-/-} KO females ($p = 0.871$). There were no significant changes in spine neck length in either thin or mushroom spine types in cerebellar neurons between WT and COX-2^{-/-} KO males or females.

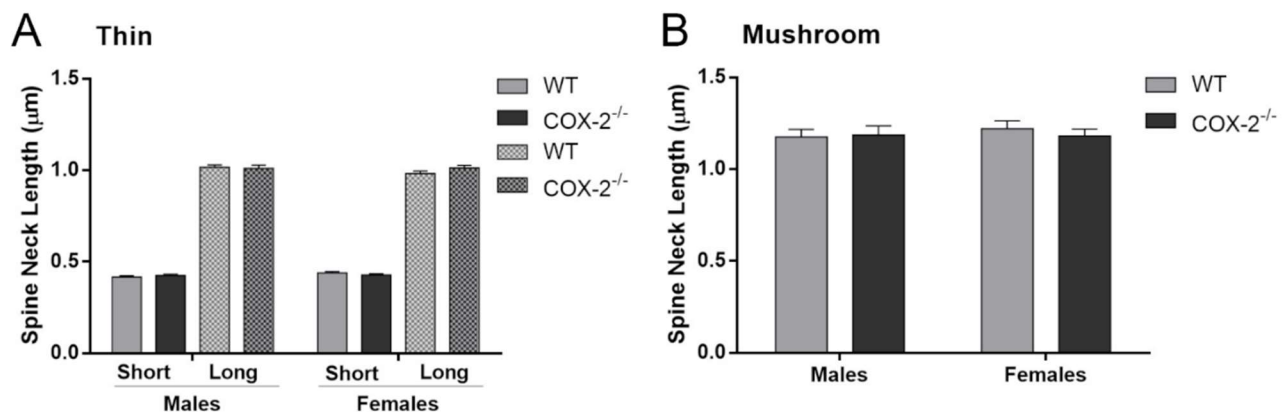


Figure 6-10. Spine neck length measurement of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. There were no changes in spine neck length of (A) Thin (short and long); or (B) Mushroom spines between WT and COX-2^{-/-} males and females at P25. $n=3$ subjects per group.

6.7.7 Spine Head Diameter

The dendritic spine head diameter measurement reflects the size of the post-synaptic density, and is related to the strength of synapses and long-term potentiation or depression (Gipson & Olive, 2017). The spine head diameter in all spines (thin, mushroom, and stubby) was quantified between WT and COX-2^{-/-} males or females. **Thin:** The average spine head diameter for short and long thin spines was 0.58 ± 0.01 and 0.63 ± 0.01 μm in WT males, 0.58 ± 0.01 and 0.63 ± 0.01 μm in COX-2^{-/-} males, 0.60 ± 0.01 and 0.62 ± 0.01 μm in WT females, and 0.62 ± 0.01 and 0.66 ± 0.02 μm in COX-2^{-/-} females (short: $p = 0.150$, long: $p = 0.308$), respectively (Figure 6-11A). **Mushroom:** The spine head diameter for mushroom spines was 0.96 ± 0.02 μm in WT males, 0.92 ± 0.02 μm in COX-2^{-/-} males, 0.95 ± 0.02 μm in WT females, and 1.01 ± 0.02 μm in COX-2^{-/-} females ($p = 0.041$) (Figure 6-11B). We found that there was a notable increase in the average of spine head diameter of mushroom spines in COX-2^{-/-} females compared to WT ($p = 0.054$). In addition, there was a significant increase in the spine head diameter of mushroom spines in COX-2^{-/-} females compared to COX-2^{-/-} males ($p = 0.005$). **Stubby:** The average spine head diameter of stubby spines was 0.75 ± 0.01 μm in WT males, 0.75 ± 0.01 μm in COX-2^{-/-} KO males, 0.76 ± 0.01 μm in WT females, and 0.77 ± 0.01 μm in COX-2^{-/-} females ($p = 0.298$) (Figure 6-11C). In conclusion, the spine head diameter of mushroom spines were significantly larger in cerebellar neurons of the COX-2^{-/-} females compared to their male counterparts.

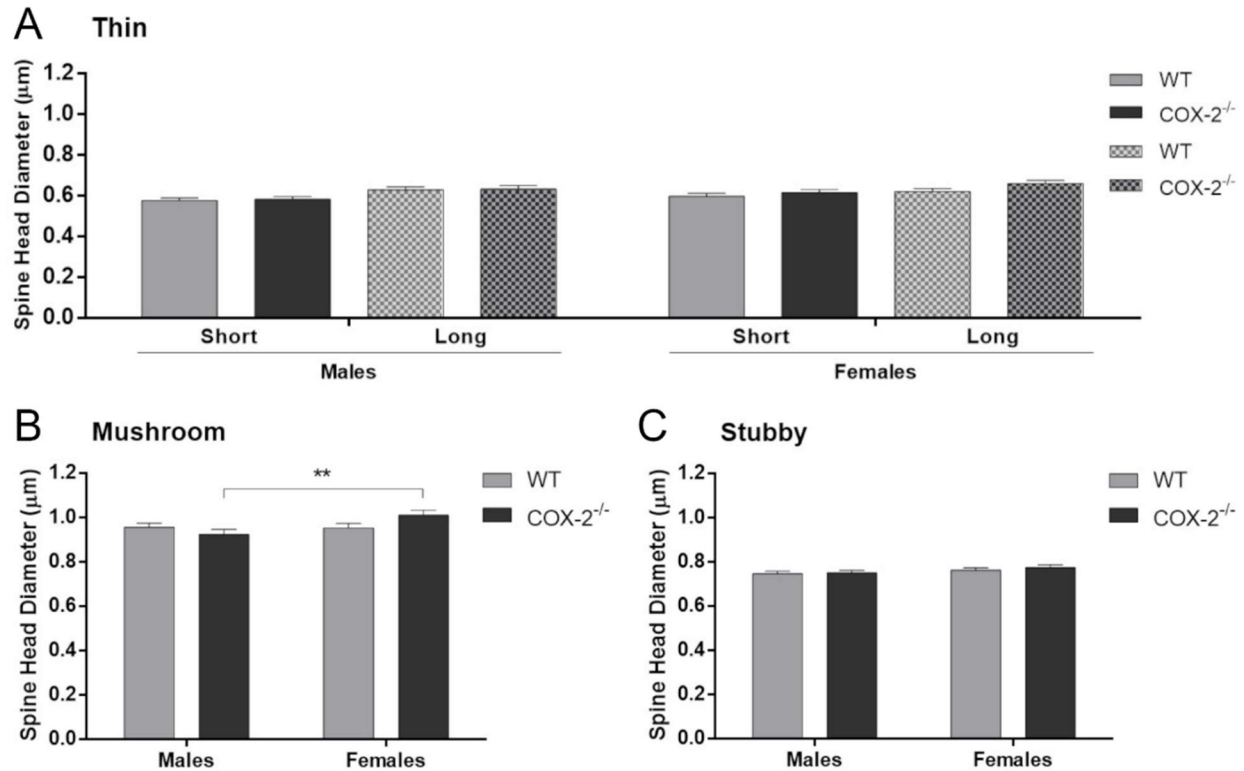


Figure 6-11. Spine head diameter measurement of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. (B) The spine head diameter of mushroom spines was significantly increased in COX-2^{-/-} females, whereas there was no change in (A) thin and (C) stubby spines. n=3 subjects per group. $p = 0.005$

6.7.8 Spine Straightness

Dendritic spine straightness is a measure of the tortuosity of a dendritic spine, which can be affected in some neurological conditions. Increased spine straightness has previously been reported in ASD (Gouder *et al.*, 2019) and increased tortuosity in humans and animal models of Fragile X syndrome (Rudelli *et al.*, 1985; Comery *et al.*, 1997). A value of 1.00 represents

complete straightness, and the lower the value, the more curvature in the spine. **Thin:** The spine straightness measurements for short and long thin spines was 0.950 ± 0.002 and 0.928 ± 0.002 μm in WT males, 0.953 ± 0.002 and 0.928 ± 0.004 μm in COX-2^{-/-} males, 0.948 ± 0.002 and 0.929 ± 0.003 μm in WT females, and 0.949 ± 0.002 and 0.932 ± 0.003 μm in COX-2^{-/-} females (short: $p = 0.158$, long: $p = 0.730$), respectively (Figure 6-12A). **Mushroom:** The spine straightness measurements for mushroom spines was 0.95 ± 0.007 in WT males, 0.94 ± 0.005 in COX-2^{-/-} males, 0.93 ± 0.007 in WT females, and 0.94 ± 0.004 in COX-2^{-/-} females ($p = 0.260$) (Figure 6-12B). **Stubby:** The spine straightness measurements for stubby spines was 0.96 ± 0.005 in WT males, 0.97 ± 0.001 in COX-2^{-/-} males, 0.97 ± 0.001 in WT females, and 0.97 ± 0.001 in COX-2^{-/-} females ($p = 0.203$) (Figure 6-12C). **Filopodia:** Lastly, the spine straightness measurements for filopodia was 0.94 ± 0.002 in WT males, 0.94 ± 0.002 in COX-2^{-/-} males, 0.94 ± 0.002 in WT females, and 0.94 ± 0.003 in COX-2^{-/-} females ($p = 0.328$) (Figure 6-12D). There were no significant changes found in the straightness of spines in either COX-2^{-/-} males or females.

6.8 Summary of Results

To summarize, we found that the COX-2^{-/-} mice had decreased mRNA levels of *spinophilin* and *β -actin* in males and females at the E19 stage, and in females only at P25. In contrast, there was sex-specific expression of spinophilin and β -actin proteins in the brain at the P25 stage only. The expression of both proteins was decreased in COX-2^{-/-} males and increased in COX-2^{-/-} females. Of all the parameters of dendritic spine morphology that were measured in cerebellar neurons of COX-2^{-/-} mice, there was a significant increase in the spine head diameter of mushroom spines in female COX-2^{-/-} mice only. This will be further explored in the discussion.

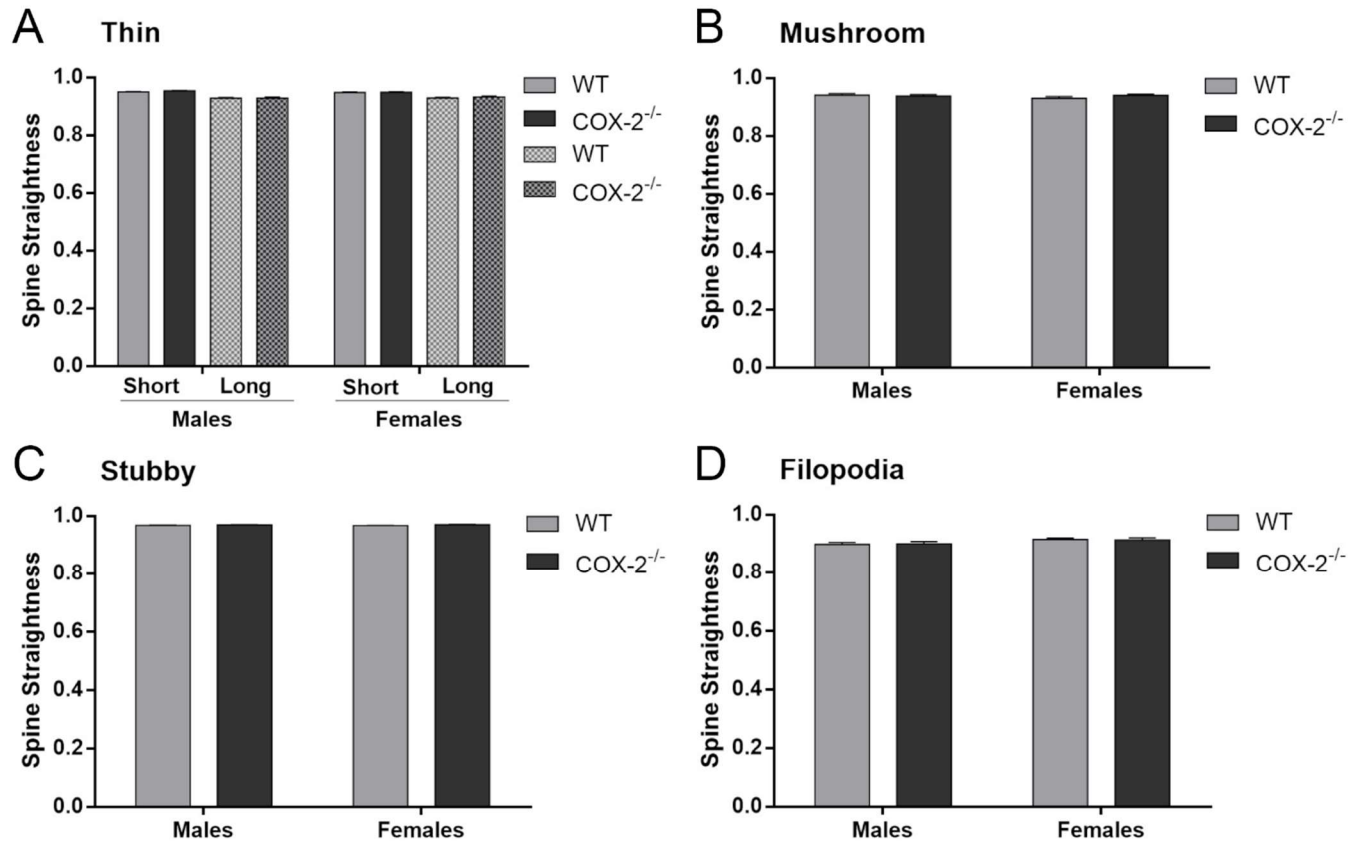


Figure 6.12. Spine straightness of dendritic spines in the cerebellum of WT and COX-2^{-/-} males and females at P25. There were no changes in spine straightness of (A) Thin (short and long); (B) Mushroom; (C) Stubby; and (D) Filopodia spines between WT and COX-2^{-/-} males and females at P25. n=3 subjects per group.

CHAPTER 7. DISCUSSION

7.1 Study 1 – Maternal Exposure to Prostaglandin E₂ Modifies Expression of Wnt Genes in Mouse Brain – An Autism Connection.

The first study provides novel data for the role of PGE₂ signaling in the prenatal brain. Using an *in vivo* mouse model, the effects of maternal exposure to the exogenous lipid mediator PGE₂ at E11, on the offspring during brain development at E16, E19 and P8.

First, the expression of all PGE₂-binding EP receptors was confirmed in the early brain throughout the developmental stages of interest. The expression profile of the EP receptors followed a similar pattern in all stages including the adult brain, with EP2 and EP3 (γ and β) having the highest expression level. Previous studies show that EP2 receptors are expressed in neurons, microglia, macrophages, and astrocytes, and its activation through the binding action of PGE₂ may lead to an increased inflammatory response by macrophages and microglial cells in the brain (Johansson *et al.*, 2013). Furthermore, PGE₂-mediated activation of the EP2 receptor leads to the phosphorylation of both isoforms of glycogen synthase kinase-3 (GSK-3), to release β -catenin from the inhibitory complex, allowing it to translocate to the nucleus and initiate gene transcription (Fujino *et al.*, 2002; Castellone *et al.*, 2005). EP3 receptors are abundantly expressed in neurons, and their activation in cells was found to be involved in increased Wnt signaling and expression of Wnt genes in cell models (Hiyama *et al.*, 2015). However, there was no *in vivo* evidence to support this interaction between the PGE₂ and Wnt pathways.

The canonical Wnt pathway is particularly interesting as current research from human genetics and animal studies show its involvement in the pathology of ASD (Tamiji & Crawford, 2010b; Zhang *et al.*, 2014b; Wong *et al.*, 2015b). The current study successfully showed that PGE₂ influenced the mRNA expression of genes from the canonical Wnt signaling pathway,

such as *Mmp7* and *Wnt2*, *in vivo* in a mouse model. The convergence of PGE₂ and the Wnt canonical pathway was also evident by increased levels of PKA-activated phosphorylated β -catenin (Ser552) and active non-phospho β -catenin (Ser33/37/Thr41), further discussed below.

Maternal exposure to PGE₂ at the onset of neurogenesis resulted in differential expression of the Wnt-regulated genes, *Mmp7* and *Wnt2*, both of which have been previously linked to neurodevelopmental disorders such as ASD. During development, the temporal expression of genes is critical to proper growth and maintenance of all structures, particularly the brain (Rakic, 2009; Ohi *et al.*, 2017). Improper expression and signalling mechanisms can wreak havoc on connectivity and function, which can lead to lasting unfavorable effects in the brain.

Under normal conditions, MMPs are expressed at low or undetectable levels in the adult brain, and are triggered during injury or upon inflammation (Vecil *et al.*, 2000), which was confirmed for *Mmp7*. In contrast, the basal expression of *Mmp7* during early development was significantly higher than in the adult, the highest at E16 and decreasing during the course of development. This strongly suggests a role for *Mmp7* in the fetal and early postnatal brain, especially at E16 which is a time period of rapid neurogenesis and synaptogenesis in most brain regions of the mouse. There was a significant PGE₂-dependent increase in expression of *Mmp7* above the normal level at E16 and a noticeable decline in the expression below the basal level at E19 that persisted to the P8 stage.

Mmp7 belongs to a family of matrix metalloproteinases that are involved in many functions such as breakdown of the extracellular matrix, development, inflammation, and synaptic plasticity (Ethell & Ethell, 2007). Moreover, *Mmp7* is also involved in increasing permeability of the BBB (Buhler *et al.*, 2009), potentially allowing for improper crossing of molecules during prenatal and postnatal development (Wong *et al.*, 2015a). It is also involved in

the release of neurotransmitters from the presynaptic membrane (Szkklarczyk *et al.*, 2007), and has caused disruption of dendritic spine structure by altering the morphology of mature mushroom spines to immature filopodia (Bilousova *et al.*, 2006). Such events can have catastrophic consequences such as improper synaptic signalling which can lead to abnormal connections and wiring of the brain.

Increased levels of Mmp7 have been found in the animal model (Bucan *et al.*, 2012) and human brain samples (Anthony *et al.*, 1997; Cossins *et al.*, 1997) of multiple sclerosis. Interestingly, Mmp7 also activates Mmp9 (von Bredow *et al.*, 1998; Wang *et al.*, 2006), which has been implicated in multiple sclerosis (Mirowska-Guzel *et al.*, 2009), epilepsy (Konopka *et al.*, 2013), fragile-X syndrome (Sidhu *et al.*, 2014), and ASD (Abdallah *et al.*, 2012). Our lab previously found elevated levels of *Mmp9* expression in neuroectodermal stem cells exposed to exogenous PGE₂ (Wong *et al.*, 2014a), and decreased levels in a COX-2^{-/-} mouse model (Wong *et al.*, 2019). Therefore, it is possible that PGE₂-dependent dysregulation of MMPs may occur during particular events or stages of development, and have unique consequences on the developing brain.

The mRNA expression of *Wnt2* significantly increased above the normal level at E19 and P8 following maternal exposure to PGE₂. Recently, our lab discovered abnormal increased expression of *Wnt2* at the P8 developmental stage in the COX-2^{-/-} mouse as well (Wong *et al.*, 2019). Together, this is strongly indicative of an association between irregular COX-2/PGE₂ signalling and the expression of *Wnt2*. Typically, *Wnt2* is expressed in the rat and mouse ventral midbrain during and after neurogenesis (Rawal *et al.*, 2006; Sousa *et al.*, 2010), and plays a role in the proliferation of dopaminergic neurons (Sousa *et al.*, 2010). *Wnt2* is also located on the widely known autism susceptibility locus on the chromosome 7q31-33 (Badner & Gershon,

2002). Mutations of the *Wnt2* gene have previously been implicated in families with ASD (Wassink *et al.*, 2001), SNP mutations in patients with autism (Marui *et al.*, 2010), and strong associations reported within a Chinese population of individuals with ASD (Chien *et al.*, 2011). Furthermore, specific polymorphisms of the *Wnt2* gene may play a potential role in one of the hallmark characteristic ASD symptoms of delayed speech (Lin *et al.*, 2012). Considering the important roles of *Wnt2* and its involvement in ASD, it is possible that PGE₂-triggered changes can alter its expression, and have a negative effect on the course of early neurodevelopment.

An interesting observation was that the protein expression levels of Wnt2 did not reflect the pattern of mRNA expression. Typically, the expression of mRNA and protein is similar and reliable when tested in experimental conditions (Koussounadis *et al.*, 2015). However, several studies have experienced a discrepancy between mRNA and protein expression, and some possible reasons will be discussed. In previous literature, the secretion level of endogenous Wnt proteins in COS cells was examined. The translated Wnt proteins successfully re-located to the endoplasmic reticulum and underwent glycosylation, but most of the protein content was not able to be secreted (Burrus & McMahon, 1995). The authors suggested that the presence of too much protein surpassed the capacity of the mechanisms to properly fold the protein and execute secretion function. There are many events and regulatory mechanisms that are involved from the transcription of mRNA to translation of the protein product. Factors such as ribosome density, rate of translation initiation and termination (Arava *et al.*, 2003; Maier *et al.*, 2009), and rate of mRNA and protein half-lives and degradation (Wang, 2008) attempt to explain the inconsistencies between mRNA and protein expression (Greenbaum *et al.*, 2003). Nonetheless, the observed changes following maternal PGE₂ exposure provide an insight into abnormal Wnt2 mRNA and protein expression in the brain during periods of neurodevelopment.

Our lab has also discovered abnormal increased expression of a third Wnt gene, *Wnt3a*, in the maternal-PGE₂-administered mouse model (Rai-Bhagal *et al.*, 2018b). PGE₂ significantly increased brain expression of *Wnt3a* in offspring at the same developmental stages – E16, E19, and P8. *Wnt3a* also plays important roles in brain development (Mattes *et al.*, 2012), during specific events such as proliferation and differentiation of neural stem cells into neurons (Yin *et al.*, 2007; Andersson *et al.*, 2011; Yang *et al.*, 2014), neurite outgrowth, neuronal polarization, intracellular calcium modulation, and neurotransmission (Avila *et al.*, 2010; Bernis *et al.*, 2013; Greer *et al.*, 2013). Interestingly, the brain regions that have been shown to be highly impacted by *Wnt3a* signaling during development—thalamus, cerebellum, and hippocampus—have also been implicated in ASD (Verhoeven *et al.*, 2010; Boucher *et al.*, 2012; Wang *et al.*, 2014b). Furthermore, *Wnt3a* increases the transcription and protein levels of strongly associated ASD-gene neuroligin-3, which is a cell-surface protein involved in synaptic transmission (Medina *et al.*, 2018). The abnormal expression of Wnt genes following PGE₂ administration provided compelling evidence towards communication between PGE₂ and Wnt pathways in the mouse brain during development.

The current study successfully validated cross-talk between the PGE₂ and Wnt developmental pathways *in vivo* in the mouse brain upon maternal PGE₂ administration through the altered expression of Wnt and Wnt-related genes. Interaction between PGE₂ and the Wnt pathway has previously been established in non-neuronal cell types (Yoshida *et al.*, 2013; Wong *et al.*, 2014a; Hiyama *et al.*, 2015), and shown *in vivo* in a zebrafish model (North *et al.*, 2010). In neuronal cell cultures, the interaction likely occurs with activation of non-phospho β -catenin (Ser33/Ser37/Thr41) (Wong *et al.*, 2014a; Wong *et al.*, 2016).

The possible point of convergence in the mouse brain is also likely through PGE₂-mediated activation of β -catenin, which is a key modulator of Wnt signalling. It is apparent through the differential expression levels of two activated phosphorylation states of β -catenin – PKA-phosphorylated β -catenin (Ser552) (Taurin *et al.*, 2006; Taurin *et al.*, 2008), and non-phosphorylated β -catenin (Ser33/37/Thr41) (Figures 3-9, 3-10). PKA is one of the major downstream components of the PGE₂ signaling pathway (Wright & McCarthy, 2009; Wong *et al.*, 2014a), and PKA-induced activation at the Serine-552 phosphorylation site has been previously linked to increased transcriptional activity of the Wnt canonical pathway (Taurin *et al.*, 2006; Zhao *et al.*, 2010). The Ser33/37/Thr41 sites can be phosphorylated by GSK-3 β , which destabilizes β -catenin and marks it for degradation (Liu *et al.*, 2002; Kimelman & Xu, 2006; Wu & He, 2006). Non-phosphorylation at these sites leads to increased levels of the active form of β -catenin, which can translocate to the nucleus to act on transcription factors and gene expression (Gao *et al.*, 2014). Furthermore, PGE₂ treatment has also shown to directly act on GSK-3 β to alter the phosphorylation processing of β -catenin (Du *et al.*, 2015; Yu *et al.*, 2015). Therefore, previous and current findings suggest that abnormal PGE₂ levels in the mouse brain during development may interfere with the Wnt pathway via phosphorylation of β -catenin at multiple sites, and lead to differential expression of crucial neurodevelopmental genes.

Another interesting finding in the current study shows the occurrence of differential regulation of gene expression between pups of the same genetic composition within a given pregnancy. As indicated by previous research, the observed differences in gene expression between offspring could be attributed to various factors such as dissimilar metabolism of exogenous PGE₂ by the mother, unequal distribution of PGE₂ to each pup due to separate placentas, or inconsistent epigenetic changes between the pups (Gareri *et al.*, 2009; Gordon *et*

al., 2011; Bui *et al.*, 2015). A previous study showed that monozygotic twins that have separate placentas (dichorionic) show more variability in epigenetic changes compared to monozygotic twins that share a placenta (monochorionic) (Bui *et al.*, 2015). Another study found variable levels of ethanol metabolites in human and guinea pig placentas following identical maternal exposure to ethanol (Gareri *et al.*, 2009). These results provide some insight into the mechanisms by which environmental factors that modulate PGE₂ levels in the brain (Tamiji & Crawford, 2010b; Wong *et al.*, 2014a; Wong *et al.*, 2015b) may influence brain development uniquely via gene expression. In addition, this may also provide a better understanding of how the external environment can influence the behavioural differences observed between human monozygotic twins affected with autism (Cook, 2009; Mitchell *et al.*, 2009). The concordance rate of ASD in monozygotic twins has been reported between 36%-88% (Ronald & Hoekstra, 2014), monozygotic-monochorionic twins having a higher concordance rate compared to monozygotic-dichorionic twins that do not share the same placenta (Bohm & Stewart, 2009). When the twin fetuses are attached to their own placentas, potential differences in environmental exposure likely lead to different ASD outcomes in twins regardless of their identical genetic makeup.

This study shows that maternal PGE₂ administration at a critical time can have an effect on expression of Wnt-target genes during prenatal (E16 and E19) brain development and continues postnatally at least till P8. Interestingly, dissimilar PGE₂ exposure between fetuses likely contributed to the differences in gene expression that was observed between pups of identical genetic composition. Nonetheless, the pattern of changes between fetuses was significant. Lastly, it also provides molecular evidence for the convergence between the PGE₂ and Wnt pathways *in vivo* in the developing mouse brain, and proposes its role in brain pathology.

7.2 Study 2 – Global Microarray Analysis in the Cyclooxygenase-1 and -2 Male Knockout Mice

In this study, the effects of COX/PGE₂ dysregulation were investigated through gene expression profiling of homozygous male COX-1^{-/-} and COX-2^{-/-} (KOs) on development of the prenatal mouse brain. It is shown for the first time that despite the differences in cellular expression in the brain, the defect in either COX enzyme can affect the expression of genes and regulation of pathways that are important for brain development and function.

Microarray technology revealed a global analysis of differentially expressed genes showing that there were nearly twice as many affected genes at the E16 stage compared to E19 in the brain of both COX-1^{-/-} and -2^{-/-} male mouse models. This suggests that the COX enzymes play a more significant role at E16 while the brain is rapidly developing, and the impact of their deletion on the expression of other developmental genes is also greater. Although neurogenesis in the developing mouse brain begins as early as E9 and continues postnatally, brain regions such as areas of the basal forebrain, striatum, thalamus, cerebral cortex, and auditory and visual systems undergo neurogenesis at E16, which is completed in most regions by E19 (Rodier, 1980; Rice & Barone, 2000). Thus the higher number of developmental genes affected at the earlier stage during rapid neurogenesis might have potential implication for the developing brain. Between the two enzymes, there was also more profound impact in the COX-2^{-/-} mouse at E16 as indicated by the greater number of affected genes, biological pathways and ASD candidate genes. This indicates that COX-2^{-/-} in particular may have a more significant influence on transcription of critical genes in the earlier stage of neurodevelopment.

There were many functional pathways affected in each COX gene KO at both developmental stages. Interestingly, many of these biological groupings encompassed ASD-risk genes, and were also found in previous functional analyses in clinical studies (Anney *et al.*,

2011; Kumar *et al.*, 2011; An *et al.*, 2014; Ning *et al.*, 2015). To date, a few studies have established ASD-like behaviours in rodent models with disrupted COX/PGE₂ signalling. Treatment with COX inhibitors in rats lead to difficulties in spatial learning (Shaw *et al.*, 2003), and changes in ASD-like behaviours of complex social interactions, sensory integration, and attention in male rats only (Dean *et al.*, 2012a), providing evidence towards ASD-like behaviours as a result of abnormal COX expression. Our lab presented for the first time validation of ASD-like behaviours of hyperactivity, anxiety, and repetitive actions in a COX-2 gene KO mouse model. (Wong *et al.*, 2019). However, there is still a considerable gap in knowledge in the cellular and molecular mechanisms that may result from a deficit in COX enzymes.

We found an enrichment of genes involved in biological processes of the regulation of synaptic transmission in each COX KO, including genes implicated in ASD. Abnormal synaptic neurobiology is one of the fundamental etiological factors of ASD pathophysiology (Ebert & Greenberg, 2013; Sala *et al.*, 2015; Lin *et al.*, 2016; Liu *et al.*, 2016c). It is well known that COX enzymes play a significant role in the modulation of synapses. Recent evidence shows that the use of COX inhibitors results in an increase in dendrite length (Dean *et al.*, 2012a) and in the expression of spinophilin protein, which is highly expressed in dendritic spines (Amateau & McCarthy, 2004).

In the COX-1^{-/-} male mouse, an enrichment of genes was found in biological processes such as long-term potentiation, and transmission of nerve impulse. Furthermore, there were 26 ASD-risk genes found throughout both developmental stages. Pathway analysis tools revealed that the ASD genes belonged to pathways such as regulation of neuron projection development, dendritic spine, synaptic transmission, and learning and memory.

COX-1 is constitutively expressed ubiquitously throughout the brain regions predominantly by microglia (Hoozemans *et al.*, 2001; Shukuri *et al.*, 2011). Between embryonic days E14 and E16, there is an explosive growth in microglia numbers in the brain (Reemst *et al.*, 2016). The development of microglia occurs through timed events regulated by the expression of specific developmental genes, and changes in chromatin. It is well-known that COX-1 expression in microglia plays a significant role in the inflammatory response (Choi *et al.*, 2008; Calvello *et al.*, 2017; Kapitanovic Vidak *et al.*, 2017). However, one of the emerging functions of microglia is the modification of synaptic networks by the removal of synapses through phagocytosis in normal brain development (Roumier *et al.*, 2004; Roumier *et al.*, 2008; Paolicelli *et al.*, 2011; Schafer *et al.*, 2013). Recent research has also shown the involvement of microglia in ASD (Edmonson *et al.*, 2016; Hanamsagar *et al.*, 2017; Lee *et al.*, 2017). This study provides further evidence that the activity of the COX-1/PGE₂ pathway in microglia may play an important role in the regulation of neural synaptic development and synapse activity during brain development. Therefore, the connection between microglia activation and COX-1/PGE₂ signalling during prenatal brain development should be investigated further.

Similarly, in the current study we found that the genes affected in the COX-2^{-/-} model were enriched in biological processes such as axon guidance or long-term depression. A particularly interesting gene dysregulated in both models involved in long-term potentiation and depression was *metabolic glutamate receptor 5 (GRM5)*, which is highly associated with ASD (Kelleher & Bear, 2008; Iossifov *et al.*, 2012; Hadley *et al.*, 2014; Ramos-Prats *et al.*, 2019). In early neurodevelopment, GRM5 is expressed in most brain regions, and is responsible for regulatory functions of excitatory and inhibitory synapses (Terbeck *et al.*, 2015). In particular, its expression in many regions of the rat brain was found to be expressed in much higher levels in

early postnatal stages following birth, compared to later time-points (Romano *et al.*, 1996). Interestingly, our lab found the expression of *GRM5* to continue to be dysregulated in brains of COX-2^{-/-} males, at a postnatal stage of day 8 after birth (P8) (Wong *et al.*, 2019), indicative of possible defects in the formation of glutamatergic signals and synaptic plasticity during critical early development. A recent study discovered *GRM5* as one of the most accurate ASD predictor genes in their diagnostic testing tool that included 237 SNPs in 146 genes (Skafidas *et al.*, 2014). In addition, treatment with ibuprofen (COX inhibitor) in older mice affected the expression of brain *GRM5* in both control and an Alzheimer's mouse model (Woodling *et al.*, 2016). The connection between COX enzymes and *GRM5* expression can allow insight into the functional role of this particular interaction which may include learning, memory, and cognition in the mouse.

Stringent pathway analysis tools using ASD-risk genes found throughout both developmental stages in the COX-2^{-/-} model, revealed affected pathways involved in dendritic spine modelling, dendrite morphogenesis, and synaptic transmission. These biological processes have been extensively reviewed as being major contributors to the etiology of ASD in human and animal models (Lai & Ip, 2013; Copf, 2016; Martinez-Cerdeno, 2017). COX-2 is also constitutively expressed but it is localized primarily in neuronal cells of the brain, particularly in dendrites and dendritic spines of the postsynaptic cell (Kaufmann *et al.*, 1996). Although COX-2 is ubiquitously expressed at various levels in the brain it is particularly enriched in the cortex and hippocampus (Yamagata *et al.*, 1993), areas that are involved in higher brain functions such as learning and memory and emotions, deficits of which lead to ASD behaviours. Previous studies suggest that COX-2 plays an important role in synaptic transmission, plasticity (Chen *et al.*, 2002; Sang *et al.*, 2005), and more specifically long-term depression, predominantly through

PGE₂ (Le *et al.*, 2010). Furthermore, COX-2 is responsible for glutamate release in the cerebellum (Tian *et al.*, 2008), cerebral cortex (Lin *et al.*, 2014), and hippocampus – most likely through signalling action of PGE₂ via the EP2 receptor (Sang *et al.*, 2005). Therefore, abnormal COX-2/PGE₂ signalling can be responsible for improper synaptic transmission, plasticity, and pruning – mechanisms which have been extensively implicated in the etiology of ASD.

Lipid function pathways, containing a number of ASD-risk genes, were also affected in both COX^{-/-} models. This was expected as COX enzymes primarily function in the metabolism of lipids for proper brain development and function. Numerous studies have confirmed abnormal lipid levels to be one of the causative factors of ASD (Meguid *et al.*, 2008; Jory, 2016; Yui *et al.*, 2016a), and supplementation with lipids to children with the disorder improved behavioural symptoms (Meguid *et al.*, 2008; Yui *et al.*, 2016a). Since defects in the COX genes can cause abnormalities in lipid formation and transportation, this could lead to unfavourable changes in the brain and deficits in mechanisms of lipid signalling.

The regulation of protein translation was also affected exclusively in the COX-2^{-/-} mouse at both stages. Defects in translation of proteins can hinder neuronal development and lead to neurodevelopment disorders (Chen *et al.*, 2019). *RBM8A*, which is as an ASD-risk gene encodes a protein that binds to mRNA and is involved in its splicing and translation (Wiegand *et al.*, 2003). In addition, it is involved in the proliferation and differentiation of glia and neurons, respectively, and its deficit can result in interruption of neurogenesis events (Mao *et al.*, 2015). When such a complex process is affected, the extent of insult that can result is inconceivable.

Next, the subset of genes that were commonly expressed between E16 and E19 in either gene knockout model were characterized for their functional roles and visualized into molecular interaction pathways using Cytoscape. In mouse brain development, E16-E19 encompasses half

of the neurogenesis period across all brain regions. Therefore, it is reasonable to assume that the genes and processes that are affected at both tested stages, were likely to have been defective throughout the entire E16-E19 period, which may provide insight into potential candidates that can be used as screening tools for neurodevelopmental disorders following birth.

In the COX-1^{-/-} model, the HOPS (homotypic fusion and protein sorting) complex was identified as an affected pathway. The role of HOPS complex in the mammalian nervous system is not well known, however, there is some interesting research emerging over the last few years in a mouse species (*buff*; *bf*), with a mutation in the *Vps33a* gene – the same gene that is also affected in the COX-1^{-/-} model. The *bf* mutant exhibited a loss of neurons in Purkinje cells of the cerebellum particularly at the young age of 6 months old, and deficits in various motor skills seen in later stages (Zhen & Li, 2015). It is also hypothesized that the HOPS complex may be involved in a neurological condition called catatonia which affects speech, motor abilities, and other behaviours, and is present in a portion of people with ASD (Dhossche, 2004; Dhossche *et al.*, 2006; Ghaziuddin *et al.*, 2020; Quilliam *et al.*, 2020), that exhibit those same deficits. This indicates the involvement of the HOPS complex in neuronal development, and should be further investigated in neurodevelopmental studies. In the COX-2^{-/-} model, there were critical interaction pathways highlighted from the set of common genes that were differentially expressed at both E16 and E19 developmental stages. An important biological process that was affected was cell-substrate adhesion, which represents the ability of cells to attach, and is critical for extracellular matrix, cell motility, cell communication, and development of synapse and pathways.

Another important finding in the current study was the confirmation of cross-talk between COX/PGE₂ and the Wnt signalling pathways in the brain, both considered as autism susceptibility pathways (Wassink *et al.*, 2001; Marui *et al.*, 2010; Tamiji & Crawford, 2010b;

Kalkman, 2012; Zhang *et al.*, 2014b; Wong *et al.*, 2015a). Wnt signalling normally induces the expression of a wide variety of regulatory pathways involved in cell fate specification, cell proliferation, migration, axon guidance and synapse formation (Okerlund & Cheyette, 2011). Some of these pathways, including neural migration, axon guidance and neural connectivity were affected in the COX^{-/-} models in the current study, and have previously been implicated in ASD pathology (Watts, 2008; Zhang *et al.*, 2014b). Communication between the COX/PGE₂ and Wnt signalling pathways has previously been established *in vitro* in non-neuronal (Yoshida *et al.*, 2013; Zhang *et al.*, 2014b; Hiyama *et al.*, 2015) and neuronal cells (Wong *et al.*, 2014b; Codocedo & Inestrosa, 2016; Davidson *et al.*, 2016), as well as *in vivo* in the zebrafish (North *et al.*, 2010). Our lab has previously shown that elevated levels of PGE₂ interferes with the canonical Wnt pathway via protein kinase A (PKA) and phosphoinositide-3 kinase (PI-3K) in neuronal cell cultures (Wong *et al.*, 2014b; Davidson *et al.*, 2016). PGE₂ increased migration and proliferation in Wnt activated cells, accelerated neuronal differentiation and altered the expression of several ASD associated Wnt-target genes, including *Ptgs2* (Wong *et al.*, 2014b; Davidson *et al.*, 2016). Expression of Wnt-target genes and β -catenin were also affected in our *in vivo* mouse model of maternal PGE₂ exposure (Rai-Bhagal *et al.*, 2018b)

There were 8 differentially expressed Wnt and Wnt-target genes, all belonging to the Wnt pathways: the canonical, PCP, and Wnt/Ca²⁺ pathway. Of these genes, *GSK3 β* has previously been implicated in animal models of autism, particularly in the functional role of synaptic plasticity (Go *et al.*, 2012; Franklin *et al.*, 2014; Caracci *et al.*, 2016; Kwan *et al.*, 2016; Wu *et al.*, 2016). Moreover, *GSK3 β* , is a crucial component of the destruction complex which regulates β -catenin activity, a key modulator of Wnt signalling (Xu *et al.*, 2009). In normal expression, binding of Wnts to their respective frizzled receptors inhibits the destruction complex, which

typically phosphorylates β -catenin and causes its degradation. β -catenin, is able to accumulate in the cell and translocate into the nucleus to trigger gene expression through various transcription factors and co-factors (Okerlund & Cheyette, 2011). Two active forms of β -catenin, P-S552 and Non-P β -catenin (S33/S37/T41) were found to be dysregulated in the COX-2^{-/-} males (Rai-Bhagal *et al.*, 2018a). The S552 phosphorylation site of β -catenin is phosphorylated by PKA, the downstream regulatory kinase of PGE₂ (Taurin *et al.*, 2006; Taurin *et al.*, 2008). Activation at this site has been previously found to increase transcriptional activity of the canonical Wnt pathway (Taurin *et al.*, 2006; Zhao *et al.*, 2010). This suggests that the increase of PKA-phosphorylated S552 β -catenin in the COX-2^{-/-} model, likely causes differential expression of the Wnt pathway genes that were dysregulated, via COX/PGE₂ signalling. Modulation of P-S552 β -catenin activity in brain tissue was also found in maternally-exposed PGE₂ mouse model (Rai-Bhagal *et al.*, 2018b). The current study strengthens our previous findings for the importance of the collaboration between the COX-2/PGE₂ and Wnt pathways in early neuronal development and that the abnormal communication between these pathways may contribute to the pathology of ASDs.

Recent studies are providing an abundance of evidence to show that there is an overlap in genes and pathways between ASD and similar neurological disorders through genome-wide studies (Cukier *et al.*, 2014; Lotan *et al.*, 2014; Glessner *et al.*, 2017; Hawi *et al.*, 2018). Differentially expressed genes from the current study were also found to be involved in the neurological disorders, Intellectual Disability (ID), Alzheimer's Disease (AD), and Schizophrenia (SZ) as well. As these conditions have a tremendous overlap of genes and incidence of comorbidity with ASD, they will be discussed below.

Intellectual disability is defined as deficits in intellectual functioning ($IQ < 70$) and adaptive skills and behaviours. The rate of co-morbidity of ID in individuals with ASD was reported to be 55.2%, further determined to be 48% prevalent in males and a high rate of 78.4% in females (Charman *et al.*, 2011). In infancy at the age of 3 months old, an association was found between head circumference and an autism diagnosis, but only when the individuals also displayed co-morbidity with an ID condition (McKeague *et al.*, 2015). Symptoms of ASD – such as poor communication, rigid repetitive behaviours, and self-injurious mannerisms – occurred more often in individuals that presented both ASD and ID (Matson & Shoemaker, 2009). Recent research has identified that at least 50% of the occurrence of an ASD condition co-morbid with ID is due to genetic factors (Balasubramanian *et al.*, 2009), particularly de novo mutations in ASD-risk genes (Jensen *et al.*, 2020). There is a considerable overlap in genetics and behaviours between ASD and ID, and perhaps an increase in severe ASD behaviours as a result of ID. We can theorize that disruptions in the COX/PGE₂ pathway may result in the manifestation of ASD and ID symptoms, which would present as a lower-functioning form of ASD.

The COX enzymes also play a key pathophysiological role in the development of Alzheimer's Disease (AD) (Hoozemans *et al.*, 2001; Hoozemans *et al.*, 2008; Guan *et al.*, 2019a; Guan & Wang, 2019). Increased expression levels of either COX enzyme may be involved in the formation of β -amyloid protein plaques, which is a defining characteristic of AD (Qin *et al.*, 2003; Hoozemans *et al.*, 2008; Dargahi *et al.*, 2011). Interestingly, increased levels of the β -amyloid precursor protein have also been found in children with ASD (Sokol *et al.*, 2006; Bailey *et al.*, 2008; Ray *et al.*, 2011; Wegiel *et al.*, 2012). Recent evidence shows alleviation of some ASD symptoms in mouse models of autism by reducing the Tau protein, which is also involved in the pathology of AD (Tai *et al.*, 2020). Furthermore, there was a significant overlap of

mutated genes found in human brains affected with AD that are also involved in ASD (Ivashko-Pachima *et al.*, 2019). One such example is the Neurotrophin Receptor P75 (p75NTR) protein which is involved in the pathogenesis of AD (Zeng *et al.*, 2011), as well as abnormalities of the cerebellum in ASD (Lotta *et al.*, 2014). The connections between ASD and AD are interesting as the two diseases occur in completely different age groups. However, it signifies the importance of the temporal expression of genes during critical brain events.

The current study also revealed a large number of dysregulated genes that are implicated in SZ, and genes that overlap both ASD and SZ. Many decades ago, autism was known as Childhood Schizophrenia until it was eventually defined as a separate disorder. These two conditions share many similar symptoms and difficult behaviours, with hallucinations being a unique hallmark symptom of SZ that allowed for easier designation. However, recent research is shedding light on the fact that ASD and SZ may occur simultaneously as co-morbid conditions. A meta-analysis of multiple studies indicated that individuals with ASD were 3.6 times more likely to also be diagnosed with SZ (Zheng *et al.*, 2018). Genome-wide studies of brain samples from individuals with ASD and SZ reveal many genes and functional pathways dysregulated in both disorders (Foss-Feig *et al.*, 2017; Gandal *et al.*, 2018). Various components of the COX/PGE₂ pathway have previously also been found in individuals with SZ (Yokota *et al.*, 2004; Muller & Schwarz, 2008; Muller *et al.*, 2012; Tang *et al.*, 2012). It is theorized that different isoforms and splice variants of genes may be one factor that determines the pathological outcome. Another factor would be the effect of defective genes on different cell types which would lead to different outcomes, depending on the cell type that was affected. Furthermore, temporal expression of genes is critical during development of the brain. Environmental events

that disrupt the proper regulation of a gene at different time points may result in unpredictable cascades of events, with a distinct set of symptoms.

Since ASD is a neurodevelopmental disorder which is present before birth, scrutinizing prenatal brain development stages is important. To our knowledge, this is the first *in vivo* study in which the effect of gene defects in COX enzymes was investigated at prenatal stages during brain development. Genome-wide studies are important in decoding multifactorial disorders, by providing a complete list of genes that are abnormally expressed. With a large number of genes associated with ASD and yet so little known about its etiology, functional annotation tools allow us to study the biological pathways that might contribute to the pathology of ASD. Due to the dysregulation of many important biological pathways and ASD associated genes in the COX-1^{-/-} or -2^{-/-} mice, the COX/PGE₂ pathway can be considered a crucial component in prenatal brain development. This could be relevant during pregnancy, as many environmental risk factors such as the drugs acetylsalicylic acid or acetaminophen can influence COX enzymes and PGE₂ levels (Ferrari *et al.*, 1990; Basselin *et al.*, 2011; Mazaud-Guittot *et al.*, 2013; Blanchard *et al.*, 2015), which may consequently affect the developing brain (Good, 2009; Gray, 2014; Philippot *et al.*, 2017). In this study, we have indicated that both COX models revealed an interaction with pathways strongly implicated in ASD, such as lipid and synaptic pathways, and the Wnt pathway only in COX-2^{-/-}. Along with the canonical pathway, genes in the PCP and Wnt/Ca²⁺ pathways were affected as well. The interaction between the COX-PGE₂ and the non-canonical Wnt pathways still needs to be investigated further. Identifying biological pathways in which a number of autism candidate genes are involved may be an effective way to get insight into the underlying molecular basis of ASDs. Based on the current data, we propose that mice with deficits in COX enzymes can be considered as potential models for studying ASD.

7.3 Study 3 – Global Microarray Analysis in the Cyclooxygenase-2 Female Knockout Mice

The current study investigated the effects of COX-2/PGE₂ signalling in the female mouse brain at embryonic stages E16 and E19, through gene expression profiling, as a complement to the previous study in males. Although the widely accepted male to female ratio in ASD is 4:1, current research is suggesting that the ratio may be close to 3:1 (Loomes *et al.*, 2017). Due to the male to female ratio in ASD, a larger number of studies are conducted in males. However, research studies in females has gained tremendous interest, as sex differences have become more apparent in the etiology of many diseases/disorders, as well as reaction to drug therapies.

The microarray data revealed a great number of genes that were differentially expressed in both developmental stages of E16 and E19 in the female COX-2^{-/-} mouse brain. An interesting observation was that at the E16 stage, more than half the differentially expressed genes were downregulated, whereas at E19, the majority of differentially expressed genes had upregulated expression. In fact, a considerable number of genes that were differentially expressed at both developmental stages, switched their expression from downregulation at E16 to upregulation at the later E19 stage. There may be a few reasons for such an occurrence: 1) it is possible that at different time-points, the demand for COX-2 activity and the subsequent genes changes substantially, and their expression (up- or down-regulation) is reflective of this need; or 2) the brain may be exerting a protective effect in the female brain by attempting to increase the expression of downregulated genes, and in turn overcompensating for the low expression at the earlier stage. It is noteworthy that this “switch” in gene expression was also observed in study 1 (Chapter 3) of the current thesis in the maternal PGE₂-exposed mice at the same developmental stages, but in both sexes (Rai-Bhogal *et al.*, 2018b). Due to distinct molecular events occurring at

those times, there may be many genes regulated by the COX-2 pathway that turn on/off, or change their expression to accommodate the current developmental needs.

Next, the genes were scrutinized for functional pathways and biological processes that using stringent analysis parameters, as described in study 2 (Chapter 4). These was a greater number of pathways/clusters of genes affected at E16 compared to E19 in the COX-2^{-/-} female mouse brain. As this is critical developmental timing during which a massive event of neurogenesis occurs in many brain regions, dysregulation of synaptic function, lipid synthesis and arachidonic acid metabolism would be injurious to the brain. All these factors have been implicated in the etiology of ASD (Tamiji & Crawford, 2010b; Bourgeron, 2015; Wong *et al.*, 2015a; Guang *et al.*, 2018), and extensively discussed in previous chapters. Further investigation also revealed a total of six ASD-risk genes that were differentially expressed in the COX-2^{-/-} female mouse brain: *Cdh10*, *Glo1*, *Gabrb1*, *Gabra2*, *Slc6a8*, and *Camk2a*. The latter 4 out of 6 ASD-related genes are implicated in neurotransmitter or synaptic functions, and will be further discussed.

COX-2 is well-known to play a significant role in regulation of the inhibitory GABA system (Kim & Alger, 2004; Toscano *et al.*, 2007; Zhang *et al.*, 2008), and differential expression of *Gabrb1* and *Gabra2* genes in the female COX-2^{-/-} mouse model provides further evidence towards their interaction. *Gabrb1* was differentially expressed at the E16 stage, whereas *Gabra2* was differentially expressed at both stages, indicating a longer period of abnormal regulation. *Gabrb1* and *Gabra2* encode subunits of the GABA-A receptor complex, which bind the major neurotransmitter in the brain, GABA. The GABAergic system is involved in inhibition of neurotransmission, and is a fundamental operation in the brain that regulates excitation and inhibition. However, during prenatal brain development, GABA-A receptors are

actually involved in excitation of immature neurons to form critical synaptic connections, which switch to an inhibitory role in the postnatal brain (Ben-Ari, 2002). It is interesting to note that, faulty processes of the GABA receptors have been heavily implicated in both animal (Tyzio *et al.*, 2014; Hou *et al.*, 2018; Zhu *et al.*, 2019a) and human studies of ASD (Ma *et al.*, 2005; Collins *et al.*, 2006; Fatemi *et al.*, 2009c; Oblak *et al.*, 2010; Robertson *et al.*, 2016). In fact, a single event of maternal infection can delay the GABA switch that initiates its inhibitory role, and lead to an abnormal excitation-inhibition ratio (Corradini *et al.*, 2018). In addition, a greater number of ASD genes and their downstream targets were found to be dysregulated in inhibitory neurons (Wang *et al.*, 2018). It is important that 2 of the 6 ASD genes found in the current study are involved in the regulation of the GABAergic system. As a future direction, it would be interesting to test *Gabra2* in the postnatal brain of COX-2^{-/-} mice to investigate if the expression of this gene continues to be abnormal. It would also be interesting to investigate the interaction mechanism between COX-2 and *Gabra2*, and furthermore whether modified *Gabra2* expression affects the GABA switch during early brain development.

Two other ASD genes found in this study were *Slc6a8* and *Camk2a* – both involved in neurotransmitter functions. The gene *Slc6a8* encodes the Slc6a8 transporter protein responsible for the transportation of creatine, which plays a considerable role in major processes such as neurotransmitter production, and recycling (uptake and release) (Allen, 2012). *Slc6a8* is an X-linked gene, and abnormal expression has been shown to cause cognitive defects in learning, memory, and language impairments (Salomons *et al.*, 2003; Udobi *et al.*, 2018). *Camk2a* is also involved in learning and memory, synaptic transmission and plasticity, and formation of dendritic spines (Stephenson *et al.*, 2017; Akita *et al.*, 2018). In addition, gene knockout systems of *Camk2a* have confirmed specific ASD-related behaviours such as abnormal social

interactions, repetitive behaviours, hyperactivity, and anxiety. Thus, the ASD-related genes found to be dysregulated in this study could cause substantial injury to the brain. Abnormalities of their cohesive functions in dendritic and synaptic development during the formation of the brain can lead to brain pathology and manifestation of neurological disorders such as ASD.

Other interesting processes that were affected at both E16 and E19 stages were translation and acetylation, which work sequentially during development. Translation is a process which encompasses coding and synthesis of proteins, followed by acetylation which occurs during post-translational modification to add acetyl groups for regulation of protein function. Tightly regulated protein translation is key to proper functioning of the brain, and more specifically found to be important for axon growth and synaptic plasticity (Glock *et al.*, 2017; Van Driesche & Martin, 2018). The impact that dysregulated translation can have is extremely complex and variable, and thus has been implicated in various neurodevelopmental disorders, including ASD (Contestabile & Sintoni, 2013; Chen *et al.*, 2019).

In conclusion, this study has successfully illustrated the impact of the COX-2^{-/-} gene mutation on the prenatal development of the female mouse brain. The genes and networks that were identified strongly suggest an impairment in development and functioning of the synaptic structure, which is a major contributor to many neurological disorders, including ASD. It was striking that 4 of the 6 ASD-related dysregulated genes were involved in some function of synaptic development. This study also provides data on prenatal genes that were abnormally expressed, but may return back to normal levels following birth. Such unique and novel data may provide insight as to what is happening at the genetic/molecular level during brain development, which leads to consequences of abhorrent synaptic function in the postnatal brain. In future directions, it is essential to further investigate COX-2 deficiency in postnatal mouse brain to

understand which genes of the synaptic network are affected compared to the prenatal stage. This study also provided genome-wide analysis in the female brain to complement the predecessor study in their male counterparts. Some sex-specific comparisons will be discussed below. Thus, it is feasible to conclude that abnormalities in COX-2, particularly during neurodevelopment can cause synaptic impairment characteristic of ASD.

7.3.1 Comparisons between Global Microarray Analyses in the COX-2^{-/-} Male and Female Mouse Brain

First, the number of genes that were differentially expressed at E16 (peak of neurogenesis) and E19 (end of neurogenesis) were identified. In the males, there was a greater number of differentially expressed genes at the earlier E16 stage, whereas in females, the number of differentially expressed genes was similar at both stages. Next, we compared the ratio of overlapping genes that are differentially regulated at both developmental stages, between males and females. In the male COX-2^{-/-} brain, the expression of 22.0% (85/386) of affected genes were dysregulated at both time-points, whereas in the female COX-2^{-/-} brain, it was 41.2% (139/337). This suggests that there may be greater risk of prolonged abnormal expression of critical genes in COX-2^{-/-} females. In addition, only in females, most downregulated genes at E16, switched their expression to upregulated by E19. Although the exact mechanisms behind this expression pattern are unclear, it can be postulated that the mechanisms by which a COX-2 deficit affects the prenatal brain, is fundamentally different between sexes.

Next, we can compare the involvement of COX-2 in ASD, by investigation of ASD-risk genes and functional pathways found in both male and female COX-2^{-/-} mice. There were 44 ASD-risk genes differentially expressed in the males, and 6 in the females. This may possibly signify a greater risk or severity of ASD pathology in the male brain. One ASD-risk gene, *Glo1*,

was of particular interest, as it was differentially expressed in both male and female COX-2^{-/-} models, but opposite regulation patterns. Interestingly, parallel studies from our lab validated differential expression of *Glo1* at P8 in both sexes as well (Wong *et al.*, 2019). *Glo1* dysregulation has shown to correlate with anxiety in mice (Hovatta *et al.*, 2005), and hence may contribute to the anxiety-like ASD behaviours observed in both male and female COX-2^{-/-} mouse models (Wong *et al.*, 2019). In addition, its dysregulation in the brain from prenatal to postnatal stages (E16 to P8) warrants further investigation as a potential marker in screening tools.

Furthermore, protein network analysis of the subset of ASD-risk genes from both males and females revealed involvement in development/functions of the dendrite and synapse, well-known factor in the outcome of ASD (Phillips & Pozzo-Miller, 2015; Martinez-Cerdeno, 2017). This finding is significant, as it suggests that despite different genes being differentially expressed in either sex, the deficit in functional outcome is similar in both. This is likely due to the localization of COX-2 in dendrites (Kaufmann *et al.*, 1996), and therefore its dysregulation can lead to various dendritic and synaptic abnormalities. Therefore, COX-2-deficiency can act on different genes and pathways in the brain between males and females, with a possibility of similar functional outcomes. Some concepts of plausible mechanisms behind sex differences in brain development will be discussed.

During prenatal development of the mouse brain, different levels of hormones and subsequent gene expression between males and females is critical to the development of sexually dimorphic regions of the mouse brain (Simerly, 1998). At embryonic day 18.5 (E18.5), typical gene expression levels of androgen-controlled hormones such as gonadotropin-release hormone, luteinizing hormone, and follicle-stimulating hormone were found to be considerably higher in the female pituitary compared to the males. Inhibition of these hormones resulted in eradication

of sex differences (Kreisman *et al.*, 2017). Interestingly, androgens also regulate COX-2 expression (Zuloaga *et al.*, 2012), deficiency of which may lead to unfavourable sex-related changes in the brain. Therefore, normal expression of COX-2 may be important during late prenatal development, particularly in females.

There is an abundance in expression of estradiol and its receptors in the developing brain. Estradiol has been linked to growth in dendrites (Lorenzo *et al.*, 1992) and axons (Blanco *et al.*, 1990; Diaz *et al.*, 1992; Cambiasso *et al.*, 1995), plays a role in dendritic spine formation and initiation of increase in glutamatergic activity, through action of the COX-2 pathway – specifically through the production of PGE₂. In turn, COX enzymes are also responsible for synthesis of estradiol, suggesting a feedback mechanism.

Interestingly, the effects of estradiol on neuronal development mirror the effects of lipids and metabolites of linoleic acid. A recent study found sex differences in the developing rat brain as a result of linoleic acid and its downstream lipid metabolites (Hennebelle *et al.*, 2019). Administration of either linoleic acid in males or 13-hydroxyoctadecadienoic acid in females caused an increase in outgrowth of axons in both sexes. Therefore, different molecules of the linoleic acid pathway can lead to the same developmental consequence in the brain of males and females. If only either linoleic acid or its metabolite had been administered to both sexes, it would have presented as having sex-specific consequences. Similarly, although the COX-2 mutation in males or females had contrasting expression patterns, and dysregulation of different genes, it presented deficits in similar functional processes (findings from Chapter 4 and 5) and presented ASD-like behaviours (Wong *et al.*, 2019).

In conclusion, whole genome analyses of COX-2 deficiency in males and females revealed ASD-risk genes and pathways during prenatal brain development. These studies

revealed that a genetic defect can present very unique expression profile of genes and pathways between sexes. It provides additional evidence towards sex-specific development of the brain. Collectively these studies are novel and unique, due to the possible comparison between sexes, particularly during prenatal development.

7.4 Study 4 – Sex Differences in Dendritic Spine Morphology of Cerebellar Neurons in the COX-2^{-/-} Mouse

There is compelling evidence for the importance of COX-2/PGE₂ signalling in brain development, and its involvement in manifestation of ASD. Parallel studies in our lab have confirmed the presentation of ASD-like symptoms in both sexes of the COX-2^{-/-} mouse (Wong *et al.*, 2019). In addition, genome-wide studies of the COX-2^{-/-} mouse models revealed dysregulation of many critical development and ASD-risk genes, as well as affected processes of dendritic spine development and morphogenesis, in addition to synaptic functions (Chapters 4 & 5) (Rai-Bhogan *et al.*, 2018a). Thus, in the current study, we investigated a deficit of COX-2 on the expression of genes that play a role in dendritic spine development.

The time-points of E19 and P25 were chosen considering the progression of spine development. The E19 stage is inclusive of the period during which dendrites and dendritic spines are beginning to form, and by P25, spine morphology and numbers are well developed. The P25 stage also marks completion of neurogenesis in the mouse cerebellum (Rodier, 1980; Koleske, 2013). Furthermore, P25 follows the second postnatal week which is particularly vulnerable to unfavourable changes in the mouse cerebellum as a result of abnormal PGE₂ levels (Dean *et al.*, 2012a).

First, the mRNA expression of *Spn* and *β-actin* – genes involved in regulation of dendritic spine function and organization – was quantified in mouse brain of WT males and

females at developmental stages of E19 and P25. Normal expression of *Spn* was higher at the postnatal stage in both sexes compared to E19, with a slightly higher level in females. Similarly, previous research in rat brain has validated the increasing expression of *Spn* throughout development from prenatal to postnatal stages (Allen *et al.*, 1997). The temporal increase of *Spn* expression from birth to the first few weeks of postnatal life, is likely due to its involvement in formation of dendritic spines (Koleske, 2013).

Spn is greatly enriched in the neck and head of dendritic spines (Allen *et al.*, 1997), and is particularly present in most dendritic spines of the cerebellum (Ouimet *et al.*, 2004). It plays a significant role in functions such as: managing spine density, regulating glutamatergic synapses, and synaptic plasticity (Feng *et al.*, 2000). Furthermore, *Spn*-deficient mice exhibited an increase in spine density at postnatal day 15.

Spn can bind actin (Sato *et al.*, 1998), which is also highly enriched in dendritic spines (Matus *et al.*, 1982; Kaech *et al.*, 1997; Fischer *et al.*, 1998). In contrast to *Spn* expression, we found higher mRNA expression of *Actb*, the gene encoding β -actin, at E19 compared to P25, in both sexes. Previous research has shown a gradual decline in *Actb* expression from birth to several postnatal stages (Fedoseeva *et al.*, 2014). β -actin is essential for embryonic and fetal survival, as it plays many important roles such as being a critical component of the cytoskeleton, cell growth, migration, and controlling gene expression (Bunnell *et al.*, 2011). Actin also makes up the structure of dendritic spines, and is critically involved in their formation and elimination, regulation of their dynamic structure by allowing change in morphology and motility, plays a role in synaptic plasticity (Fischer *et al.*, 1998), and learning and memory (Basu & Lamprecht, 2018).

Next, the protein expression of Spn and β -actin was quantified in the brains of COX-2^{-/-} males and females. First, it was interesting that the mRNA expression levels of *Spn* and *Actb* did not reflect the expression patterns of the proteins. Similarly, Lee et al (2004) found that changes observed in Spn immunoreactivity did not reflect mRNA expression in either males or females in young rats (Lee *et al.*, 2004). This validates the results found in the current study, and suggests that in abnormal conditions, Spn protein levels may not mirror the expression of mRNA (Lee *et al.*, 2004). Previous studies also confirm increased levels of Spn in the cerebellum following administration of COX-2 inhibitors (Dean *et al.*, 2012a). In contrast, direct administration of PGE₂ to the cerebellum caused a decrease in the levels of Spn protein (Dean *et al.*, 2012a), and reduction in the dendrite length of Purkinje cells in the cerebellum (Hoffman *et al.*, 2016).

Next, we found striking sex-dependent expression of both Spn and β -actin at the P25 stage. The expression level of these proteins was significantly reduced in the males, contrasting a substantial increase in females. This same expression pattern of Spn immunoreactivity between sexes was also reported by Lee et al (2004), following estrogen administration (Lee *et al.*, 2004). Interestingly, the COX pathway is involved in the synthesis of estradiol in the brain, particularly in the cerebellum during early postnatal development (Dean *et al.*, 2012a; Hoffman *et al.*, 2016; Wright *et al.*, 2019). Recent animal studies have shown that abnormal estradiol synthesis in the hippocampus, resulted in loss of synapse and impairments in cognition, in females only (Vierk *et al.*, 2014), implicating COX-2 signalling via estradiol in sex-dependent gene expression in the cerebellum. Administration of COX-2 inhibitors to young rats (aged P14) also caused an increase in dendrite length and number of dendritic spines in Purkinje cells of the cerebellum (Dean *et al.*, 2012a).

In the current study, various parameters of dendritic spines were quantified in the cerebellum of COX-2^{-/-} males and females at P25. We found the max diameter of mushroom spines was larger in COX-2^{-/-} females, compared to COX-2^{-/-} males. Spines were characterized as mushroom-shaped when spine head diameter was two times the size of neck diameter. Mushroom spines are considered mature spines with stable synaptic connections, and retaining shape and morphology (Holtmaat *et al.*, 2005). The increase in head diameter size can affect the size of the postsynaptic density, alter the number of presynaptic vesicles and glutamate receptors, or modify the dynamics of neurotransmitter or Ca²⁺ flux (Bell *et al.*, 2019) – all properties that can lead to abnormalities in synaptic transmission and plasticity (Harris & Kater, 1994). Therefore, increase in max diameter of mushroom spine head can alter signal transmission in Purkinje cells of COX-2^{-/-} females, and lead to cerebellar pathology.

The cerebellum was once known as the part of the brain largely responsible for motor functions, but over the years research studies have confirmed that it also plays a critical role in cognition, social development, and language (Levisohn *et al.*, 2000; Schmahmann, 2019). Studies of cerebellar lesions in children exhibit motor delays, and behavioural challenges in cognitive functions such as various language skills, task planning and organization, social behaviours, and memory (Levisohn *et al.*, 2000; Riva & Giorgi, 2000; Scott *et al.*, 2001; Limperopoulos *et al.*, 2007; Tavano *et al.*, 2007; Bolduc *et al.*, 2011). Furthermore, there was a correlation between the type/location of lesion and severity of deficits, resulting in a spectrum of symptoms between individuals. These studies signify the role of the cerebellum in higher-order cognitive functions.

The cerebellum is one of the structures of the central nervous system that continues to develop postnatally, in both mammals and rodents (Rodier, 1980; Rice & Barone, 2000). In

mice, cerebellar development begins around embryonic days 9-12 (Sgaier *et al.*, 2005), is formed structurally with an onset of immature synaptic connections by birth (embryonic days 19-21), and is completed around postnatal day 16 (Sillitoe & Joyner, 2007). In humans, growth of the cerebellum peaks at the postnatal age of approximately 7 months old (Koop *et al.*, 1986; Rice & Barone, 2000). This opens up the potential for the cerebellum to be vulnerable to insults for a very long developmental period – embryonic to postnatal.

Recent emerging evidence from the human brain suggests that there may be a critically vulnerable period during which various medical conditions that cause inflammation in the body result in increased levels of *Ptgs2* (COX-2) mRNA in the cerebellum (Wright *et al.*, 2019). The experimental age group of deceased children ranged from 0-9 years old. Interestingly, the increase in *Ptgs2* (COX-2) mRNA was seen in ages 1-9 years, but not younger than 1 year old. This concluded that in children with medical conditions, the cerebellum may become vulnerable to inflammation and produce abnormal COX-2 levels after the age of 1 year old (Wright *et al.*, 2019). Coincidentally, many ASD symptoms begin to present themselves approximately between the age of 1 and 2. It is also possible that an infant who has a genetic predisposition may be particularly vulnerable to further insults during cerebellar development.

The pathology of ASD has been well-characterized as a dysfunction of synapses, ranging from abnormalities in long-range synaptic connections, to the tiny protrusions that are dendritic spines (Ebert & Greenberg, 2013; Lin *et al.*, 2014; Sala *et al.*, 2015; Liu *et al.*, 2016c). Abnormalities in structure and function of spines have been associated with many pathological conditions such as Down's syndrome, Angelman's syndrome, schizophrenia, epilepsy, fragile X syndrome, Rett's syndrome, and ASD (Wong, 2005; Glausier & Lewis, 2013; Xu *et al.*, 2014; Phillips & Pozzo-Miller, 2015; Joensuu *et al.*, 2018; Torres *et al.*, 2018; Booker *et al.*, 2019;

Wang *et al.*, 2019). These structures could be impacted by various factors ranging from genetic to environmental, timing of exposure, critical developmental windows in different brain regions, etc. There is still a gap in knowledge in the involvement of the cerebellum in ASD, and in particular the impact caused by dendritic spines on dendrites of Purkinje cells.

In conclusion, the current study presented sex-specific changes in the COX-2^{-/-} mouse brain at P25. There was contrasting expression pattern of Spinophilin and β -actin between males and females, with the latter expressing very high levels of both proteins. In addition, there was an increase in head diameter size of mushroom spines, exclusively in females. First, this change in morphology may modify the strength of signals leading to abnormal synaptic plasticity. Second, the sex-specific changes seen in females complemented the analyses from the genome-wide study in Chapter 5. Some limitations of the study include the methodology that was used to obtain z-stacks and images. They were captured with the intention of providing information on dendritic spine density and morphology only. Currently, a parallel study in the lab has explored other properties of dendrites (unpublished) in the same samples. It would be interesting to investigate earlier postnatal stages while the cerebellum is developing, during which abnormal spine changes may also be present in males.

CHAPTER 8. CONCLUSION

8.1 Key Findings and Future Directions

Over the last few decades, a vast amount of research has been dedicated to understanding the complex etiology of ASD. Many genes and pathways along with environmental factors have been associated with its manifestation. Although some roles of COX/PGE₂ signalling in brain development and diseases have been established, there is still a considerable gap in the understanding of its involvement in ASD. The aim of the studies in this dissertation were to investigate the impact of abnormal COX/PGE₂ levels (high or low) during critical prenatal and postnatal periods of brain development, on gene and protein expression, developmental pathways, sex-specific changes, and implications in autism spectrum disorder (ASD). We investigated these key aims in two established mouse models of ASD in our lab: 1) PGE₂-injected and 2) lacking the PGE₂ producing enzymes (COX-2^{-/-}). Both models have been assessed to have sex-dependent manifestation of typical autism-related symptoms such as impairment in social interaction, motor deficits, anxiety and hyperactive behaviours (Wong *et al.*, 2019). Below, the overall key findings of the studies will be summarized.

1. *Genetic or environmental disruption to the COX/PGE₂ signalling pathway can lead to modification of gene and protein expression during brain development.* Abnormal COX/PGE₂ signalling through one-time maternal PGE₂ administration (environmental) (Chapter 3) or gene-knockout models (genetic) (Chapters 4-6) caused differential expression of critical developmental genes and proteins involved in key functions such as synaptic spine development, synaptic transmission, lipid metabolism, and regulation of Wnt genes, in the prenatal and postnatal mouse brain. Therefore, any perturbations of the pathway,

whether resulting in increased or decreased levels of PGE₂, can lead to harmful changes in gene expression that take part in key biological pathways during prenatal development.

2. *Many ASD-associated genes were differentially expressed in both PGE₂-injected and COX-deficient mouse models.* Many ASD genes that were found in the studies are well associated in clinical populations. A few examples are *Wnt2* (Chapter 3), *Grm5*, *Camk4*, *Nrxn1*, *Nrxn2*, *Nrxn3*, *Gsk3B* (Chapter 4), *Gabrb1*, *Gabra2*, *Camk2a*, and *Glo1* (Chapters 4 and 5) – all previously found in cases of ASD. The COX/PGE₂ signalling pathway cross-talks with various developmental pathways (such as Wnt) and can influence a cascade of events that lead to differential expression of various genes involved in many biological processes. It is clear from our studies that abnormal levels of PGE₂ lead to variable outcomes, as is characteristic of ASD. Therefore, although there are currently hundreds of genes associated with ASD, the expression of many may be linked to similar deficits of initial brain insult.
3. *Maternal PGE₂ exposure can result in different outcomes in gene expression levels between individual pups of the same pregnancy (Chapter 3).* The design of this study was unique, as typically samples are pooled together to generate a standard mean value for each condition. We found that there are indeed differences in gene expression between genetically identical pups following increased PGE₂ levels (environment) during gestation, supporting the role of the environment in the observed differences. Studies show that even identical twins can have distinct differences in epigenetic changes, and these differences are more pronounced in dichorionic twins (Bui *et al.*, 2015). Maternal ethanol exposure also resulted in dissimilar ethanol metabolites in placenta samples of humans and guinea pigs (Gareri *et al.*, 2009). Clinical ASD studies show that identical twins differ in severity of ASD symptoms, particularly social traits. Our study provides evidence that a single environmental exposure

can cause varying levels of differential gene expression between twins in the same pregnancy, and may be related to the degree of severity of behavioural symptoms. In future studies, it would be interesting to test PGE₂ concentration in placenta samples shortly after administration, to validate if there are any variances, as detected in gene expression in the brain.

4. *The COX/PGE₂ signalling pathway is involved in dendrite/synaptic development and functions.* All our studies provide evidence that COX/PGE₂ is involved in synaptic development/functions. Increase in PGE₂ levels and lack of COX enzymes all lead to changes in expression of genes involved in synaptic plasticity, release of neurotransmitters, or dendritic spine development. COX-2-deficiency also caused a sex-specific increase in spine head diameter size in mushroom spines of cerebellar neurons of female mice only (Chapter 6). Altogether, the current studies provide evidence that COX/PGE₂ signalling is involved in normal synaptic functioning during early brain development. Abnormalities in the development and function of synaptic structures have been well researched and confirmed for their involvement in ASD and other neurological disorders. Future studies should focus on scrutinizing other dendritic and synaptic properties in these mouse models. It may also be of great interest to explore other time-points during neurodevelopment as well as analyze dendritic spine morphology in other brain regions using similar methodologies.
5. *An interaction between COX-2/PGE₂ and the Wnt pathway was confirmed in vivo in the prenatal mouse brain, following both increased PGE₂ exposure (Chapter 3) and lack of COX-2 enzyme (Chapter 4).* The interaction was confirmed by the expression of Wnt and Wnt-target genes (Chapters 3 and 4), and irregular expression of β -catenin (Chapter 3) – the key modulator of the Wnt pathway. It is important that both mouse models (genetic and

environmental) used in our studies confirmed an interaction between the two pathways, particularly at the E16 time-point during a period of neurogenesis and development in most brain regions. Not only is regulation of the Wnt pathway critically important during development, its strongly associated with ASD as well. Therefore, atypical COX-2 activity or PGE₂ expression can interfere with Wnts and Wnt targets to cause defects in various functions and lead to abnormal neurodevelopment.

6. *The COX/PGE₂ pathway causes sex-specific changes in the developing brain.* Global analyses between male and female COX-2^{-/-} gene KOs revealed a greater number of downregulated genes and ASD-risk genes in the males. In females, a majority of the downregulated genes at E16 switched to upregulation by the E19 stage. Therefore, it is evident that the regulation of developmental genes by COX/PGE₂ is sex-specific.

Interestingly, the ASD genes found in both males and females reveal their involvement in synaptic functions. Therefore, as previously suggested by other studies, COX/PGE₂ leads to abnormalities in synaptic functions in both sexes, although the mechanism and severity of deficits are likely different. COX-2^{-/-} females also exhibited dendritic spine changes, accompanied by abnormal SPN and β -actin expression at P25. Future studies should investigate other postnatal time-points, because it is probable that there may be changes in the male brain at a different time-point. As the male and female brain is so different, particularly during development, there is a fundamental need to continue to study males and females in ASD and other neurological disorders.

7. *Long-term gene expression spanning from prenatal to postnatal brain development of highly associated ASD-risk genes such as *Glo1* (Chapters 4 and 5) and *Gabra2* (Chapter 5) were identified, and warrant further investigation as potential candidate markers for ASD*

diagnostic tools. Genes that are abnormally expressed for a long developmental period may provide some insight into what malformation may have occurred prenatally. Furthermore, such studies add to a repertoire of genes that can be characterized in future studies to be used possibly for early screening and diagnostic tools, and therapeutic research. Currently, although children can be diagnosed as early as age 2, most don't get a diagnosis till they are 4-5 years old on average. Therefore, early genetic testing accompanied with initial ASD-like behaviours can lead to critical early intervention strategies.

8.2 Study Limitations

Some limitations of the collection of studies in this dissertation will be outlined. In study 1, although placentas were initially collected for analysis, we were unable to properly extract DNA. It may be worth while to conduct genetic analysis and investigate the amounts of PGE₂ in placentas from all pups in one pregnancy. In studies 2-4, we used mouse model KO's for COX enzymes that are responsible for prostanoid production. Although sufficient evidence shows that PGE₂ is the major prostanoid expressed in the brain, and many studies support brain PGE₂ upregulation by COX enzymes, effects of other downstream metabolites cannot be completely ruled out. In study 4, all collected brain samples from the E19 developmental stage were damaged by a tissue processing facility and could not be used for dendritic spine analysis, leaving only the P25 stage. Another time-point for comparison of how dendritic spines change over time would have been an important addition to the study. Furthermore, the dendrite image Z-stacks were taken in 0.15µm increments. To increase the accuracy of measurements, the slides were re-imaged with increased number of Z-stacks, and various parameters were analyzed by another student.

8.3 Final Conclusions

Thus, through maternal PGE₂ exposure and COX-deficient mouse models, we provided convincing evidence for the important role that COX/PGE₂ signalling plays in prenatal development, and its potential involvement in neurodevelopmental disorders such as ASD. These studies are significant for the scientific community as they provide further knowledge on the potential effects of irregular COX/PGE₂ exposure during pregnancy. As one of the major functions of COX/PGE₂ is in inflammation, activation of the immune system during sensitive embryonic developmental periods could have lasting effects on the brain. PGE₂ levels can also increase as a result of various environmental factors (as discussed in Chapter 1) and cross the blood-brain barrier, allowing it to interfere with various pathways and act on many genes and functions. Many future studies can emerge from our findings, such as further exploration of the molecular mechanisms and interactions of COX/PGE₂, its involvement in abnormal development of synaptic structures and functions, and observation at different time-points to identify whether there are associations between timing of exposure and consequence.

REFERENCES

- Aavani, T., Rana, S.A., Hawkes, R. & Pittman, Q.J. (2015) Maternal immune activation produces cerebellar hyperplasia and alterations in motor and social behaviors in male and female mice. *Cerebellum*, **14**, 491-505.
- Abdallah, M.W., Pearce, B.D., Larsen, N., Greaves-Lord, K., Norgaard-Pedersen, B., Hougaard, D.M., Mortensen, E.L. & Grove, J. (2012) Amniotic fluid MMP-9 and neurotrophins in autism spectrum disorders: an exploratory study. *Autism Res*, **5**, 428-433.
- Abramovitz, M., Adam, M., Boie, Y., Carriere, M., Denis, D., Godbout, C., Lamontagne, S., Rochette, C., Sawyer, N., Tremblay, N.M., Belley, M., Gallant, M., Dufresne, C., Gareau, Y., Ruel, R., Juteau, H., Labelle, M., Ouimet, N. & Metters, K.M. (2000) The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochimica et biophysica acta*, **1483**, 285-293.
- Adams, J., Collaco-Moraes, Y. & de Belleruche, J. (1996) Cyclooxygenase-2 induction in cerebral cortex: an intracellular response to synaptic excitation. *Journal of neurochemistry*, **66**, 6-13.
- Adrian, M., Kusters, R., Storm, C., Hoogenraad, C.C. & Kapitein, L.C. (2017) Probing the Interplay between Dendritic Spine Morphology and Membrane-Bound Diffusion. *Biophysical journal*, **113**, 2261-2270.
- Aerts, R., Van Overmeire, I., Colles, A., Andjelkovic, M., Malarvannan, G., Poma, G., Den Hond, E., Van de Mieroop, E., Dewolf, M.C., Charlet, F., Van Nieuwenhuyse, A., Van Loco, J. & Covaci, A. (2019) Determinants of persistent organic pollutant (POP) concentrations in human breast milk of a cross-sectional sample of primiparous mothers in Belgium. *Environment international*, **131**, 104979.
- Agay-Shay, K., Martinez, D., Valvi, D., Garcia-Esteban, R., Basagana, X., Robinson, O., Casas, M., Sunyer, J. & Vrijheid, M. (2015) Exposure to Endocrine-Disrupting Chemicals during Pregnancy and Weight at 7 Years of Age: A Multi-pollutant Approach. *Environmental health perspectives*, **123**, 1030-1037.
- Akita, T., Aoto, K., Kato, M., Shiina, M., Mutoh, H., Nakashima, M., Kuki, I., Okazaki, S., Magara, S., Shiihara, T., Yokochi, K., Aiba, K., Tohyama, J., Ohba, C., Miyatake, S., Miyake, N., Ogata, K., Fukuda, A., Matsumoto, N. & Saitsu, H. (2018) De novo variants in CAMK2A and CAMK2B cause neurodevelopmental disorders. *Annals of clinical and translational neurology*, **5**, 280-296.

- Al-Absi, A.R., Christensen, H.S., Sanchez, C. & Nyengaard, J.R. (2018) Evaluation of semi-automatic 3D reconstruction for studying geometry of dendritic spines. *Journal of chemical neuroanatomy*, **94**, 119-124.
- Alfawaz, H., Al-Onazi, M., Bukhari, S.I., Binobead, M., Othman, N., Algahtani, N., Bhat, R.S., Moubayed, N.M.S., Alzeer, H.S. & El-Ansary, A. (2018) The Independent and Combined Effects of Omega-3 and Vitamin B12 in Ameliorating Propionic Acid Induced Biochemical Features in Juvenile Rats as Rodent Model of Autism. *Journal of molecular neuroscience : MN*, **66**, 403-413.
- Alix, E., Schmitt, C., Strazielle, N. & Gherzi-Egea, J.F. (2008) Prostaglandin E2 metabolism in rat brain: Role of the blood-brain interfaces. *Cerebrospinal fluid research*, **5**, 5.
- Allen, P.B., Ouimet, C.C. & Greengard, P. (1997) Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9956-9961.
- Allen, P.J. (2012) Creatine metabolism and psychiatric disorders: Does creatine supplementation have therapeutic value? *Neuroscience and biobehavioral reviews*, **36**, 1442-1462.
- Amateau, S.K. & McCarthy, M.M. (2002) A novel mechanism of dendritic spine plasticity involving estradiol induction of prostaglandin-E2. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **22**, 8586-8596.
- Amateau, S.K. & McCarthy, M.M. (2004) Induction of PGE2 by estradiol mediates developmental masculinization of sex behavior. *Nature neuroscience*, **7**, 643-650.
- Amodeo, D.A., Pahua, A.E., Zarate, M., Taylor, J.A., Peterson, S., Posadas, R., Oliver, B.L. & Amodeo, L.R. (2019) Differences in the expression of restricted repetitive behaviors in female and male BTBR T+tf/J mice. *Behavioural brain research*, **372**, 112028.
- An, J.Y., Cristino, A.S., Zhao, Q., Edson, J., Williams, S.M., Ravine, D., Wray, J., Marshall, V.M., Hunt, A., Whitehouse, A.J. & Claudianos, C. (2014) Towards a molecular characterization of autism spectrum disorders: an exome sequencing and systems approach. *Translational psychiatry*, **4**, e394.
- Andersson, T., Duckworth, J.K., Fritz, N., Lewicka, M., Sodersten, E., Uhlen, P. & Hermanson, O. (2011) Noggin and Wnt3a enable BMP4-dependent differentiation of telencephalic stem cells into GluR-agonist responsive neurons. *Molecular and cellular neurosciences*, **47**, 10-18.

- Andrade, C. (2016) Use of acetaminophen (paracetamol) during pregnancy and the risk of autism spectrum disorder in the offspring. *The Journal of clinical psychiatry*, **77**, e152-154.
- Anney, R.J., Kenny, E.M., O'Dushlaine, C., Yaspan, B.L., Parkhomenka, E., Buxbaum, J.D., Sutcliffe, J., Gill, M., Gallagher, L., Autism Genome, P., Buxbaum, J.D., Sutcliffe, J., Gill, M. & Gallagher, L. (2011) Gene-ontology enrichment analysis in two independent family-based samples highlights biologically plausible processes for autism spectrum disorders. *European journal of human genetics : EJHG*, **19**, 1082-1089.
- Anthony, D.C., Ferguson, B., Matyzak, M.K., Miller, K.M., Esiri, M.M. & Perry, V.H. (1997) Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. *Neuropathology and applied neurobiology*, **23**, 406-415.
- Arava, Y., Wang, Y., Storey, J.D., Liu, C.L., Brown, P.O. & Herschlag, D. (2003) Genome-wide analysis of mRNA translation profiles in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 3889-3894.
- Araya, R., Jiang, J., Eisenthal, K.B. & Yuste, R. (2006) The spine neck filters membrane potentials. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 17961-17966.
- Ardinger, H.H., Atkin, J.F., Blackston, R.D., Elsas, L.J., Clarren, S.K., Livingstone, S., Flannery, D.B., Pellock, J.M., Harrod, M.J., Lammer, E.J. & et al. (1988) Verification of the fetal valproate syndrome phenotype. *Am J Med Genet*, **29**, 171-185.
- Arellano, J.I., Benavides-Piccione, R., Defelipe, J. & Yuste, R. (2007) Ultrastructure of dendritic spines: correlation between synaptic and spine morphologies. *Frontiers in neuroscience*, **1**, 131-143.
- Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J.B., Nusbaum, J.D., Corcoran, D.L., Langlois, C., Munschauer, M., Dewell, S., Hafner, M., Williams, Z., Ohler, U. & Tuschl, T. (2012) FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature*, **492**, 382-386.
- Atladdottir, H.O., Henriksen, T.B., Schendel, D.E. & Parner, E.T. (2012) Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics*, **130**, e1447-1454.
- Atladdottir, H.O., Thorsen, P., Ostergaard, L., Schendel, D.E., Lemcke, S., Abdallah, M. & Parner, E.T. (2010) Maternal infection requiring hospitalization during pregnancy and

- autism spectrum disorders. *Journal of autism and developmental disorders*, **40**, 1423-1430.
- Avella-Garcia, C.B., Julvez, J., Fortuny, J., Rebordosa, C., Garcia-Esteban, R., Galan, I.R., Tardon, A., Rodriguez-Bernal, C.L., Iniguez, C., Andiaarena, A., Santa-Marina, L. & Sunyer, J. (2016) Acetaminophen use in pregnancy and neurodevelopment: attention function and autism spectrum symptoms. *International journal of epidemiology*, **45**, 1987-1996.
- Avila, M.E., Sepulveda, F.J., Burgos, C.F., Moraga-Cid, G., Parodi, J., Moon, R.T., Aguayo, L.G., Opazo, C. & De Ferrari, G.V. (2010) Canonical Wnt3a modulates intracellular calcium and enhances excitatory neurotransmission in hippocampal neurons. *The Journal of biological chemistry*, **285**, 18939-18947.
- Badner, J.A. & Gershon, E.S. (2002) Regional meta-analysis of published data supports linkage of autism with markers on chromosome 7. *Molecular psychiatry*, **7**, 56-66.
- Bae, S.M. & Hong, J.Y. (2018) The Wnt Signaling Pathway and Related Therapeutic Drugs in Autism Spectrum Disorder. *Clinical psychopharmacology and neuroscience : the official scientific journal of the Korean College of Neuropsychopharmacology*, **16**, 129-135.
- Bagni, C. & Zukin, R.S. (2019) A Synaptic Perspective of Fragile X Syndrome and Autism Spectrum Disorders. *Neuron*, **101**, 1070-1088.
- Bailey, A.R., Giunta, B.N., Obregon, D., Nikolic, W.V., Tian, J., Sanberg, C.D., Sutton, D.T. & Tan, J. (2008) Peripheral biomarkers in Autism: secreted amyloid precursor protein-alpha as a probable key player in early diagnosis. *International journal of clinical and experimental medicine*, **1**, 338-344.
- Balasubramanian, B., C, V.B. & N, A.G. (2009) Genetic studies in children with intellectual disability and autistic spectrum of disorders. *Indian journal of human genetics*, **15**, 103-107.
- Bandim, J.M., Ventura, L.O., Miller, M.T., Almeida, H.C. & Costa, A.E. (2003) Autism and Mobius sequence: an exploratory study of children in northeastern Brazil. *Arquivos de neuro-psiquiatria*, **61**, 181-185.
- Baronio, D., Bauer-Negrini, G., Castro, K., Della-Flora Nunes, G., Riesgo, R., Mendes-da-Cruz, D.A., Savino, W., Gottfried, C. & Bambini-Junior, V. (2018) Reduced CD4 T Lymphocytes in Lymph Nodes of the Mouse Model of Autism Induced by Valproic Acid. *Neuroimmunomodulation*, **25**, 280-284.

- Basselin, M., Ramadan, E., Chen, M. & Rapoport, S.I. (2011) Anti-inflammatory effects of chronic aspirin on brain arachidonic acid metabolites. *Neurochemical research*, **36**, 139-145.
- Bassell, G.J. & Warren, S.T. (2008) Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. *Neuron*, **60**, 201-214.
- Basu, S. & Lamprecht, R. (2018) The Role of Actin Cytoskeleton in Dendritic Spines in the Maintenance of Long-Term Memory. *Frontiers in molecular neuroscience*, **11**, 143.
- Basu, S.N., Kollu, R. & Banerjee-Basu, S. (2009) AutDB: a gene reference resource for autism research. *Nucleic Acids Res*, **37**, D832-836.
- Bauer, A.Z., Kriebel, D., Herbert, M.R., Bornehag, C.G. & Swan, S.H. (2018) Prenatal paracetamol exposure and child neurodevelopment: A review. *Hormones and behavior*, **101**, 125-147.
- Baumgart, D.C. & Carding, S.R. (2007) Inflammatory bowel disease: cause and immunobiology. *Lancet*, **369**, 1627-1640.
- Bell, J.G., MacKinlay, E.E., Dick, J.R., MacDonald, D.J., Boyle, R.M. & Glen, A.C. (2004) Essential fatty acids and phospholipase A2 in autistic spectrum disorders. *Prostaglandins, leukotrienes, and essential fatty acids*, **71**, 201-204.
- Bell, J.G., Miller, D., MacDonald, D.J., MacKinlay, E.E., Dick, J.R., Cheseldine, S., Boyle, R.M., Graham, C. & O'Hare, A.E. (2010) The fatty acid compositions of erythrocyte and plasma polar lipids in children with autism, developmental delay or typically developing controls and the effect of fish oil intake. *The British journal of nutrition*, **103**, 1160-1167.
- Bell, M., Bartol, T., Sejnowski, T. & Rangamani, P. (2019) Dendritic spine geometry and spine apparatus organization govern the spatiotemporal dynamics of calcium. *The Journal of general physiology*, **151**, 1017-1034.
- Ben-Ari, Y. (2002) Excitatory actions of gaba during development: the nature of the nurture. *Nature reviews*, **3**, 728-739.
- Benn, C.L., Fox, H. & Bates, G.P. (2008) Optimisation of region-specific reference gene selection and relative gene expression analysis methods for pre-clinical trials of Huntington's disease. *Molecular neurodegeneration*, **3**, 17.

- Berkel, S., Marshall, C.R., Weiss, B., Howe, J., Roeth, R., Moog, U., Endris, V., Roberts, W., Szatmari, P., Pinto, D., Bonin, M., Riess, A., Engels, H., Sprengel, R., Scherer, S.W. & Rappold, G.A. (2010) Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nature genetics*, **42**, 489-491.
- Bernis, M.E., Oksdath, M., Dupraz, S., Nieto Guil, A., Fernandez, M.M., Malchiodi, E.L., Rosso, S.B. & Quiroga, S. (2013) Wingless-type family member 3A triggers neuronal polarization via cross-activation of the insulin-like growth factor-1 receptor pathway. *Frontiers in cellular neuroscience*, **7**, 194.
- Bharadwaj, S.K., Vishnu Bhat, B., Vickneswaran, V., Adhisivam, B., Bobby, Z. & Habeebullah, S. (2018) Oxidative Stress, Antioxidant Status and Neurodevelopmental Outcome in Neonates Born to Pre-eclamptic Mothers. *Indian journal of pediatrics*, **85**, 351-357.
- Bilousova, T.V., Rusakov, D.A., Ethell, D.W. & Ethell, I.M. (2006) Matrix metalloproteinase-7 disrupts dendritic spines in hippocampal neurons through NMDA receptor activation. *Journal of neurochemistry*, **97**, 44-56.
- Bittker, S.S. & Bell, K.R. (2018) Acetaminophen, antibiotics, ear infection, breastfeeding, vitamin D drops, and autism: an epidemiological study. *Neuropsychiatric disease and treatment*, **14**, 1399-1414.
- Bittker, S.S. & Bell, K.R. (2020) Postnatal Acetaminophen and Potential Risk of Autism Spectrum Disorder among Males. *Behavioral sciences*, **10**.
- Bjorklund, G., Meguid, N.A., El-Bana, M.A., Tinkov, A.A., Saad, K., Dadar, M., Hemimi, M., Skalny, A.V., Hosnedlova, B., Kizek, R., Osredkar, J., Urbina, M.A., Fabjan, T., El-Houfey, A.A., Kaluzna-Czaplinska, J., Gatarek, P. & Chirumbolo, S. (2020) Oxidative Stress in Autism Spectrum Disorder. *Molecular neurobiology*, **57**, 2314-2332.
- Blanchard, H.C., Taha, A.Y., Rapoport, S.I. & Yuan, Z.X. (2015) Low-dose aspirin (acetylsalicylate) prevents increases in brain PGE2, 15-epi-lipoxin A4 and 8-isoprostane concentrations in 9 month-old HIV-1 transgenic rats, a model for HIV-1 associated neurocognitive disorders. *Prostaglandins, leukotrienes, and essential fatty acids*, **96**, 25-30.
- Blanco, G., Diaz, H., Carrer, H.F. & Beauge, L. (1990) Differentiation of rat hippocampal neurons induced by estrogen in vitro: effects on neuritogenesis and Na, K-ATPase activity. *Journal of neuroscience research*, **27**, 47-54.

- Blue, M.E. & Parnavelas, J.G. (1983) The formation and maturation of synapses in the visual cortex of the rat. II. Quantitative analysis. *Journal of neurocytology*, **12**, 697-712.
- Boccuto, L., Lauri, M., Sarasua, S.M., Skinner, C.D., Buccella, D., Dwivedi, A., Orteschi, D., Collins, J.S., Zollino, M., Visconti, P., Dupont, B., Tiziano, D., Schroer, R.J., Neri, G., Stevenson, R.E., Gurrieri, F. & Schwartz, C.E. (2013) Prevalence of SHANK3 variants in patients with different subtypes of autism spectrum disorders. *European journal of human genetics : EJHG*, **21**, 310-316.
- Boda, E., Pini, A., Hoxha, E., Parolisi, R. & Tempia, F. (2009) Selection of reference genes for quantitative real-time RT-PCR studies in mouse brain. *Journal of molecular neuroscience : MN*, **37**, 238-253.
- Bohm, H.V. & Stewart, M.G. (2009) Brief report: on the concordance percentages for Autistic Spectrum Disorder of twins. *Journal of autism and developmental disorders*, **39**, 806-808.
- Bohm, H.V., Stewart, M.G. & Healy, A.M. (2013) On the Autistic Spectrum Disorder concordance rates of twins and non-twin siblings. *Medical hypotheses*, **81**, 789-791.
- Boie, Y., Stocco, R., Sawyer, N., Slipetz, D.M., Ungrin, M.D., Neuschafer-Rube, F., Puschel, G.P., Metters, K.M. & Abramovitz, M. (1997) Molecular cloning and characterization of the four rat prostaglandin E2 prostanoid receptor subtypes. *European journal of pharmacology*, **340**, 227-241.
- Bolduc, M.E., Du Plessis, A.J., Sullivan, N., Khwaja, O.S., Zhang, X., Barnes, K., Robertson, R.L. & Limperopoulos, C. (2011) Spectrum of neurodevelopmental disabilities in children with cerebellar malformations. *Developmental medicine and child neurology*, **53**, 409-416.
- Bolivar, V.J., Walters, S.R. & Phoenix, J.L. (2007) Assessing autism-like behavior in mice: variations in social interactions among inbred strains. *Behavioural brain research*, **176**, 21-26.
- Booker, S.A., Domanski, A.P.F., Dando, O.R., Jackson, A.D., Isaac, J.T.R., Hardingham, G.E., Wyllie, D.J.A. & Kind, P.C. (2019) Altered dendritic spine function and integration in a mouse model of fragile X syndrome. *Nature communications*, **10**, 4813.
- Borczyk, M., Sliwinska, M.A., Caly, A., Bernas, T. & Radwanska, K. (2019) Neuronal plasticity affects correlation between the size of dendritic spine and its postsynaptic density. *Sci Rep*, **9**, 1693.

- Bouchard, M.F., Chevrier, J., Harley, K.G., Kogut, K., Vedar, M., Calderon, N., Trujillo, C., Johnson, C., Bradman, A., Barr, D.B. & Eskenazi, B. (2011) Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. *Environmental health perspectives*, **119**, 1189-1195.
- Boucher, J., Mayes, A. & Bigham, S. (2012) Memory in autistic spectrum disorder. *Psychological bulletin*, **138**, 458-496.
- Boukhris, T., Sheehy, O., Mottron, L. & Berard, A. (2016) Antidepressant Use During Pregnancy and the Risk of Autism Spectrum Disorder in Children. *JAMA pediatrics*, **170**, 117-124.
- Bourgeron, T. (2015) From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nature reviews*, **16**, 551-563.
- Bozek, K., Wei, Y., Yan, Z., Liu, X., Xiong, J., Sugimoto, M., Tomita, M., Paabo, S., Sherwood, C.C., Hof, P.R., Ely, J.J., Li, Y., Steinhauser, D., Willmitzer, L., Giavalisco, P. & Khaitovich, P. (2015) Organization and evolution of brain lipidome revealed by large-scale analysis of human, chimpanzee, macaque, and mouse tissues. *Neuron*, **85**, 695-702.
- Bradman, A., Barr, D.B., Claus Henn, B.G., Drumheller, T., Curry, C. & Eskenazi, B. (2003) Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study. *Environmental health perspectives*, **111**, 1779-1782.
- Brandlistuen, R.E., Ystrom, E., Nulman, I., Koren, G. & Nordeng, H. (2013) Prenatal paracetamol exposure and child neurodevelopment: a sibling-controlled cohort study. *International journal of epidemiology*, **42**, 1702-1713.
- Braun, J.M., Kalkbrenner, A.E., Calafat, A.M., Yolton, K., Ye, X., Dietrich, K.N. & Lanphear, B.P. (2011) Impact of early-life bisphenol A exposure on behavior and executive function in children. *Pediatrics*, **128**, 873-882.
- Braun, J.M., Yolton, K., Dietrich, K.N., Hornung, R., Ye, X., Calafat, A.M. & Lanphear, B.P. (2009) Prenatal bisphenol A exposure and early childhood behavior. *Environmental health perspectives*, **117**, 1945-1952.
- Brigandi, S.A., Shao, H., Qian, S.Y., Shen, Y., Wu, B.L. & Kang, J.X. (2015) Autistic children exhibit decreased levels of essential Fatty acids in red blood cells. *International journal of molecular sciences*, **16**, 10061-10076.

- Brodkin, E.S. (2007) BALB/c mice: low sociability and other phenotypes that may be relevant to autism. *Behavioural brain research*, **176**, 53-65.
- Brodkin, E.S., Hagemann, A., Nemetski, S.M. & Silver, L.M. (2004) Social approach-avoidance behavior of inbred mouse strains towards DBA/2 mice. *Brain research*, **1002**, 151-157.
- Brown, A.S., Cheslack-Postava, K., Rantakokko, P., Kiviranta, H., Hinkka-Yli-Salomaki, S., McKeague, I.W., Surcel, H.M. & Sourander, A. (2018) Association of Maternal Insecticide Levels With Autism in Offspring From a National Birth Cohort. *The American journal of psychiatry*, **175**, 1094-1101.
- Brueggemeier, R.W., Richards, J.A., Joomprabutra, S., Bhat, A.S. & Whetstone, J.L. (2001) Molecular pharmacology of aromatase and its regulation by endogenous and exogenous agents. *The Journal of steroid biochemistry and molecular biology*, **79**, 75-84.
- Bucan, V., Mandel, K., Bertram, C., Lazaridis, A., Reimers, K., Park-Simon, T.W., Vogt, P.M. & Hass, R. (2012) LEF-1 regulates proliferation and MMP-7 transcription in breast cancer cells. *Genes to cells : devoted to molecular & cellular mechanisms*, **17**, 559-567.
- Buhler, L.A., Samara, R., Guzman, E., Wilson, C.L., Krizanac-Bengez, L., Janigro, D. & Ethell, D.W. (2009) Matrix metalloproteinase-7 facilitates immune access to the CNS in experimental autoimmune encephalomyelitis. *BMC neuroscience*, **10**, 17.
- Bui, M., Benyamin, B., Shah, S., Henders, A.K., Martin, N.G., Montgomery, G.W. & McRae, A.F. (2015) Sharing a Placenta is Associated With a Greater Similarity in DNA Methylation in Monozygotic Versus Dizygotic Twin Pairs in Blood at Age 14. *Twin research and human genetics : the official journal of the International Society for Twin Studies*, **18**, 680-685.
- Bunnell, T.M., Burbach, B.J., Shimizu, Y. & Ervasti, J.M. (2011) beta-Actin specifically controls cell growth, migration, and the G-actin pool. *Molecular biology of the cell*, **22**, 4047-4058.
- Burke, J.E. & Dennis, E.A. (2009) Phospholipase A2 structure/function, mechanism, and signaling. *Journal of lipid research*, **50 Suppl**, S237-242.
- Burket, J.A., Cannon, W.R., Jacome, L.F. & Deutsch, S.I. (2010) MK-801, a noncompetitive NMDA receptor antagonist, elicits circling behavior in the genetically inbred Balb/c mouse strain. *Brain research bulletin*, **83**, 337-339.

- Burks, S.R., Wright, C.L. & McCarthy, M.M. (2007) Exploration of prostanoid receptor subtype regulating estradiol and prostaglandin E2 induction of spinophilin in developing preoptic area neurons. *Neuroscience*, **146**, 1117-1127.
- Burrus, L.W. & McMahon, A.P. (1995) Biochemical analysis of murine Wnt proteins reveals both shared and distinct properties. *Experimental cell research*, **220**, 363-373.
- Buse, E. & Krisch, B. (1987) The mouse neural plate as starting material for studying neuronal differentiation in vitro. *Anatomy and embryology*, **175**, 331-340.
- Cage, E. & Burton, H. (2019) Gender Differences in the First Impressions of Autistic Adults. *Autism Res.*
- Calderon-Garciduenas, L., Solt, A.C., Henriquez-Roldan, C., Torres-Jardon, R., Nuse, B., Herritt, L., Villarreal-Calderon, R., Osnaya, N., Stone, I., Garcia, R., Brooks, D.M., Gonzalez-Maciel, A., Reynoso-Robles, R., Delgado-Chavez, R. & Reed, W. (2008) Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. *Toxicologic pathology*, **36**, 289-310.
- Calderon, F. & Kim, H.Y. (2004) Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *Journal of neurochemistry*, **90**, 979-988.
- Calvello, R., Lofrumento, D.D., Perrone, M.G., Cianciulli, A., Salvatore, R., Vitale, P., De Nuccio, F., Giannotti, L., Nicolardi, G., Panaro, M.A. & Scilimati, A. (2017) Highly Selective Cyclooxygenase-1 Inhibitors P6 and Mofezolac Counteract Inflammatory State both In Vitro and In Vivo Models of Neuroinflammation. *Frontiers in neurology*, **8**, 251.
- Cambiasso, M.J., Diaz, H., Caceres, A. & Carrer, H.F. (1995) Neuritogenic effect of estradiol on rat ventromedial hypothalamic neurons co-cultured with homotopic or heterotopic glia. *Journal of neuroscience research*, **42**, 700-709.
- Candelario-Jalil, E., Slawik, H., Ridelis, I., Waschbisch, A., Akundi, R.S., Hull, M. & Fiebich, B.L. (2005) Regional distribution of the prostaglandin E2 receptor EP1 in the rat brain: accumulation in Purkinje cells of the cerebellum. *Journal of molecular neuroscience : MN*, **27**, 303-310.
- Caracci, M.O., Avila, M.E. & De Ferrari, G.V. (2016) Synaptic Wnt/GSK3beta Signaling Hub in Autism. *Neural plasticity*, **2016**, 9603751.

- Carlezon, W.A., Jr., Kim, W., Missig, G., Finger, B.C., Landino, S.M., Alexander, A.J., Mokler, E.L., Robbins, J.O., Li, Y., Bolshakov, V.Y., McDougale, C.J. & Kim, K.S. (2019) Maternal and early postnatal immune activation produce sex-specific effects on autism-like behaviors and neuroimmune function in mice. *Sci Rep*, **9**, 16928.
- Carlson, S.E. (2001) Docosahexaenoic acid and arachidonic acid in infant development. *Seminars in neonatology* : *SN*, **6**, 437-449.
- Castellone, M.D., Teramoto, H., Williams, B.O., Druey, K.M. & Gutkind, J.S. (2005) Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science*, **310**, 1504-1510.
- Cellot, G., Maggi, L., Di Castro, M.A., Catalano, M., Migliore, R., Migliore, M., Scattoni, M.L., Calamandrei, G. & Cherubini, E. (2016) Premature changes in neuronal excitability account for hippocampal network impairment and autistic-like behavior in neonatal BTBR T+tf/J mice. *Sci Rep*, **6**, 31696.
- Chang, M.C., Lin, S.I., Lin, L.D., Chan, C.P., Lee, M.S., Wang, T.M., Jeng, P.Y., Yeung, S.Y. & Jeng, J.H. (2016) Prostaglandin E2 Stimulates EP2, Adenylate Cyclase, Phospholipase C, and Intracellular Calcium Release to Mediate Cyclic Adenosine Monophosphate Production in Dental Pulp Cells. *Journal of endodontics*, **42**, 584-588.
- Charman, T., Pickles, A., Simonoff, E., Chandler, S., Loucas, T. & Baird, G. (2011) IQ in children with autism spectrum disorders: data from the Special Needs and Autism Project (SNAP). *Psychological medicine*, **41**, 619-627.
- Chaste, P. & Leboyer, M. (2012) Autism risk factors: genes, environment, and gene-environment interactions. *Dialogues in clinical neuroscience*, **14**, 281-292.
- Chatterjee, M., Kurup, P.K., Lundbye, C.J., Hugger Toft, A.K., Kwon, J., Benedict, J., Kamceva, M., Banke, T.G. & Lombroso, P.J. (2018) STEP inhibition reverses behavioral, electrophysiologic, and synaptic abnormalities in Fmr1 KO mice. *Neuropharmacology*, **128**, 43-53.
- Chen, C. & Bazan, N.G. (2005) Lipid signaling: Sleep, synaptic plasticity, and neuroprotection. *Prostag Oth Lipid M*, **77**, 65-76.
- Chen, C., Magee, J.C. & Bazan, N.G. (2002) Cyclooxygenase-2 regulates prostaglandin E2 signaling in hippocampal long-term synaptic plasticity. *J Neurophysiol*, **87**, 2851-2857.

- Chen, Y.C., Chang, Y.W. & Huang, Y.S. (2019) Dysregulated Translation in Neurodevelopmental Disorders: An Overview of Autism-Risk Genes Involved in Translation. *Developmental neurobiology*, **79**, 60-74.
- Cheslack-Postava, K., Rantakokko, P.V., Hinkka-Yli-Salomaki, S., Surcel, H.M., McKeague, I.W., Kiviranta, H.A., Sourander, A. & Brown, A.S. (2013) Maternal serum persistent organic pollutants in the Finnish Prenatal Study of Autism: A pilot study. *Neurotoxicology and teratology*, **38**, 1-5.
- Chien, Y.L., Wu, Y.Y., Chiu, Y.N., Liu, S.K., Tsai, W.C., Lin, P.I., Chen, C.H., Gau, S.S. & Chien, W.H. (2011) Association study of the CNS patterning genes and autism in Han Chinese in Taiwan. *Progress in neuro-psychopharmacology & biological psychiatry*, **35**, 1512-1517.
- Chih, B., Afridi, S.K., Clark, L. & Scheiffele, P. (2004) Disorder-associated mutations lead to functional inactivation of neuroligins. *Human molecular genetics*, **13**, 1471-1477.
- Chikazu, D., Fujikawa, Y., Fujihara, H., Suenaga, H., Saijo, H., Ohkubo, K., Ogasawara, T., Mori, Y., Iino, M. & Takato, T. (2011) Cyclooxygenase-2 activity is important in craniofacial fracture repair. *International journal of oral and maxillofacial surgery*, **40**, 322-326.
- Choi, B.H. (1979) Mechanism of neuronal migration in human foetal cerebrum in vitro. *Yonsei medical journal*, **20**, 92-104.
- Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffler, C.A., Littman, D.R. & Huh, J.R. (2016) The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science*, **351**, 933-939.
- Choi, S.H., Langenbach, R. & Bosetti, F. (2008) Genetic deletion or pharmacological inhibition of cyclooxygenase-1 attenuate lipopolysaccharide-induced inflammatory response and brain injury. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, **22**, 1491-1501.
- Christensen, J., Gronborg, T.K., Sorensen, M.J., Schendel, D., Parner, E.T., Pedersen, L.H. & Vestergaard, M. (2013) Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *Jama*, **309**, 1696-1703.
- Chung, S.W., Gould, B., Zhang, R., Hu, Y., Levy, G.A. & Gorczynski, R.M. (1998) Pretreatment of donor stimulator cells by 16,16 dimethyl prostaglandin E2 influences the recipient immune response. *Surgery*, **123**, 171-180.

- Ciani, L. & Salinas, P.C. (2005) WNTs in the vertebrate nervous system: from patterning to neuronal connectivity. *Nature reviews*, **6**, 351-362.
- Cina, C., Bechberger, J.F., Ozog, M.A. & Naus, C.C. (2007) Expression of connexins in embryonic mouse neocortical development. *The Journal of comparative neurology*, **504**, 298-313.
- Clancy, B., Darlington, R.B. & Finlay, B.L. (2001) Translating developmental time across mammalian species. *Neuroscience*, **105**, 7-17.
- Clancy, B., Finlay, B.L., Darlington, R.B. & Anand, K.J. (2007) Extrapolating brain development from experimental species to humans. *Neurotoxicology*, **28**, 931-937.
- Clandinin, M.T., Chappell, J.E., Leong, S., Heim, T., Swyer, P.R. & Chance, G.W. (1980) Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early human development*, **4**, 121-129.
- Codocedo, J.F. & Inestrosa, N.C. (2016) Wnt-5a-regulated miR-101b controls COX2 expression in hippocampal neurons. *Biological research*, **49**, 9.
- Collins, A.L., Ma, D., Whitehead, P.L., Martin, E.R., Wright, H.H., Abramson, R.K., Hussman, J.P., Haines, J.L., Cuccaro, M.L., Gilbert, J.R. & Pericak-Vance, M.A. (2006) Investigation of autism and GABA receptor subunit genes in multiple ethnic groups. *Neurogenetics*, **7**, 167-174.
- Comery, T.A., Harris, J.B., Willems, P.J., Oostra, B.A., Irwin, S.A., Weiler, I.J. & Greenough, W.T. (1997) Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 5401-5404.
- Comoletti, D., De Jaco, A., Jennings, L.L., Flynn, R.E., Gaietta, G., Tsigelny, I., Ellisman, M.H. & Taylor, P. (2004) The Arg451Cys-neurologin-3 mutation associated with autism reveals a defect in protein processing. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 4889-4893.
- Constantino, J.N., Todorov, A., Hilton, C., Law, P., Zhang, Y., Molloy, E., Fitzgerald, R. & Geschwind, D. (2013) Autism recurrence in half siblings: strong support for genetic mechanisms of transmission in ASD. *Molecular psychiatry*, **18**, 137-138.

- Constantino, J.N., Zhang, Y., Frazier, T., Abbacchi, A.M. & Law, P. (2010) Sibling recurrence and the genetic epidemiology of autism. *The American journal of psychiatry*, **167**, 1349-1356.
- Contestabile, A. & Sintoni, S. (2013) Histone acetylation in neurodevelopment. *Current pharmaceutical design*, **19**, 5043-5050.
- Cook, E.H., Jr. (2009) Literature review: gastrointestinal symptoms in ASD, brain structure of identical twins with ASD. *Autism Res*, **2**, 285-286.
- Copf, T. (2016) Impairments in dendrite morphogenesis as etiology for neurodevelopmental disorders and implications for therapeutic treatments. *Neuroscience and biobehavioral reviews*, **68**, 946-978.
- Cordero, C., Windham, G.C., Schieve, L.A., Fallin, M.D., Croen, L.A., Siega-Riz, A.M., Engel, S.M., Herring, A.H., Stuebe, A.M., Vladutiu, C.J. & Daniels, J.L. (2019) Maternal diabetes and hypertensive disorders in association with autism spectrum disorder. *Autism Res*, **12**, 967-975.
- Corradini, I., Focchi, E., Rasile, M., Morini, R., Desiato, G., Tomasoni, R., Lizier, M., Ghirardini, E., Fesce, R., Morone, D., Barajon, I., Antonucci, F., Pozzi, D. & Matteoli, M. (2018) Maternal Immune Activation Delays Excitatory-to-Inhibitory Gamma-Aminobutyric Acid Switch in Offspring. *Biological psychiatry*, **83**, 680-691.
- Corthals, K., Heukamp, A.S., Kossen, R., Grosshennig, I., Hahn, N., Gras, H., Gopfert, M.C., Heinrich, R. & Geurten, B.R.H. (2017) Neuroligins Nlg2 and Nlg4 Affect Social Behavior in *Drosophila melanogaster*. *Frontiers in psychiatry*, **8**, 113.
- Cossins, J.A., Clements, J.M., Ford, J., Miller, K.M., Pigott, R., Vos, W., Van der Valk, P. & De Groot, C.J. (1997) Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions. *Acta neuropathologica*, **94**, 590-598.
- Cotney, J., Muhle, R.A., Sanders, S.J., Liu, L., Willsey, A.J., Niu, W., Liu, W., Klei, L., Lei, J., Yin, J., Reilly, S.K., Tebbenkamp, A.T., Bichsel, C., Pletikos, M., Sestan, N., Roeder, K., State, M.W., Devlin, B. & Noonan, J.P. (2015) The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. *Nature communications*, **6**, 6404.
- Craig, A.M. & Kang, Y. (2007) Neurexin-neuroligin signaling in synapse development. *Current opinion in neurobiology*, **17**, 43-52.

- Croen, L.A., Qian, Y., Ashwood, P., Zerbo, O., Schendel, D., Pinto-Martin, J., Daniele Fallin, M., Levy, S., Schieve, L.A., Yeargin-Allsopp, M., Sabourin, K.R. & Ames, J.L. (2019) Infection and Fever in Pregnancy and Autism Spectrum Disorders: Findings from the Study to Explore Early Development. *Autism Res*, **12**, 1551-1561.
- Cruz-Martin, A., Crespo, M. & Portera-Cailliau, C. (2010) Delayed stabilization of dendritic spines in fragile X mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 7793-7803.
- Cukier, H.N., Dueker, N.D., Slifer, S.H., Lee, J.M., Whitehead, P.L., Lalanne, E., Leyva, N., Konidari, I., Gentry, R.C., Hulme, W.F., Booven, D.V., Mayo, V., Hofmann, N.K., Schmidt, M.A., Martin, E.R., Haines, J.L., Cuccaro, M.L., Gilbert, J.R. & Pericak-Vance, M.A. (2014) Exome sequencing of extended families with autism reveals genes shared across neurodevelopmental and neuropsychiatric disorders. *Molecular autism*, **5**, 1.
- D'Mello, A.M. & Stoodley, C.J. (2015) Cerebro-cerebellar circuits in autism spectrum disorder. *Frontiers in neuroscience*, **9**, 408.
- Dalvi, S., Nguyen, H.H., On, N., Mitchell, R.W., Aukema, H.M., Miller, D.W. & Hatch, G.M. (2015) Exogenous arachidonic acid mediates permeability of human brain microvessel endothelial cells through prostaglandin E2 activation of EP3 and EP4 receptors. *Journal of neurochemistry*, **135**, 867-879.
- Damodaran, L.P. & Arumugam, G. (2011) Urinary oxidative stress markers in children with autism. *Redox report : communications in free radical research*, **16**, 216-222.
- Dargahi, L., Nasiraei-Moghadam, S., Abdi, A., Khalaj, L., Moradi, F. & Ahmadiani, A. (2011) Cyclooxygenase (COX)-1 activity precedes the COX-2 induction in Abeta-induced neuroinflammation. *Journal of molecular neuroscience : MN*, **45**, 10-21.
- Darnell, J.C., Van Driesche, S.J., Zhang, C., Hung, K.Y., Mele, A., Fraser, C.E., Stone, E.F., Chen, C., Fak, J.J., Chi, S.W., Licatalosi, D.D., Richter, J.D. & Darnell, R.B. (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell*, **146**, 247-261.
- Davenport, E.C., Szulc, B.R., Drew, J., Taylor, J., Morgan, T., Higgs, N.F., Lopez-Domenech, G. & Kittler, J.T. (2019) Autism and Schizophrenia-Associated CYFIP1 Regulates the Balance of Synaptic Excitation and Inhibition. *Cell reports*, **26**, 2037-2051 e2036.

- Davidson, J.M., Wong, C.T., Rai-Bhogal, R., Li, H. & Crawford, D.A. (2016) Prostaglandin E2 elevates calcium in differentiated neuroectodermal stem cells. *Molecular and cellular neurosciences*, **74**, 71-77.
- Davies, N.M., Longstreth, J. & Jamali, F. (2001) Misoprostol therapeutics revisited. *Pharmacotherapy*, **21**, 60-73.
- de Cock, M., Maas, Y.G. & van de Bor, M. (2012) Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. *Acta paediatrica*, **101**, 811-818.
- Dean, J.C., Hailey, H., Moore, S.J., Lloyd, D.J., Turnpenny, P.D. & Little, J. (2002) Long term health and neurodevelopment in children exposed to antiepileptic drugs before birth. *Journal of medical genetics*, **39**, 251-259.
- Dean, S.L., Knutson, J.F., Krebs-Kraft, D.L. & McCarthy, M.M. (2012a) Prostaglandin E2 is an endogenous modulator of cerebellar development and complex behavior during a sensitive postnatal period. *The European journal of neuroscience*, **35**, 1218-1229.
- Dean, S.L., Wright, C.L., Hoffman, J.F., Wang, M., Alger, B.E. & McCarthy, M.M. (2012b) Prostaglandin E2 stimulates estradiol synthesis in the cerebellum postnatally with associated effects on Purkinje neuron dendritic arbor and electrophysiological properties. *Endocrinology*, **153**, 5415-5427.
- Deininger, M.H., Weller, M., Streffer, J., Mittelbronn, M. & Meyermann, R. (1999) Patterns of cyclooxygenase-1 and -2 expression in human gliomas in vivo. *Acta neuropathologica*, **98**, 240-244.
- Deutsch, S.I., Mastropaolo, J., Powell, D.G., Rosse, R.B. & Bachus, S.E. (1998) Inbred mouse strains differ in their sensitivity to an antiseizure effect of MK-801. *Clinical neuropharmacology*, **21**, 255-257.
- Deutsch, S.I., Rosse, R.B., Paul, S.M., Riggs, R.L. & Mastropaolo, J. (1997) Inbred mouse strains differ in sensitivity to "popping" behavior elicited by MK-801. *Pharmacology, biochemistry, and behavior*, **57**, 315-317.
- Dewan, P., Jain, V., Gupta, P. & Banerjee, B.D. (2013) Organochlorine pesticide residues in maternal blood, cord blood, placenta, and breastmilk and their relation to birth size. *Chemosphere*, **90**, 1704-1710.

- Dhossche, D.M. (2004) Autism as early expression of catatonia. *Medical science monitor : international medical journal of experimental and clinical research*, **10**, RA31-39.
- Dhossche, D.M., Carroll, B.T. & Carroll, T.D. (2006) Is there a common neuronal basis for autism and catatonia? *International review of neurobiology*, **72**, 151-164.
- Di Cesare, A., Del Piccolo, P., Zacchetti, D. & Grohovaz, F. (2006) EP2 receptor stimulation promotes calcium responses in astrocytes via activation of the adenylyl cyclase pathway. *Cellular and molecular life sciences : CMLS*, **63**, 2546-2553.
- Diaz, H., Lorenzo, A., Carrer, H.F. & Caceres, A. (1992) Time lapse study of neurite growth in hypothalamic dissociated neurons in culture: sex differences and estrogen effects. *Journal of neuroscience research*, **33**, 266-281.
- Dinchuk, J.E., Car, B.D., Focht, R.J., Johnston, J.J., Jaffee, B.D., Covington, M.B., Contel, N.R., Eng, V.M., Collins, R.J., Czerniak, P.M. & et al. (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature*, **378**, 406-409.
- DiPietro, J.A., Davis, M.F., Costigan, K.A. & Barr, D.B. (2014) Fetal heart rate and motor activity associations with maternal organochlorine levels: results of an exploratory study. *Journal of exposure science & environmental epidemiology*, **24**, 474-481.
- Dong, Z., Chen, W., Chen, C., Wang, H., Cui, W., Tan, Z., Robinson, H., Gao, N., Luo, B., Zhang, L., Zhao, K., Xiong, W.C. & Mei, L. (2020) CUL3 Deficiency Causes Social Deficits and Anxiety-like Behaviors by Impairing Excitation-Inhibition Balance through the Promotion of Cap-Dependent Translation. *Neuron*, **105**, 475-490 e476.
- Du, M., Shi, F., Zhang, H., Xia, S., Zhang, M., Ma, J., Bai, X., Zhang, L., Wang, Y., Cheng, S., Yang, Q. & Leng, J. (2015) Prostaglandin E2 promotes human cholangiocarcinoma cell proliferation, migration and invasion through the upregulation of beta-catenin expression via EP3-4 receptor. *Oncology reports*, **34**, 715-726.
- Dumanis, S.B., Cha, H.J., Song, J.M., Trotter, J.H., Spitzer, M., Lee, J.Y., Weeber, E.J., Turner, R.S., Pak, D.T., Rebeck, G.W. & Hoe, H.S. (2011) ApoE receptor 2 regulates synapse and dendritic spine formation. *PLoS One*, **6**, e17203.
- Dumont, I., Peri, K.G., Hardy, P., Hou, X., Martinez-Bermudez, A.K., Molotchnikoff, S., Varma, D.R. & Chemtob, S. (1998) PGE2, via EP3 receptors, regulates brain nitric oxide synthase in the perinatal period. *The American journal of physiology*, **275**, R1812-1821.

- Durand, C.M., Betancur, C., Boeckers, T.M., Bockmann, J., Chaste, P., Fauchereau, F., Nygren, G., Rastam, M., Gillberg, I.C., Anckarsater, H., Sponheim, E., Goubran-Botros, H., Delorme, R., Chabane, N., Mouren-Simeoni, M.C., de Mas, P., Bieth, E., Roge, B., Heron, D., Burglen, L., Gillberg, C., Leboyer, M. & Bourgeron, T. (2007) Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nature genetics*, **39**, 25-27.
- Durkin, M.S., Maenner, M.J., Newschaffer, C.J., Lee, L.C., Cunniff, C.M., Daniels, J.L., Kirby, R.S., Leavitt, L., Miller, L., Zahorodny, W. & Schieve, L.A. (2008) Advanced parental age and the risk of autism spectrum disorder. *American journal of epidemiology*, **168**, 1268-1276.
- Ebert, D.H. & Greenberg, M.E. (2013) Activity-dependent neuronal signalling and autism spectrum disorder. *Nature*, **493**, 327-337.
- Echeverria, V., Clerman, A. & Dore, S. (2005) Stimulation of PGE receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following beta-amyloid exposure. *The European journal of neuroscience*, **22**, 2199-2206.
- Edmonson, C.A., Ziats, M.N. & Rennert, O.M. (2016) A Non-inflammatory Role for Microglia in Autism Spectrum Disorders. *Frontiers in neurology*, **7**, 9.
- Ehninger, D., Sano, Y., de Vries, P.J., Dies, K., Franz, D., Geschwind, D.H., Kaur, M., Lee, Y.S., Li, W., Lowe, J.K., Nakagawa, J.A., Sahin, M., Smith, K., Whittemore, V. & Silva, A.J. (2012) Gestational immune activation and Tsc2 haploinsufficiency cooperate to disrupt fetal survival and may perturb social behavior in adult mice. *Molecular psychiatry*, **17**, 62-70.
- El-Ansary, A., Bjorklund, G., Chirumbolo, S. & Alnakhli, O.M. (2017) Predictive value of selected biomarkers related to metabolism and oxidative stress in children with autism spectrum disorder. *Metabolic brain disease*, **32**, 1209-1221.
- El-Ansary, A., Hassan, W.M., Qasem, H. & Das, U.N. (2016) Identification of Biomarkers of Impaired Sensory Profiles among Autistic Patients. *PLoS One*, **11**, e0164153.
- El-Ansary, A.K., Bacha, A.G. & Al-Ayahdi, L.Y. (2011) Impaired plasma phospholipids and relative amounts of essential polyunsaturated fatty acids in autistic patients from Saudi Arabia. *Lipids in health and disease*, **10**, 63.
- El-Kordi, A., Winkler, D., Hammerschmidt, K., Kastner, A., Krueger, D., Ronnenberg, A., Ritter, C., Jatho, J., Radyushkin, K., Bourgeron, T., Fischer, J., Brose, N. & Ehrenreich,

- H. (2013) Development of an autism severity score for mice using Nlgn4 null mutants as a construct-valid model of heritable monogenic autism. *Behavioural brain research*, **251**, 41-49.
- Engel, S.M., Berkowitz, G.S., Barr, D.B., Teitelbaum, S.L., Siskind, J., Meisel, S.J., Wetmur, J.G. & Wolff, M.S. (2007) Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. *American journal of epidemiology*, **165**, 1397-1404.
- Engman, M.L., Sundin, M., Miniscalco, C., Westerlund, J., Lewensohn-Fuchs, I., Gillberg, C. & Fernell, E. (2015) Prenatal acquired cytomegalovirus infection should be considered in children with autism. *Acta paediatrica*, **104**, 792-795.
- Esclassan, F., Francois, J., Phillips, K.G., Loomis, S. & Gilmour, G. (2015) Phenotypic characterization of nonsocial behavioral impairment in neurexin 1alpha knockout rats. *Behavioral neuroscience*, **129**, 74-85.
- Eskenazi, B., Harley, K., Bradman, A., Weltzien, E., Jewell, N.P., Barr, D.B., Furlong, C.E. & Holland, N.T. (2004) Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environmental health perspectives*, **112**, 1116-1124.
- Ethell, I.M. & Ethell, D.W. (2007) Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. *Journal of neuroscience research*, **85**, 2813-2823.
- Etherton, M., Foldy, C., Sharma, M., Tabuchi, K., Liu, X., Shamloo, M., Malenka, R.C. & Sudhof, T.C. (2011) Autism-linked neuroligin-3 R451C mutation differentially alters hippocampal and cortical synaptic function. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 13764-13769.
- Etherton, M.R., Blaiss, C.A., Powell, C.M. & Sudhof, T.C. (2009) Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 17998-18003.
- Evans, J.C., Robinson, C.M., Shi, M. & Webb, D.J. (2015) The guanine nucleotide exchange factor (GEF) Asef2 promotes dendritic spine formation via Rac activation and spinophilin-dependent targeting. *The Journal of biological chemistry*, **290**, 10295-10308.

- Ey, E., Yang, M., Katz, A.M., Woldeyohannes, L., Silverman, J.L., Leblond, C.S., Faure, P., Torquet, N., Le Sourd, A.M., Bourgeron, T. & Crawley, J.N. (2012) Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking *neurologin4*. *Genes, brain, and behavior*, **11**, 928-941.
- Fairless, A.H., Dow, H.C., Kreibich, A.S., Torre, M., Kuruvilla, M., Gordon, E., Morton, E.A., Tan, J., Berrettini, W.H., Li, H., Abel, T. & Brodtkin, E.S. (2012) Sociability and brain development in BALB/cJ and C57BL/6J mice. *Behavioural brain research*, **228**, 299-310.
- Fairless, A.H., Dow, H.C., Toledo, M.M., Malkus, K.A., Edelmann, M., Li, H., Talbot, K., Arnold, S.E., Abel, T. & Brodtkin, E.S. (2008) Low sociability is associated with reduced size of the corpus callosum in the BALB/cJ inbred mouse strain. *Brain research*, **1230**, 211-217.
- Fan, H.W., Liu, G.Y., Zhao, C.F., Li, X.F. & Yang, X.Y. (2015) Differential expression of COX-2 in osteoarthritis and rheumatoid arthritis. *Genetics and molecular research : GMR*, **14**, 12872-12879.
- Fang, S.Y., Wang, S., Huang, N., Yeh, H.H. & Chen, C.Y. (2015) Prenatal Infection and Autism Spectrum Disorders in Childhood: A Population-Based Case-Control Study in Taiwan. *Paediatric and perinatal epidemiology*, **29**, 307-316.
- Fatemi, S.H., Folsom, T.D., Reutiman, T.J., Abu-Odeh, D., Mori, S., Huang, H. & Oishi, K. (2009a) Abnormal expression of myelination genes and alterations in white matter fractional anisotropy following prenatal viral influenza infection at E16 in mice. *Schizophrenia research*, **112**, 46-53.
- Fatemi, S.H., Folsom, T.D., Reutiman, T.J., Huang, H., Oishi, K. & Mori, S. (2009b) Prenatal viral infection of mice at E16 causes changes in gene expression in hippocampi of the offspring. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, **19**, 648-653.
- Fatemi, S.H., Reutiman, T.J., Folsom, T.D., Huang, H., Oishi, K., Mori, S., Smee, D.F., Pearce, D.A., Winter, C., Sohr, R. & Juckel, G. (2008) Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. *Schizophrenia research*, **99**, 56-70.
- Fatemi, S.H., Reutiman, T.J., Folsom, T.D. & Thuras, P.D. (2009c) GABA(A) receptor downregulation in brains of subjects with autism. *Journal of autism and developmental disorders*, **39**, 223-230.

- Fedoseeva, L.A., Shevelev, O.B., Kolosova, N.G. & Dymshits, G.M. (2014) MS2 phage ribonucleoproteins as exogenous internal control for RT-qPCR data normalization in gene expression study of developing rat brain. *Biochemistry. Biokhimiia*, **79**, 706-716.
- Feng, J., Yan, Z., Ferreira, A., Tomizawa, K., Liauw, J.A., Zhuo, M., Allen, P.B., Ouimet, C.C. & Greengard, P. (2000) Spinophilin regulates the formation and function of dendritic spines. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9287-9292.
- Fernandez de Cossio, L., Guzman, A., van der Veldt, S. & Luheshi, G.N. (2017) Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. *Brain, behavior, and immunity*, **63**, 88-98.
- Ferrari, R.A., Ward, S.J., Zobre, C.M., Van Liew, D.K., Perrone, M.H., Connell, M.J. & Haubrich, D.R. (1990) Estimation of the in vivo effect of cyclooxygenase inhibitors on prostaglandin E2 levels in mouse brain. *European journal of pharmacology*, **179**, 25-34.
- Fifkova, E. & Delay, R.J. (1982) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. *The Journal of cell biology*, **95**, 345-350.
- Fischer, M., Kaech, S., Knutti, D. & Matus, A. (1998) Rapid actin-based plasticity in dendritic spines. *Neuron*, **20**, 847-854.
- Folstein, S. & Rutter, M. (1977) Infantile autism: a genetic study of 21 twin pairs. *Journal of child psychology and psychiatry, and allied disciplines*, **18**, 297-321.
- Foss-Feig, J.H., Adkinson, B.D., Ji, J.L., Yang, G., Srihari, V.H., McPartland, J.C., Krystal, J.H., Murray, J.D. & Anticevic, A. (2017) Searching for Cross-Diagnostic Convergence: Neural Mechanisms Governing Excitation and Inhibition Balance in Schizophrenia and Autism Spectrum Disorders. *Biological psychiatry*, **81**, 848-861.
- Franklin, A.V., King, M.K., Palomo, V., Martinez, A., McMahon, L.L. & Jope, R.S. (2014) Glycogen synthase kinase-3 inhibitors reverse deficits in long-term potentiation and cognition in fragile X mice. *Biological psychiatry*, **75**, 198-206.
- Frankowski, J.C., DeMars, K.M., Ahmad, A.S., Hawkins, K.E., Yang, C., Leclerc, J.L., Dore, S. & Candelario-Jalil, E. (2015) Detrimental role of the EP1 prostanoid receptor in blood-brain barrier damage following experimental ischemic stroke. *Sci Rep*, **5**, 17956.

- Frazier, T.W., Georgiades, S., Bishop, S.L. & Hardan, A.Y. (2014) Behavioral and cognitive characteristics of females and males with autism in the Simons Simplex Collection. *Journal of the American Academy of Child and Adolescent Psychiatry*, **53**, 329-340 e321-323.
- Freire, C., Amaya, E., Fernandez, M.F., Gonzalez-Galarzo, M.C., Ramos, R., Molina-Molina, J.M., Arrebola, J.P. & Olea, N. (2011) Relationship between occupational social class and exposure to organochlorine pesticides during pregnancy. *Chemosphere*, **83**, 831-838.
- Fuentes, A., Spaziani, E.P. & O'Brien, W.F. (1996) The expression of cyclooxygenase-2 (COX-2) in amnion and decidua following spontaneous labor. *Prostaglandins*, **52**, 261-267.
- Fujino, H., West, K.A. & Regan, J.W. (2002) Phosphorylation of glycogen synthase kinase-3 and stimulation of T-cell factor signaling following activation of EP2 and EP4 prostanoid receptors by prostaglandin E2. *The Journal of biological chemistry*, **277**, 2614-2619.
- Full, F. & Gack, M.U. (2014) Prostaglandin E2: the villain in the host response to influenza virus. *Immunity*, **40**, 453-454.
- Funk, C.D. (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*, **294**, 1871-1875.
- Furnari, M.A., Saw, C.L., Kong, A.N. & Wagner, G.C. (2014) Altered behavioral development in Nrf2 knockout mice following early postnatal exposure to valproic acid. *Brain research bulletin*, **109**, 132-142.
- Furuyashiki, T. & Narumiya, S. (2011) Stress responses: the contribution of prostaglandin E(2) and its receptors. *Nat Rev Endocrinol*, **7**, 163-175.
- Galvez, R. & Greenough, W.T. (2005) Sequence of abnormal dendritic spine development in primary somatosensory cortex of a mouse model of the fragile X mental retardation syndrome. *American journal of medical genetics. Part A*, **135**, 155-160.
- Gandal, M.J., Zhang, P., Hadjimichael, E., Walker, R.L., Chen, C., Liu, S., Won, H., van Bakel, H., Varghese, M., Wang, Y., Shieh, A.W., Haney, J., Parhami, S., Belmont, J., Kim, M., Moran Losada, P., Khan, Z., Mleczko, J., Xia, Y., Dai, R., Wang, D., Yang, Y.T., Xu, M., Fish, K., Hof, P.R., Warrell, J., Fitzgerald, D., White, K., Jaffe, A.E., Psych, E.C., Peters, M.A., Gerstein, M., Liu, C., Iakoucheva, L.M., Pinto, D. & Geschwind, D.H. (2018) Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science*, **362**.

- Gao, C., Chen, G., Romero, G., Moschos, S., Xu, X. & Hu, J. (2014) Induction of Gsk3 β - β -TrCP interaction is required for late phase stabilization of β -catenin in canonical Wnt signaling. *The Journal of biological chemistry*, **289**, 7099-7108.
- Gao, R. & Penzes, P. (2015) Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders. *Current molecular medicine*, **15**, 146-167.
- Garber, K., Smith, K.T., Reines, D. & Warren, S.T. (2006) Transcription, translation and fragile X syndrome. *Current opinion in genetics & development*, **16**, 270-275.
- Gareri, J., Brien, J., Reynolds, J. & Koren, G. (2009) Potential role of the placenta in fetal alcohol spectrum disorder. *Paediatric drugs*, **11**, 26-29.
- Garrido, M.P., Hurtado, I., Valenzuela-Valderrama, M., Salvatierra, R., Hernandez, A., Vega, M., Selman, A., Quest, A.F.G. & Romero, C. (2019) NGF-Enhanced Vasculogenic Properties of Epithelial Ovarian Cancer Cells Is Reduced by Inhibition of the COX-2/PGE2 Signaling Axis. *Cancers*, **11**.
- Gauthier, J., Spiegelman, D., Piton, A., Lafreniere, R.G., Laurent, S., St-Onge, J., Lapointe, L., Hamdan, F.F., Cossette, P., Mottron, L., Fombonne, E., Joobar, R., Marineau, C., Drapeau, P. & Rouleau, G.A. (2009) Novel de novo SHANK3 mutation in autistic patients. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, **150B**, 421-424.
- Gemzell-Danielsson, K. & Lalitkumar, S. (2008) Second trimester medical abortion with mifepristone-misoprostol and misoprostol alone: a review of methods and management. *Reproductive health matters*, **16**, 162-172.
- Gersting, J.A., Schaub, C.E. & Wood, C.E. (2009) Development of prostaglandin endoperoxide synthase expression in the ovine fetal central nervous system and pituitary. *Gene expression patterns : GEP*, **9**, 603-611.
- Ghasemi Firouzabadi, S., Vameghi, R., Kariminejad, R., Darvish, H., Banihashemi, S., Firouzkouhi Moghaddam, M., Jamali, P., Farbod Mofidi Tehrani, H., Dehghani, H., Raeisoon, M.R., Narooie-Nejad, M., Jamshidi, J., Tafakhori, A., Sadabadi, S. & Behjati, F. (2016) Analysis of Copy Number Variations in Patients with Autism Using Cytogenetic and MLPA Techniques: Report of 16p13.1p13.3 and 10q26.3 Duplications. *International journal of molecular and cellular medicine*, **5**, 236-245.

- Ghaziuddin, N., Andersen, L. & Ghaziuddin, M. (2020) Catatonia in Patients with Autism Spectrum Disorder. *Child and adolescent psychiatric clinics of North America*, **29**, 443-454.
- Gipson, C.D. & Olive, M.F. (2017) Structural and functional plasticity of dendritic spines - root or result of behavior? *Genes, brain, and behavior*, **16**, 101-117.
- Glausier, J.R. & Lewis, D.A. (2013) Dendritic spine pathology in schizophrenia. *Neuroscience*, **251**, 90-107.
- Glessner, J.T., Li, J., Wang, D., March, M., Lima, L., Desai, A., Hadley, D., Kao, C., Gur, R.E., Cohen, N., Sleiman, P.M.A., Li, Q., Hakonarson, H. & Janssen, C.N.G.W.G. (2017) Copy number variation meta-analysis reveals a novel duplication at 9p24 associated with multiple neurodevelopmental disorders. *Genome medicine*, **9**, 106.
- Glock, C., Heumuller, M. & Schuman, E.M. (2017) mRNA transport & local translation in neurons. *Current opinion in neurobiology*, **45**, 169-177.
- Go, H.S., Kim, K.C., Choi, C.S., Jeon, S.J., Kwon, K.J., Han, S.H., Lee, J., Cheong, J.H., Ryu, J.H., Kim, C.H., Ko, K.H. & Shin, C.Y. (2012) Prenatal exposure to valproic acid increases the neural progenitor cell pool and induces macrocephaly in rat brain via a mechanism involving the GSK-3 β /beta-catenin pathway. *Neuropharmacology*, **63**, 1028-1041.
- Goessling, W., North, T.E., Loewer, S., Lord, A.M., Lee, S., Stoick-Cooper, C.L., Weidinger, G., Puder, M., Daley, G.Q., Moon, R.T. & Zon, L.I. (2009) Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. *Cell*, **136**, 1136-1147.
- Good, P. (2009) Did acetaminophen provoke the autism epidemic? *Alternative medicine review : a journal of clinical therapeutic*, **14**, 364-372.
- Gordon, L., Joo, J.H., Andronikos, R., Ollikainen, M., Wallace, E.M., Umstad, M.P., Permezel, M., Oshlack, A., Morley, R., Carlin, J.B., Saffery, R., Smyth, G.K. & Craig, J.M. (2011) Expression discordance of monozygotic twins at birth: effect of intrauterine environment and a possible mechanism for fetal programming. *Epigenetics*, **6**, 579-592.
- Gouder, L., Vitrac, A., Goubran-Botros, H., Danckaert, A., Tinevez, J.Y., Andre-Leroux, G., Atanasova, E., Lemièrre, N., Biton, A., Leblond, C.S., Poulet, A., Boland, A., Deleuze, J.F., Benchoua, A., Delorme, R., Bourgeron, T. & Cloez-Tayarani, I. (2019) Altered

- spinogenesis in iPSC-derived cortical neurons from patients with autism carrying de novo SHANK3 mutations. *Sci Rep*, **9**, 94.
- Gray, E.G. (1959) Electron microscopy of synaptic contacts on dendrite spines of the cerebral cortex. *Nature*, **183**, 1592-1593.
- Gray, P.H. (2014) In infants born extremely preterm, aspirin or NSAID use during pregnancy are associated with increased risk of quadriparetic cerebral palsy. *Evidence-based nursing*, **17**, 16-17.
- Grayton, H.M., Missler, M., Collier, D.A. & Fernandes, C. (2013) Altered social behaviours in neurexin 1alpha knockout mice resemble core symptoms in neurodevelopmental disorders. *PLoS One*, **8**, e67114.
- Greenbaum, D., Colangelo, C., Williams, K. & Gerstein, M. (2003) Comparing protein abundance and mRNA expression levels on a genomic scale. *Genome biology*, **4**, 117.
- Greenberg, D.A., Hodge, S.E., Sowinski, J. & Nicoll, D. (2001) Excess of twins among affected sibling pairs with autism: implications for the etiology of autism. *American journal of human genetics*, **69**, 1062-1067.
- Greer, Y.E., Fields, A.P., Brown, A.M. & Rubin, J.S. (2013) Atypical protein kinase Ciota is required for Wnt3a-dependent neurite outgrowth and binds to phosphorylated dishevelled 2. *The Journal of biological chemistry*, **288**, 9438-9446.
- Grossman, A.W., Aldridge, G.M., Weiler, I.J. & Greenough, W.T. (2006) Local protein synthesis and spine morphogenesis: Fragile X syndrome and beyond. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **26**, 7151-7155.
- Guan, P.P., Liang, Y.Y., Cao, L.L., Yu, X. & Wang, P. (2019a) Cyclooxygenase-2 Induced the beta-Amyloid Protein Deposition and Neuronal Apoptosis Via Upregulating the Synthesis of Prostaglandin E2 and 15-Deoxy-Delta(12,14)-prostaglandin J2. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics*.
- Guan, P.P. & Wang, P. (2019) Integrated communications between cyclooxygenase-2 and Alzheimer's disease. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, **33**, 13-33.

- Guan, P.P., Yu, X., Zou, Y.H. & Wang, P. (2019b) Cyclooxygenase-2 is critical for the propagation of beta-amyloid protein and reducing the glycosylation of tau in Alzheimer's disease. *Cellular & molecular immunology*, **16**, 892-894.
- Guang, S., Pang, N., Deng, X., Yang, L., He, F., Wu, L., Chen, C., Yin, F. & Peng, J. (2018) Synaptopathology Involved in Autism Spectrum Disorder. *Frontiers in cellular neuroscience*, **12**, 470.
- Gupta, A., Chatree, S., Buo, A.M., Moorer, M.C. & Stains, J.P. (2019) Connexin43 enhances Wnt and PGE2-dependent activation of beta-catenin in osteoblasts. *Pflugers Archiv : European journal of physiology*, **471**, 1235-1243.
- Ha, S., Lee, D., Cho, Y.S., Chung, C., Yoo, Y.E., Kim, J., Lee, J., Kim, W., Kim, H., Bae, Y.C., Tanaka-Yamamoto, K. & Kim, E. (2016) Cerebellar Shank2 Regulates Excitatory Synapse Density, Motor Coordination, and Specific Repetitive and Anxiety-Like Behaviors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **36**, 12129-12143.
- Hadley, D., Wu, Z.L., Kao, C., Kini, A., Mohamed-Hadley, A., Thomas, K., Vazquez, L., Qiu, H., Mentch, F., Pellegrino, R., Kim, C., Connolly, J., Consortium, A.G.P., Glessner, J. & Hakonarson, H. (2014) The impact of the metabotropic glutamate receptor and other gene family interaction networks on autism. *Nature communications*, **5**, 4074.
- Hammer, M., Krueger-Burg, D., Tuffy, L.P., Cooper, B.H., Taschenberger, H., Goswami, S.P., Ehrenreich, H., Jonas, P., Varoqueaux, F., Rhee, J.S. & Brose, N. (2015) Perturbed Hippocampal Synaptic Inhibition and gamma-Oscillations in a Neuroligin-4 Knockout Mouse Model of Autism. *Cell reports*, **13**, 516-523.
- Hanamsagar, R., Alter, M.D., Block, C.S., Sullivan, H., Bolton, J.L. & Bilbo, S.D. (2017) Generation of a microglial developmental index in mice and in humans reveals a sex difference in maturation and immune reactivity. *Glia*.
- Hara, Y., Ago, Y., Higuchi, M., Hasebe, S., Nakazawa, T., Hashimoto, H., Matsuda, T. & Takuma, K. (2017) Oxytocin attenuates deficits in social interaction but not recognition memory in a prenatal valproic acid-induced mouse model of autism. *Hormones and behavior*, **96**, 130-136.
- Harris, K.M. & Kater, S.B. (1994) Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. *Annual review of neuroscience*, **17**, 341-371.

- Hatanaka, Y., Kabuta, T. & Wada, K. (2017) Disturbance in Maternal Environment Leads to Abnormal Synaptic Instability during Neuronal Circuitry Development. *Frontiers in neuroscience*, **11**, 35.
- Hawi, Z., Tong, J., Dark, C., Yates, H., Johnson, B. & Bellgrove, M.A. (2018) The role of cadherin genes in five major psychiatric disorders: A literature update. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, **177**, 168-180.
- Hayek, J., Cervellati, C., Crivellari, I., Pecorelli, A. & Valacchi, G. (2017) Lactonase Activity and Lipoprotein-Phospholipase A2 as Possible Novel Serum Biomarkers for the Differential Diagnosis of Autism Spectrum Disorders and Rett Syndrome: Results from a Pilot Study. *Oxidative medicine and cellular longevity*, **2017**, 5694058.
- He, G.L., Luo, Z., Shen, T.T., Li, P., Yang, J., Luo, X., Chen, C.H., Gao, P. & Yang, X.S. (2016) Inhibition of STAT3- and MAPK-dependent PGE2 synthesis ameliorates phagocytosis of fibrillar beta-amyloid peptide (1-42) via EP2 receptor in EMF-stimulated N9 microglial cells. *Journal of neuroinflammation*, **13**, 296.
- Hemond, S.G. & Glover, J.C. (1993) Clonal patterns of cell proliferation, migration, and dispersal in the brainstem of the chicken embryo. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **13**, 1387-1402.
- Hennebelle, M., Morgan, R.K., Sethi, S., Zhang, Z., Chen, H., Grodzki, A.C., Lein, P.J. & Taha, A.Y. (2019) Linoleic acid-derived metabolites constitute the majority of oxylipins in the rat pup brain and stimulate axonal growth in primary rat cortical neuron-glia co-cultures in a sex-dependent manner. *Journal of neurochemistry*.
- Hering, H. & Sheng, M. (2001) Dendritic spines: structure, dynamics and regulation. *Nature reviews*, **2**, 880-888.
- Hernandez, C.J., Baez-Becerra, C., Contreras-Zarate, M.J., Arboleda, H. & Arboleda, G. (2019) PINK1 Silencing Modifies Dendritic Spine Dynamics of Mouse Hippocampal Neurons. *Journal of molecular neuroscience : MN*.
- Hewett, S.J., Shi, J., Gong, Y., Dhandapani, K., Pilbeam, C. & Hewett, J.A. (2016) Spontaneous Glutamatergic Synaptic Activity Regulates Constitutive COX-2 Expression in Neurons: OPPOSING ROLES FOR THE TRANSCRIPTION FACTORS CREB (cAMP RESPONSE ELEMENT BINDING) PROTEIN AND Sp1 (STIMULATORY PROTEIN-1). *The Journal of biological chemistry*, **291**, 27279-27288.

- Heyer, D.B. & Meredith, R.M. (2017) Environmental toxicology: Sensitive periods of development and neurodevelopmental disorders. *Neurotoxicology*, **58**, 23-41.
- Hinz, B. & Brune, K. (2002) Cyclooxygenase-2--10 years later. *The Journal of pharmacology and experimental therapeutics*, **300**, 367-375.
- Hiyama, A., Yokoyama, K., Nukaga, T., Sakai, D. & Mochida, J. (2015) Response to tumor necrosis factor-alpha mediated inflammation involving activation of prostaglandin E2 and Wnt signaling in nucleus pulposus cells. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*, **33**, 1756-1768.
- Hoffman, J.F., Wright, C.L. & McCarthy, M.M. (2016) A Critical Period in Purkinje Cell Development Is Mediated by Local Estradiol Synthesis, Disrupted by Inflammation, and Has Enduring Consequences Only for Males. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **36**, 10039-10049.
- Holtmaat, A.J., Trachtenberg, J.T., Wilbrecht, L., Shepherd, G.M., Zhang, X., Knott, G.W. & Svoboda, K. (2005) Transient and persistent dendritic spines in the neocortex in vivo. *Neuron*, **45**, 279-291.
- Homma, S., Yaginuma, H. & Oppenheim, R.W. (1994) Programmed cell death during the earliest stages of spinal cord development in the chick embryo: a possible means of early phenotypic selection. *The Journal of comparative neurology*, **345**, 377-395.
- Hoover, S.M. (1999) Exposure to persistent organochlorines in Canadian breast milk: a probabilistic assessment. *Risk analysis : an official publication of the Society for Risk Analysis*, **19**, 527-545.
- Hoozemans, J.J., Rozemuller, A.J., Janssen, I., De Groot, C.J., Veerhuis, R. & Eikelenboom, P. (2001) Cyclooxygenase expression in microglia and neurons in Alzheimer's disease and control brain. *Acta neuropathologica*, **101**, 2-8.
- Hoozemans, J.J., Rozemuller, J.M., van Haastert, E.S., Veerhuis, R. & Eikelenboom, P. (2008) Cyclooxygenase-1 and -2 in the different stages of Alzheimer's disease pathology. *Current pharmaceutical design*, **14**, 1419-1427.
- Hornig, M., Bresnahan, M.A., Che, X., Schultz, A.F., Ukaigwe, J.E., Eddy, M.L., Hirtz, D., Gunnes, N., Lie, K.K., Magnus, P., Mjaaland, S., Reichborn-Kjennerud, T., Schjolberg, S., Oyen, A.S., Levin, B., Susser, E.S., Stoltenberg, C. & Lipkin, W.I. (2018) Prenatal fever and autism risk. *Molecular psychiatry*, **23**, 759-766.

- Hosie, S., Ellis, M., Swaminathan, M., Ramalhosa, F., Seger, G.O., Balasuriya, G.K., Gillberg, C., Rastam, M., Churilov, L., McKeown, S.J., Yalcinkaya, N., Urvil, P., Savidge, T., Bell, C.A., Bodin, O., Wood, J., Franks, A.E., Bornstein, J.C. & Hill-Yardin, E.L. (2019) Gastrointestinal dysfunction in patients and mice expressing the autism-associated R451C mutation in neuroligin-3. *Autism Res*, **12**, 1043-1056.
- Hou, Q., Wang, Y., Li, Y., Chen, D., Yang, F. & Wang, S. (2018) A Developmental Study of Abnormal Behaviors and Altered GABAergic Signaling in the VPA-Treated Rat Model of Autism. *Frontiers in behavioral neuroscience*, **12**, 182.
- Hovatta, I., Tennant, R.S., Helton, R., Marr, R.A., Singer, O., Redwine, J.M., Ellison, J.A., Schadt, E.E., Verma, I.M., Lockhart, D.J. & Barlow, C. (2005) Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. *Nature*, **438**, 662-666.
- Hsu, P.S., Lin, C.M., Chang, J.F., Wu, C.S., Sia, K.C., Lee, I.T., Huang, K.Y. & Lin, W.N. (2019) Participation of NADPH Oxidase-Related Reactive Oxygen Species in Leptin-Promoted Pulmonary Inflammation: Regulation of cPLA2alpha and COX-2 Expression. *International journal of molecular sciences*, **20**.
- Hu, X.L., Bergstrom, S.A., Brink, M., Ronnback, A. & Dahlqvist, P. (2010) Enriched environment increases spinophilin mRNA expression and spinophilin immunoreactive dendritic spines in hippocampus and cortex. *Neuroscience letters*, **476**, 79-83.
- Huang, C., Chen, Y., Liu, H., Yang, J., Song, X., Zhao, J., He, N., Zhou, C.J., Wang, Y., Huang, C. & Dong, Q. (2017) Celecoxib targets breast cancer stem cells by inhibiting the synthesis of prostaglandin E2 and down-regulating the Wnt pathway activity. *Oncotarget*, **8**, 115254-115269.
- Huang da, W., Sherman, B.T. & Lempicki, R.A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, **4**, 44-57.
- Huber, K.M., Gallagher, S.M., Warren, S.T. & Bear, M.F. (2002) Altered synaptic plasticity in a mouse model of fragile X mental retardation. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7746-7750.
- Hung, A.Y., Futai, K., Sala, C., Valtschanoff, J.G., Ryu, J., Woodworth, M.A., Kidd, F.L., Sung, C.C., Miyakawa, T., Bear, M.F., Weinberg, R.J. & Sheng, M. (2008) Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **28**, 1697-1708.

- Hwang, S.R., Kim, C.Y., Shin, K.M., Jo, J.H., Kim, H.A. & Heo, Y. (2015) Altered expression levels of neurodevelopmental proteins in fetal brains of BTBR T+tf/J mice with autism-like behavioral characteristics. *Journal of toxicology and environmental health. Part A*, **78**, 516-523.
- Idborg, H., Pawelzik, S.C., Perez-Manso, M., Bjork, L., Hamrin, J., Herlenius, E. & Jakobsson, P.J. (2014) Evaluation of urinary prostaglandin E2 metabolite as a biomarker in infants with fever due to viral infection. *Prostaglandins, leukotrienes, and essential fatty acids*, **91**, 269-275.
- Ikeda, Y. & Nagai, A. (2006) Differential expression of the estrogen receptors alpha and beta during postnatal development of the rat cerebellum. *Brain research*, **1083**, 39-49.
- Ille, F. & Sommer, L. (2005) Wnt signaling: multiple functions in neural development. *Cellular and molecular life sciences : CMLS*, **62**, 1100-1108.
- Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., Smith, J.D., Paerper, B., Nickerson, D.A., Dea, J., Dong, S., Gonzalez, L.E., Mandell, J.D., Mane, S.M., Murtha, M.T., Sullivan, C.A., Walker, M.F., Waqar, Z., Wei, L., Willsey, A.J., Yamrom, B., Lee, Y.H., Grabowska, E., Dalkic, E., Wang, Z., Marks, S., Andrews, P., Leotta, A., Kendall, J., Hakker, I., Rosenbaum, J., Ma, B., Rodgers, L., Troge, J., Narzisi, G., Yoon, S., Schatz, M.C., Ye, K., McCombie, W.R., Shendure, J., Eichler, E.E., State, M.W. & Wigler, M. (2014) The contribution of de novo coding mutations to autism spectrum disorder. *Nature*, **515**, 216-221.
- Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., Kendall, J., Grabowska, E., Ma, B., Marks, S., Rodgers, L., Stepansky, A., Troge, J., Andrews, P., Bekritsky, M., Pradhan, K., Ghiban, E., Kramer, M., Parla, J., Demeter, R., Fulton, L.L., Fulton, R.S., Magrini, V.J., Ye, K., Darnell, J.C., Darnell, R.B., Mardis, E.R., Wilson, R.K., Schatz, M.C., McCombie, W.R. & Wigler, M. (2012) De novo gene disruptions in children on the autistic spectrum. *Neuron*, **74**, 285-299.
- Ivashko-Pachima, Y., Hadar, A., Grigg, I., Korenkova, V., Kapitansky, O., Karmon, G., Gershovits, M., Sayas, C.L., Kooy, R.F., Attems, J., Gurwitz, D. & Gozes, I. (2019) Discovery of autism/intellectual disability somatic mutations in Alzheimer's brains: mutated ADNP cytoskeletal impairments and repair as a case study. *Molecular psychiatry*.
- Jablonska-Trypuc, A., Wolejko, E., Wydro, U. & Butarewicz, A. (2017) The impact of pesticides on oxidative stress level in human organism and their activity as an endocrine disruptor.

- Journal of environmental science and health. Part. B, Pesticides, food contaminants, and agricultural wastes*, **52**, 483-494.
- Jacome, L.F., Burket, J.A., Herndon, A.L. & Deutsch, S.I. (2011) Genetically inbred Balb/c mice differ from outbred Swiss Webster mice on discrete measures of sociability: relevance to a genetic mouse model of autism spectrum disorders. *Autism Res*, **4**, 393-400.
- Jacquemont, S., Coe, B.P., Hersch, M., Duyzend, M.H., Krumm, N., Bergmann, S., Beckmann, J.S., Rosenfeld, J.A. & Eichler, E.E. (2014) A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *American journal of human genetics*, **94**, 415-425.
- Jakab, R.L., Wong, J.K. & Belcher, S.M. (2001) Estrogen receptor beta immunoreactivity in differentiating cells of the developing rat cerebellum. *The Journal of comparative neurology*, **430**, 396-409.
- Jalal, N., Surendranath, A.R., Pathak, J.L., Yu, S. & Chung, C.Y. (2018) Bisphenol A (BPA) the mighty and the mutagenic. *Toxicology reports*, **5**, 76-84.
- Jamain, S., Quach, H., Betancur, C., Rastam, M., Colineaux, C., Gillberg, I.C., Soderstrom, H., Giros, B., Leboyer, M., Gillberg, C., Bourgeron, T. & Paris Autism Research International Sibpair, S. (2003) Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nature genetics*, **34**, 27-29.
- Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., Ramanantsoa, N., Gallego, J., Ronnenberg, A., Winter, D., Frahm, J., Fischer, J., Bourgeron, T., Ehrenreich, H. & Brose, N. (2008) Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1710-1715.
- Jensen, M., Smolen, C. & Girirajan, S. (2020) Gene discoveries in autism are biased towards comorbidity with intellectual disability. *Journal of medical genetics*.
- Jensen, T.K., Mustieles, V., Bleses, D., Frederiksen, H., Trecca, F., Schoeters, G., Andersen, H.R., Grandjean, P., Kyhl, H.B., Juul, A., Bilenberg, N. & Andersson, A.M. (2019) Prenatal bisphenol A exposure is associated with language development but not with ADHD-related behavior in toddlers from the Odense Child Cohort. *Environmental research*, **170**, 398-405.
- Jin, J., Shie, F.S., Liu, J., Wang, Y., Davis, J., Schantz, A.M., Montine, K.S., Montine, T.J. & Zhang, J. (2007) Prostaglandin E2 receptor subtype 2 (EP2) regulates microglial

- activation and associated neurotoxicity induced by aggregated alpha-synuclein. *Journal of neuroinflammation*, **4**, 2.
- Joensuu, M., Lanoue, V. & Hotulainen, P. (2018) Dendritic spine actin cytoskeleton in autism spectrum disorder. *Progress in neuro-psychopharmacology & biological psychiatry*, **84**, 362-381.
- Johannessen, C.U. & Johannessen, S.I. (2003) Valproate: past, present, and future. *CNS drug reviews*, **9**, 199-216.
- Johansson, J.U., Pradhan, S., Lokteva, L.A., Woodling, N.S., Ko, N., Brown, H.D., Wang, Q., Loh, C., Cekanaviciute, E., Buckwalter, M., Manning-Bog, A.B. & Andreasson, K.I. (2013) Suppression of inflammation with conditional deletion of the prostaglandin E2 EP2 receptor in macrophages and brain microglia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **33**, 16016-16032.
- Jones, V.C., Birrell, M.A., Maher, S.A., Griffiths, M., Grace, M., O'Donnell, V.B., Clark, S.R. & Belvisi, M.G. (2015) Role of Ep and Ep receptors in airway microvascular leak induced by prostaglandin E. *British journal of pharmacology*.
- Jones, V.C., Birrell, M.A., Maher, S.A., Griffiths, M., Grace, M., O'Donnell, V.B., Clark, S.R. & Belvisi, M.G. (2016) Role of EP2 and EP4 receptors in airway microvascular leak induced by prostaglandin E2. *British journal of pharmacology*, **173**, 992-1004.
- Jory, J. (2016) Abnormal fatty acids in Canadian children with autism. *Nutrition*, **32**, 474-477.
- Ju, A., Hammerschmidt, K., Tantra, M., Krueger, D., Brose, N. & Ehrenreich, H. (2014) Juvenile manifestation of ultrasound communication deficits in the neuroligin-4 null mutant mouse model of autism. *Behavioural brain research*, **270**, 159-164.
- Kaech, S., Fischer, M., Doll, T. & Matus, A. (1997) Isoform specificity in the relationship of actin to dendritic spines. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **17**, 9565-9572.
- Kalkman, H.O. (2012) A review of the evidence for the canonical Wnt pathway in autism spectrum disorders. *Molecular autism*, **3**, 10.
- Kapitanovic Vidak, H., Catela Ivkovic, T., Vidak, Z. & Kapitanovic, S. (2017) COX-1 and COX-2 polymorphisms in susceptibility to cerebral palsy in very preterm infants. *Molecular neurobiology*, **54**, 930-938.

- Kasai, H., Fukuda, M., Watanabe, S., Hayashi-Takagi, A. & Noguchi, J. (2010) Structural dynamics of dendritic spines in memory and cognition. *Trends in neurosciences*, **33**, 121-129.
- Kaufmann, W.E., Worley, P.F., Pegg, J., Bremer, M. & Isakson, P. (1996) COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 2317-2321.
- Kaufmann, W.E., Worley, P.F., Taylor, C.V., Bremer, M. & Isakson, P.C. (1997) Cyclooxygenase-2 expression during rat neocortical development and in Rett syndrome. *Brain & development*, **19**, 25-34.
- Kawahara, K., Hohjoh, H., Inazumi, T., Tsuchiya, S. & Sugimoto, Y. (2015) Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. *Biochimica et biophysica acta*, **1851**, 414-421.
- Kawano, T., Anrather, J., Zhou, P., Park, L., Wang, G., Frys, K.A., Kunz, A., Cho, S., Orio, M. & Iadecola, C. (2006) Prostaglandin E2 EP1 receptors: downstream effectors of COX-2 neurotoxicity. *Nature medicine*, **12**, 225-229.
- Kelleher, R.J., 3rd & Bear, M.F. (2008) The autistic neuron: troubled translation? *Cell*, **135**, 401-406.
- Kim, D.K. & Jang, T.J. (2006) Cyclooxygenase-2 expression and effect of celecoxib in flurothyl-induced neonatal seizure. *International journal of experimental pathology*, **87**, 73-78.
- Kim, H.G., Kishikawa, S., Higgins, A.W., Seong, I.S., Donovan, D.J., Shen, Y., Lally, E., Weiss, L.A., Najm, J., Kutsche, K., Descartes, M., Holt, L., Braddock, S., Troxell, R., Kaplan, L., Volkmar, F., Klin, A., Tsatsanis, K., Harris, D.J., Noens, I., Pauls, D.L., Daly, M.J., MacDonald, M.E., Morton, C.C., Quade, B.J. & Gusella, J.F. (2008) Disruption of neurexin 1 associated with autism spectrum disorder. *American journal of human genetics*, **82**, 199-207.
- Kim, J. & Alger, B.E. (2004) Inhibition of cyclooxygenase-2 potentiates retrograde endocannabinoid effects in hippocampus. *Nature neuroscience*, **7**, 697-698.
- Kim, S.S., Meeker, J.D., Keil, A.P., Aung, M.T., Bommarito, P.A., Cantonwine, D.E., McElrath, T.F. & Ferguson, K.K. (2019) Exposure to 17 trace metals in pregnancy and associations with urinary oxidative stress biomarkers. *Environmental research*, **179**, 108854.

- Kimelman, D. & Xu, W. (2006) beta-catenin destruction complex: insights and questions from a structural perspective. *Oncogene*, **25**, 7482-7491.
- Kirkby, N.S., Lundberg, M.H., Harrington, L.S., Leadbeater, P.D., Milne, G.L., Potter, C.M., Al-Yamani, M., Adeyemi, O., Warner, T.D. & Mitchell, J.A. (2012) Cyclooxygenase-1, not cyclooxygenase-2, is responsible for physiological production of prostacyclin in the cardiovascular system. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 17597-17602.
- Kitaoka, S., Furuyashiki, T., Nishi, A., Shuto, T., Koyasu, S., Matsuoka, T., Miyasaka, M., Greengard, P. & Narumiya, S. (2007) Prostaglandin E2 acts on EP1 receptor and amplifies both dopamine D1 and D2 receptor signaling in the striatum. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **27**, 12900-12907.
- Koch, H., Huh, S.E., Elsen, F.P., Carroll, M.S., Hodge, R.D., Bedogni, F., Turner, M.S., Hevner, R.F. & Ramirez, J.M. (2010) Prostaglandin E2-induced synaptic plasticity in neocortical networks of organotypic slice cultures. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 11678-11687.
- Koleske, A.J. (2013) Molecular mechanisms of dendrite stability. *Nature reviews*, **14**, 536-550.
- Komprda, J., Komprdova, K., Dominguez-Romero, E., Mikes, O., Rihackova, K., Cupr, P., Cerna, M. & Scheringer, M. (2019) Dynamics of PCB exposure in the past 50years and recent high concentrations in human breast milk: Analysis of influencing factors using a physiologically based pharmacokinetic model. *The Science of the total environment*, **690**, 388-399.
- Konopka, A., Grajkowska, W., Ziemianska, K., Roszkowski, M., Daszkiewicz, P., Rysz, A., Marchel, A., Koperski, L., Wilczynski, G.M. & Dzwonek, J. (2013) Matrix metalloproteinase-9 (MMP-9) in human intractable epilepsy caused by focal cortical dysplasia. *Epilepsy research*, **104**, 45-58.
- Koop, M., Rilling, G., Herrmann, A. & Kretschmann, H.J. (1986) Volumetric development of the fetal telencephalon, cerebral cortex, diencephalon, and rhombencephalon including the cerebellum in man. *Bibliotheca anatomica*, 53-78.
- Koppen, G., Den Hond, E., Nelen, V., Van De Mieroop, E., Bruckers, L., Bilau, M., Keune, H., Van Larebeke, N., Covaci, A., Van De Weghe, H., Schroijs, C., Desager, K., Stalpaert, M., Baeyens, W. & Schoeters, G. (2009) Organochlorine and heavy metals in newborns: results from the Flemish Environment and Health Survey (FLEHS 2002-2006). *Environment international*, **35**, 1015-1022.

- Kosuge, Y., Miyagishi, H., Yoneoka, Y., Yoneda, K., Nango, H., Ishige, K. & Ito, Y. (2018) Pathophysiological role of prostaglandin E2-induced up-regulation of the EP2 receptor in motor neuron-like NSC-34 cells and lumbar motor neurons in ALS model mice. *Neurochemistry international*, **119**, 132-139.
- Koudelova, J. & Mourek, J. (1994) The lipid peroxidation in various parts of the rat brain: effect of age, hypoxia and hyperoxia. *Physiological research*, **43**, 169-173.
- Koussounadis, A., Langdon, S.P., Um, I.H., Harrison, D.J. & Smith, V.A. (2015) Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Sci Rep*, **5**, 10775.
- Koyama, R. & Ikegaya, Y. (2015) Microglia in the pathogenesis of autism spectrum disorders. *Neurosci Res*, **100**, 1-5.
- Koyama, Y. & Tohyama, M. (2012) A modified and highly sensitive Golgi-Cox method to enable complete and stable impregnation of embryonic neurons. *Journal of neuroscience methods*, **209**, 58-61.
- Kreisman, M.J., Song, C.I., Yip, K., Natale, B.V., Natale, D.R. & Breen, K.M. (2017) Androgens Mediate Sex-Dependent Gonadotropin Expression During Late Prenatal Development in the Mouse. *Endocrinology*, **158**, 2884-2894.
- Kumar, A., Swanwick, C.C., Johnson, N., Menashe, I., Basu, S.N., Bales, M.E. & Banerjee-Basu, S. (2011) A brain region-specific predictive gene map for autism derived by profiling a reference gene set. *PLoS One*, **6**, e28431.
- Kumar, S., Reynolds, K., Ji, Y., Gu, R., Rai, S. & Zhou, C.J. (2019) Impaired neurodevelopmental pathways in autism spectrum disorder: a review of signaling mechanisms and crosstalk. *J Neurodev Disord*, **11**, 10.
- Kwan, V., Unda, B.K. & Singh, K.K. (2016) Wnt signaling networks in autism spectrum disorder and intellectual disability. *J Neurodev Disord*, **8**, 45.
- Lai, J.K., Lerch, J.P., Doering, L.C., Foster, J.A. & Ellegood, J. (2016) Regional brain volumes changes in adult male FMR1-KO mouse on the FVB strain. *Neuroscience*, **318**, 12-21.
- Lai, K.O. & Ip, N.Y. (2013) Structural plasticity of dendritic spines: the underlying mechanisms and its dysregulation in brain disorders. *Biochimica et biophysica acta*, **1832**, 2257-2263.

- Lai, M.C., Lombardo, M.V. & Baron-Cohen, S. (2014) Autism. *Lancet*, **383**, 896-910.
- Langenbach, R., Morham, S.G., Tiano, H.F., Loftin, C.D., Ghanayem, B.I., Chulada, P.C., Mahler, J.F., Lee, C.A., Goulding, E.H., Kluckman, K.D., Kim, H.S. & Smithies, O. (1995) Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*, **83**, 483-492.
- Lante, F., Meunier, J., Guiramand, J., Maurice, T., Cavalier, M., de Jesus Ferreira, M.C., Aimar, R., Cohen-Solal, C., Vignes, M. & Barbanel, G. (2007) Neurodevelopmental damage after prenatal infection: role of oxidative stress in the fetal brain. *Free radical biology & medicine*, **42**, 1231-1245.
- Laumonnier, F., Bonnet-Brilhault, F., Gomot, M., Blanc, R., David, A., Moizard, M.P., Raynaud, M., Ronce, N., Lemonnier, E., Calvas, P., Laudier, B., Chelly, J., Fryns, J.P., Ropers, H.H., Hamel, B.C., Andres, C., Barthelemy, C., Moraine, C. & Briault, S. (2004) X-linked mental retardation and autism are associated with a mutation in the NLGN4 gene, a member of the neuroligin family. *American journal of human genetics*, **74**, 552-557.
- Lavaque, E., Mayen, A., Azcoitia, I., Tena-Sempere, M. & Garcia-Segura, L.M. (2006) Sex differences, developmental changes, response to injury and cAMP regulation of the mRNA levels of steroidogenic acute regulatory protein, cytochrome p450scc, and aromatase in the olivocerebellar system. *Journal of neurobiology*, **66**, 308-318.
- Le, T.D., Shirai, Y., Okamoto, T., Tatsukawa, T., Nagao, S., Shimizu, T. & Ito, M. (2010) Lipid signaling in cytosolic phospholipase A2 α -cyclooxygenase-2 cascade mediates cerebellar long-term depression and motor learning. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 3198-3203.
- LeBlanc, J.J. & Fagiolini, M. (2011) Autism: a "critical period" disorder? *Neural plasticity*, **2011**, 921680.
- Leblond, C.S., Heinrich, J., Delorme, R., Proepper, C., Betancur, C., Huguet, G., Konyukh, M., Chaste, P., Ey, E., Rastam, M., Anckarsater, H., Nygren, G., Gillberg, I.C., Melke, J., Toro, R., Regnault, B., Fauchereau, F., Mercati, O., Lemiere, N., Skuse, D., Poot, M., Holt, R., Monaco, A.P., Jarvela, I., Kantojarvi, K., Vanhala, R., Curran, S., Collier, D.A., Bolton, P., Chiocchetti, A., Klauck, S.M., Poustka, F., Freitag, C.M., Waltes, R., Kopp, M., Duketis, E., Bacchelli, E., Minopoli, F., Ruta, L., Battaglia, A., Mazzone, L., Maestrini, E., Sequeira, A.F., Oliveira, B., Vicente, A., Oliveira, G., Pinto, D., Scherer, S.W., Zelenika, D., Delepine, M., Lathrop, M., Bonneau, D., Guinchat, V., Devillard, F., Assouline, B., Mouren, M.C., Leboyer, M., Gillberg, C., Boeckers, T.M. & Bourgeron, T.

- (2012) Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. *PLoS genetics*, **8**, e1002521.
- Leclerc, J.L., Lampert, A.S., Diller, M.A. & Dore, S. (2015a) Genetic deletion of the prostaglandin E2 EP2 receptor subtype 3 improves anatomical and functional outcomes after intracerebral hemorrhage. *The European journal of neuroscience*, **41**, 1381-1391.
- Leclerc, J.L., Lampert, A.S., Diller, M.A., Immergluck, J.B. & Dore, S. (2015b) Prostaglandin E2 EP2 receptor deletion attenuates intracerebral hemorrhage-induced brain injury and improves functional recovery. *ASN neuro*, **7**.
- Lee, A.S., Azmitia, E.C. & Whitaker-Azmitia, P.M. (2017) Developmental microglial priming in postmortem autism spectrum disorder temporal cortex. *Brain, behavior, and immunity*, **62**, 193-202.
- Lee, F.H.F., Lai, T.K.Y., Su, P. & Liu, F. (2019) Altered cortical Cytoarchitecture in the Fmr1 knockout mouse. *Molecular brain*, **12**, 56.
- Lee, J.J., Wedow, R., Okbay, A., Kong, E., Maghzian, O., Zacher, M., Nguyen-Viet, T.A., Bowers, P., Sidorenko, J., Karlsson Linner, R., Fontana, M.A., Kundu, T., Lee, C., Li, H., Li, R., Royer, R., Timshel, P.N., Walters, R.K., Willoughby, E.A., Yengo, L., and Me Research, T., Cogent, Social Science Genetic Association, C., Alver, M., Bao, Y., Clark, D.W., Day, F.R., Furlotte, N.A., Joshi, P.K., Kemper, K.E., Kleinman, A., Langenberg, C., Magi, R., Trampush, J.W., Verma, S.S., Wu, Y., Lam, M., Zhao, J.H., Zheng, Z., Boardman, J.D., Campbell, H., Freese, J., Harris, K.M., Hayward, C., Herd, P., Kumari, M., Lencz, T., Luan, J., Malhotra, A.K., Metspalu, A., Milani, L., Ong, K.K., Perry, J.R.B., Porteous, D.J., Ritchie, M.D., Smart, M.C., Smith, B.H., Tung, J.Y., Wareham, N.J., Wilson, J.F., Beauchamp, J.P., Conley, D.C., Esko, T., Lehrer, S.F., Magnusson, P.K.E., Oskarsson, S., Pers, T.H., Robinson, M.R., Thom, K., Watson, C., Chabris, C.F., Meyer, M.N., Laibson, D.I., Yang, J., Johannesson, M., Koellinger, P.D., Turley, P., Visscher, P.M., Benjamin, D.J. & Cesarini, D. (2018) Gene discovery and polygenic prediction from a genome-wide association study of educational attainment in 1.1 million individuals. *Nature genetics*, **50**, 1112-1121.
- Lee, S.J., Romeo, R.D., Svenningsson, P., Campomanes, C.R., Allen, P.B., Greengard, P. & McEwen, B.S. (2004) Estradiol affects spinophilin protein differently in gonadectomized males and females. *Neuroscience*, **127**, 983-988.
- Lenz, K.M., Nugent, B.M., Haliyur, R. & McCarthy, M.M. (2013) Microglia are essential to masculinization of brain and behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **33**, 2761-2772.

- Leutner, S., Eckert, A. & Muller, W.E. (2001) ROS generation, lipid peroxidation and antioxidant enzyme activities in the aging brain. *Journal of neural transmission*, **108**, 955-967.
- Levin-Arama, M., Abraham, L., Waner, T., Harmelin, A., Steinberg, D.M., Lahav, T. & Harlev, M. (2016) Subcutaneous Compared with Intraperitoneal KetamineXylazine for Anesthesia of Mice. *Journal of the American Association for Laboratory Animal Science : JAALAS*, **55**, 794-800.
- Levisohn, L., Cronin-Golomb, A. & Schmahmann, J.D. (2000) Neuropsychological consequences of cerebellar tumour resection in children: cerebellar cognitive affective syndrome in a paediatric population. *Brain : a journal of neurology*, **123** (Pt 5), 1041-1050.
- Li, J., Serafin, E. & Baccei, M.L. (2018a) Prostaglandin Signaling Governs Spike Timing-Dependent Plasticity at Sensory Synapses onto Mouse Spinal Projection Neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **38**, 6628-6639.
- Li, L., Kim, H.T., Nellore, A., Patsoukis, N., Petkova, V., McDonough, S., Politikos, I., Nikiforow, S., Soiffer, R., Antin, J.H., Ballen, K., Cutler, C., Ritz, J. & Boussiotis, V.A. (2014) Prostaglandin E2 promotes survival of naive UCB T cells via the Wnt/beta-catenin pathway and alters immune reconstitution after UCBT. *Blood cancer journal*, **4**, e178.
- Li, M., Fallin, M.D., Riley, A., Landa, R., Walker, S.O., Silverstein, M., Caruso, D., Pearson, C., Kiang, S., Dahm, J.L., Hong, X., Wang, G., Wang, M.C., Zuckerman, B. & Wang, X. (2016) The Association of Maternal Obesity and Diabetes With Autism and Other Developmental Disabilities. *Pediatrics*, **137**, e20152206.
- Li, Q., Bozek, K., Xu, C., Guo, Y., Sun, J., Paabo, S., Sherwood, C.C., Hof, P.R., Ely, J.J., Li, Y., Willmitzer, L., Giavalisco, P. & Khaitovich, P. (2017) Changes in Lipidome Composition during Brain Development in Humans, Chimpanzees, and Macaque Monkeys. *Molecular biology and evolution*, **34**, 1155-1166.
- Li, Y., Missig, G., Finger, B.C., Landino, S.M., Alexander, A.J., Mokler, E.L., Robbins, J.O., Manasian, Y., Kim, W., Kim, K.S., McDougale, C.J., Carlezon, W.A., Jr. & Bolshakov, V.Y. (2018b) Maternal and Early Postnatal Immune Activation Produce Dissociable Effects on Neurotransmission in mPFC-Amygdala Circuits. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **38**, 3358-3372.

- Liang, X., Lin, L., Woodling, N.S., Wang, Q., Anacker, C., Pan, T., Merchant, M. & Andreasson, K. (2011) Signaling via the prostaglandin E(2) receptor EP4 exerts neuronal and vascular protection in a mouse model of cerebral ischemia. *The Journal of clinical investigation*, **121**, 4362-4371.
- Liang, X., Wang, Q., Hand, T., Wu, L., Breyer, R.M., Montine, T.J. & Andreasson, K. (2005) Deletion of the prostaglandin E2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 10180-10187.
- Liang, X., Wang, Q., Shi, J., Lokteva, L., Breyer, R.M., Montine, T.J. & Andreasson, K. (2008) The prostaglandin E2 EP2 receptor accelerates disease progression and inflammation in a model of amyotrophic lateral sclerosis. *Annals of neurology*, **64**, 304-314.
- Libbey, J.E., Sweeten, T.L., McMahon, W.M. & Fujinami, R.S. (2005) Autistic disorder and viral infections. *Journal of neurovirology*, **11**, 1-10.
- Liew, Z., Ritz, B., Virk, J. & Olsen, J. (2016) Maternal use of acetaminophen during pregnancy and risk of autism spectrum disorders in childhood: A Danish national birth cohort study. *Autism Res*, **9**, 951-958.
- Lim, C.S., Kim, H., Yu, N.K., Kang, S.J., Kim, T., Ko, H.G., Lee, J., Yang, J.E., Ryu, H.H., Park, T., Gim, J., Nam, H.J., Baek, S.H., Wegener, S., Schmitz, D., Boeckers, T.M., Lee, M.G., Kim, E., Lee, J.H., Lee, Y.S. & Kaang, B.K. (2017) Enhancing inhibitory synaptic function reverses spatial memory deficits in Shank2 mutant mice. *Neuropharmacology*, **112**, 104-112.
- Lim, H., Paria, B.C., Das, S.K., Dinchuk, J.E., Langenbach, R., Trzaskos, J.M. & Dey, S.K. (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell*, **91**, 197-208.
- Limperopoulos, C., Bassan, H., Gauvreau, K., Robertson, R.L., Jr., Sullivan, N.R., Benson, C.B., Avery, L., Stewart, J., Soul, J.S., Ringer, S.A., Volpe, J.J. & duPlessis, A.J. (2007) Does cerebellar injury in premature infants contribute to the high prevalence of long-term cognitive, learning, and behavioral disability in survivors? *Pediatrics*, **120**, 584-593.
- Lin, P.I., Chien, Y.L., Wu, Y.Y., Chen, C.H., Gau, S.S., Huang, Y.S., Liu, S.K., Tsai, W.C. & Chiu, Y.N. (2012) The WNT2 gene polymorphism associated with speech delay inherent to autism. *Research in developmental disabilities*, **33**, 1533-1540.

- Lin, T.Y., Lu, C.W., Wang, C.C., Huang, S.K. & Wang, S.J. (2014) Cyclooxygenase 2 inhibitor celecoxib inhibits glutamate release by attenuating the PGE2/EP2 pathway in rat cerebral cortex endings. *The Journal of pharmacology and experimental therapeutics*, **351**, 134-145.
- Lin, Y.C., Frei, J.A., Kilander, M.B., Shen, W. & Blatt, G.J. (2016) A Subset of Autism-Associated Genes Regulate the Structural Stability of Neurons. *Frontiers in cellular neuroscience*, **10**, 263.
- Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.H., Tan, Y., Zhang, Z., Lin, X. & He, X. (2002) Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell*, **108**, 837-847.
- Liu, L., Cao, Z., Chen, J., Li, R., Cao, Y., Zhu, C., Wu, K., Wu, J., Liu, F. & Zhu, Y. (2012a) Influenza A virus induces interleukin-27 through cyclooxygenase-2 and protein kinase A signaling. *The Journal of biological chemistry*, **287**, 11899-11910.
- Liu, P., Wu, C., Chang, X., Qi, X., Zheng, M. & Zhou, Z. (2016a) Adverse Associations of both Prenatal and Postnatal Exposure to Organophosphorous Pesticides with Infant Neurodevelopment in an Agricultural Area of Jiangsu Province, China. *Environmental health perspectives*, **124**, 1637-1643.
- Liu, S., Qiu, S., Lu, Y., Kanu, J.S., Li, R., Bai, Y., Zhu, X., Lei, J., Xu, N., Yu, Y., Liu, Y. & Jiang, H. (2016b) The rs251684 Variant of PLA2G4C Is Associated with Autism Spectrum Disorder in the Northeast Han Chinese Population. *Genetic testing and molecular biomarkers*, **20**, 747-752.
- Liu, X., Han, D., Somel, M., Jiang, X., Hu, H., Gujjarro, P., Zhang, N., Mitchell, A., Halene, T., Ely, J.J., Sherwood, C.C., Hof, P.R., Qiu, Z., Paabo, S., Akbarian, S. & Khaitovich, P. (2016c) Disruption of an Evolutionarily Novel Synaptic Expression Pattern in Autism. *PLoS biology*, **14**, e1002558.
- Liu, X.H., Kirschenbaum, A., Weinstein, B.M., Zaidi, M., Yao, S. & Levine, A.C. (2010) Prostaglandin E2 modulates components of the Wnt signaling system in bone and prostate cancer cells. *Biochem Biophys Res Commun*, **394**, 715-720.
- Liu, Y., Hu, Z., Xun, G., Peng, Y., Lu, L., Xu, X., Xiong, Z., Xia, L., Liu, D., Li, W., Zhao, J. & Xia, K. (2012b) Mutation analysis of the NRXN1 gene in a Chinese autism cohort. *Journal of psychiatric research*, **46**, 630-634.

- Liu, Z.H., Chuang, D.M. & Smith, C.B. (2011) Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. *Int J Neuropsychopharmacol*, **14**, 618-630.
- Loftin, C.D., Tiano, H.F. & Langenbach, R. (2002) Phenotypes of the COX-deficient mice indicate physiological and pathophysiological roles for COX-1 and COX-2. *Prostaglandins Other Lipid Mediat*, **68-69**, 177-185.
- Loftin, C.D., Trivedi, D.B., Tiano, H.F., Clark, J.A., Lee, C.A., Epstein, J.A., Morham, S.G., Breyer, M.D., Nguyen, M., Hawkins, B.M., Goulet, J.L., Smithies, O., Koller, B.H. & Langenbach, R. (2001) Failure of ductus arteriosus closure and remodeling in neonatal mice deficient in cyclooxygenase-1 and cyclooxygenase-2. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 1059-1064.
- Long, M., Ghisari, M., Kjeldsen, L., Wielsoe, M., Norgaard-Pedersen, B., Mortensen, E.L., Abdallah, M.W. & Bonfeld-Jorgensen, E.C. (2019) Autism spectrum disorders, endocrine disrupting compounds, and heavy metals in amniotic fluid: a case-control study. *Molecular autism*, **10**, 1.
- Loomes, R., Hull, L. & Mandy, W.P.L. (2017) What Is the Male-to-Female Ratio in Autism Spectrum Disorder? A Systematic Review and Meta-Analysis. *Journal of the American Academy of Child and Adolescent Psychiatry*, **56**, 466-474.
- Lord, C., Elsabbagh, M., Baird, G. & Veenstra-Vanderweele, J. (2018) Autism spectrum disorder. *Lancet*, **392**, 508-520.
- Lorenzo, A., Diaz, H., Carrer, H. & Caceres, A. (1992) Amygdala neurons in vitro: neurite growth and effects of estradiol. *Journal of neuroscience research*, **33**, 418-435.
- Lotan, A., Fenckova, M., Bralten, J., Alttoa, A., Dixon, L., Williams, R.W. & van der Voet, M. (2014) Neuroinformatic analyses of common and distinct genetic components associated with major neuropsychiatric disorders. *Frontiers in neuroscience*, **8**, 331.
- Loth, E., Murphy, D.G. & Spooren, W. (2016) Defining Precision Medicine Approaches to Autism Spectrum Disorders: Concepts and Challenges. *Frontiers in psychiatry*, **7**, 188.
- Lotta, L.T., Conrad, K., Cory-Slechta, D. & Schor, N.F. (2014) Cerebellar Purkinje cell p75 neurotrophin receptor and autistic behavior. *Translational psychiatry*, **4**, e416.
- Loynes, C.A., Lee, J.A., Robertson, A.L., Steel, M.J., Ellett, F., Feng, Y., Levy, B.D., Whyte, M.K.B. & Renshaw, S.A. (2018) PGE2 production at sites of tissue injury promotes an

- anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution in vivo. *Science advances*, **4**, eaar8320.
- Lu, D., Wang, D., Ni, R., Lin, Y., Feng, C., Xu, Q., Jia, X., Wang, G. & Zhou, Z. (2015) Organochlorine pesticides and their metabolites in human breast milk from Shanghai, China. *Environmental science and pollution research international*, **22**, 9293-9306.
- Lu, J.W., Wang, W.S., Zhou, Q., Gan, X.W., Myatt, L. & Sun, K. (2019) Activation of prostaglandin EP4 receptor attenuates the induction of cyclooxygenase-2 expression by EP2 receptor activation in human amnion fibroblasts: implications for parturition. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, **33**, 8148-8160.
- Luo, X., Zhu, Q., Zhang, J., Huang, Q., Xie, Z. & Cheng, Y. (2017) The Double Roles of the Prostaglandin E2 EP2 Receptor in Intracerebral Hemorrhage. *Current drug targets*, **18**, 1377-1385.
- Luzardo, O.P., Mahtani, V., Troyano, J.M., Alvarez de la Rosa, M., Padilla-Perez, A.I., Zumbado, M., Almeida, M., Burillo-Putze, G., Boada, C. & Boada, L.D. (2009) Determinants of organochlorine levels detectable in the amniotic fluid of women from Tenerife Island (Canary Islands, Spain). *Environmental research*, **109**, 607-613.
- Ma, D.Q., Whitehead, P.L., Menold, M.M., Martin, E.R., Ashley-Koch, A.E., Mei, H., Ritchie, M.D., DeLong, G.R., Abramson, R.K., Wright, H.H., Cuccaro, M.L., Hussman, J.P., Gilbert, J.R. & Pericak-Vance, M.A. (2005) Identification of significant association and gene-gene interaction of GABA receptor subunit genes in autism. *American journal of human genetics*, **77**, 377-388.
- Maenner, M.J., Shaw, K.A., Baio, J., EdS, Washington, A., Patrick, M., DiRienzo, M., Christensen, D.L., Wiggins, L.D., Pettygrove, S., Andrews, J.G., Lopez, M., Hudson, A., Baroud, T., Schwenk, Y., White, T., Rosenberg, C.R., Lee, L.C., Harrington, R.A., Huston, M., Hewitt, A., PhD, Esler, A., Hall-Lande, J., Poynter, J.N., Hallas-Muchow, L., Constantino, J.N., Fitzgerald, R.T., Zahorodny, W., Shenouda, J., Daniels, J.L., Warren, Z., Vehorn, A., Salinas, A., Durkin, M.S. & Dietz, P.M. (2020) Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. *Morbidity and mortality weekly report. Surveillance summaries*, **69**, 1-12.
- Maier, T., Guell, M. & Serrano, L. (2009) Correlation of mRNA and protein in complex biological samples. *FEBS letters*, **583**, 3966-3973.

- Maingret, V., Barthelet, G., Deforges, S., Jiang, N., Mulle, C. & Amedee, T. (2017) PGE2-EP3 signaling pathway impairs hippocampal presynaptic long-term plasticity in a mouse model of Alzheimer's disease. *Neurobiology of aging*, **50**, 13-24.
- Malkova, N.V., Yu, C.Z., Hsiao, E.Y., Moore, M.J. & Patterson, P.H. (2012) Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain, behavior, and immunity*, **26**, 607-616.
- Mancini, A.D. & Di Battista, J.A. (2011) The cardinal role of the phospholipase A(2)/cyclooxygenase-2/prostaglandin E synthase/prostaglandin E(2) (PCPP) axis in inflammostasis. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]*, **60**, 1083-1092.
- Manzari, N., Matvienko-Sikar, K., Baldoni, F., O'Keeffe, G.W. & Khashan, A.S. (2019) Prenatal maternal stress and risk of neurodevelopmental disorders in the offspring: a systematic review and meta-analysis. *Social psychiatry and psychiatric epidemiology*, **54**, 1299-1309.
- Mao, H., Pilaz, L.J., McMahon, J.J., Golzio, C., Wu, D., Shi, L., Katsanis, N. & Silver, D.L. (2015) Rbm8a haploinsufficiency disrupts embryonic cortical development resulting in microcephaly. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **35**, 7003-7018.
- Marks, A.R., Harley, K., Bradman, A., Kogut, K., Barr, D.B., Johnson, C., Calderon, N. & Eskenazi, B. (2010) Organophosphate pesticide exposure and attention in young Mexican-American children: the CHAMACOS study. *Environmental health perspectives*, **118**, 1768-1774.
- Martinez-Cerdeno, V. (2017) Dendrite and spine modifications in autism and related neurodevelopmental disorders in patients and animal models. *Developmental neurobiology*, **77**, 393-404.
- Martinez, M. (1992) Tissue levels of polyunsaturated fatty acids during early human development. *The Journal of pediatrics*, **120**, S129-138.
- Marui, T., Funatogawa, I., Koishi, S., Yamamoto, K., Matsumoto, H., Hashimoto, O., Jinde, S., Nishida, H., Sugiyama, T., Kasai, K., Watanabe, K., Kano, Y. & Kato, N. (2010) Association between autism and variants in the wingless-type MMTV integration site family member 2 (WNT2) gene. *Int J Neuropsychopharmacol*, **13**, 443-449.

- Maslinska, D., Kaliszek, A., Opertowska, J., Toborowicz, J., Deregowski, K. & Szukiewicz, D. (1999) Constitutive expression of cyclooxygenase-2 (COX-2) in developing brain. A. Choroid plexus in human fetuses. *Folia neuropathologica*, **37**, 287-291.
- Massicotte, G. (2000) Modification of glutamate receptors by phospholipase A2: its role in adaptive neural plasticity. *Cellular and molecular life sciences : CMLS*, **57**, 1542-1550.
- Matson, J.L. & Shoemaker, M. (2009) Intellectual disability and its relationship to autism spectrum disorders. *Research in developmental disabilities*, **30**, 1107-1114.
- Mattes, B., Weber, S., Peres, J., Chen, Q., Davidson, G., Houart, C. & Scholpp, S. (2012) Wnt3 and Wnt3a are required for induction of the mid-diencephalic organizer in the caudal forebrain. *Neural development*, **7**, 12.
- Matus, A., Ackermann, M., Pehling, G., Byers, H.R. & Fujiwara, K. (1982) High actin concentrations in brain dendritic spines and postsynaptic densities. *Proceedings of the National Academy of Sciences of the United States of America*, **79**, 7590-7594.
- Mawson, A.R. & Croft, A.M. (2019) Rubella Virus Infection, the Congenital Rubella Syndrome, and the Link to Autism. *International journal of environmental research and public health*, **16**.
- Mazaud-Guittot, S., Nicolas Nicolaz, C., Desdoits-Lethimonier, C., Coiffec, I., Ben Maamar, M., Balaguer, P., Kristensen, D.M., Chevrier, C., Lavoue, V., Poulain, P., Dejucq-Rainsford, N. & Jegou, B. (2013) Paracetamol, aspirin, and indomethacin induce endocrine disturbances in the human fetal testis capable of interfering with testicular descent. *The Journal of clinical endocrinology and metabolism*, **98**, E1757-1767.
- McCanlies, E.C., Fekedulegn, D., Mnatsakanova, A., Burchfiel, C.M., Sanderson, W.T., Charles, L.E. & Hertz-Picciotto, I. (2012) Parental occupational exposures and autism spectrum disorder. *Journal of autism and developmental disorders*, **42**, 2323-2334.
- McCullough, L., Wu, L., Haughey, N., Liang, X., Hand, T., Wang, Q., Breyer, R.M. & Andreasson, K. (2004) Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 257-268.
- McFarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.L., Bolivar, V.J. & Crawley, J.N. (2008) Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes, brain, and behavior*, **7**, 152-163.

- McKeague, I.W., Brown, A.S., Bao, Y., Hinkka-Yli-Salomaki, S., Huttunen, J. & Sourander, A. (2015) Autism with intellectual disability related to dynamics of head circumference growth during early infancy. *Biological psychiatry*, **77**, 833-840.
- McKinney, B.C., Grossman, A.W., Elisseou, N.M. & Greenough, W.T. (2005) Dendritic spine abnormalities in the occipital cortex of C57BL/6 Fmr1 knockout mice. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, **136B**, 98-102.
- McNaughton, C.H., Moon, J., Strawderman, M.S., Maclean, K.N., Evans, J. & Strupp, B.J. (2008) Evidence for social anxiety and impaired social cognition in a mouse model of fragile X syndrome. *Behavioral neuroscience*, **122**, 293-300.
- Medina, M.A., Andrade, V.M., Caracci, M.O., Avila, M.E., Verdugo, D.A., Vargas, M.F., Ugarte, G.D., Reyes, A.E., Opazo, C. & De Ferrari, G.V. (2018) Wnt/beta-catenin signaling stimulates the expression and synaptic clustering of the autism-associated Neuroligin 3 gene. *Translational psychiatry*, **8**, 45.
- Meguid, N.A., Atta, H.M., Gouda, A.S. & Khalil, R.O. (2008) Role of polyunsaturated fatty acids in the management of Egyptian children with autism. *Clinical biochemistry*, **41**, 1044-1048.
- Meier, C. (1976) Some observations on early myelination in the human spinal cord. Light and electron microscope study. *Brain research*, **104**, 21-32.
- Mercier, F., Kwon, Y.C. & Douet, V. (2012) Hippocampus/amygdala alterations, loss of heparan sulfates, fractones and ventricle wall reduction in adult BTBR T+ tf/J mice, animal model for autism. *Neuroscience letters*, **506**, 208-213.
- Merico, D., Isserlin, R., Stueker, O., Emili, A. & Bader, G.D. (2010) Enrichment map: a network-based method for gene-set enrichment visualization and interpretation. *PloS one*, **5**, e13984.
- Mi, Z., Abrahamson, E.E., Ryu, A.Y., Fish, K.N., Sweet, R.A., Mufson, E.J. & Ikonomic, M.D. (2017) Loss of precuneus dendritic spines immunopositive for spinophilin is related to cognitive impairment in early Alzheimer's disease. *Neurobiology of aging*, **55**, 159-166.
- Miller, V.M., Zhu, Y., Bucher, C., McGinnis, W., Ryan, L.K., Siegel, A. & Zalcman, S. (2013) Gestational flu exposure induces changes in neurochemicals, affiliative hormones and

- brainstem inflammation, in addition to autism-like behaviors in mice. *Brain, behavior, and immunity*, **33**, 153-163.
- Mineur, Y.S., Huynh, L.X. & Crusio, W.E. (2006) Social behavior deficits in the Fmr1 mutant mouse. *Behavioural brain research*, **168**, 172-175.
- Mineur, Y.S., Sluyter, F., de Wit, S., Oostra, B.A. & Crusio, W.E. (2002) Behavioral and neuroanatomical characterization of the Fmr1 knockout mouse. *Hippocampus*, **12**, 39-46.
- Minghetti, L. (2004) Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. *Journal of neuropathology and experimental neurology*, **63**, 901-910.
- Miodovnik, A., Engel, S.M., Zhu, C., Ye, X., Soorya, L.V., Silva, M.J., Calafat, A.M. & Wolff, M.S. (2011) Endocrine disruptors and childhood social impairment. *Neurotoxicology*, **32**, 261-267.
- Mirowska-Guzel, D., Gromadzka, G., Czlonkowski, A. & Czlonkowska, A. (2009) Association of MMP1, MMP3, MMP9, and MMP12 polymorphisms with risk and clinical course of multiple sclerosis in a Polish population. *Journal of neuroimmunology*, **214**, 113-117.
- Mitchell, S.R., Reiss, A.L., Tatusko, D.H., Ikuta, I., Kazmerski, D.B., Botti, J.A., Burnette, C.P. & Kates, W.R. (2009) Neuroanatomic alterations and social and communication deficits in monozygotic twins discordant for autism disorder. *The American journal of psychiatry*, **166**, 917-925.
- Mizutani, Y., Ohi, Y., Kimura, S., Miyazawa, K., Goto, S. & Haji, A. (2015) Effects of prostaglandin E2 on synaptic transmission in the rat spinal trigeminal subnucleus caudalis. *Brain research*, **1625**, 29-38.
- Moessner, R., Marshall, C.R., Sutcliffe, J.S., Skaug, J., Pinto, D., Vincent, J., Zwaigenbaum, L., Fernandez, B., Roberts, W., Szatmari, P. & Scherer, S.W. (2007) Contribution of SHANK3 mutations to autism spectrum disorder. *American journal of human genetics*, **81**, 1289-1297.
- Moldrich, R.X., Leanage, G., She, D., Dolan-Evans, E., Nelson, M., Reza, N. & Reutens, D.C. (2013) Inhibition of histone deacetylase in utero causes sociability deficits in postnatal mice. *Behavioural brain research*, **257**, 253-264.
- Morgan, J.T., Chana, G., Pardo, C.A., Achim, C., Semendeferi, K., Buckwalter, J., Courchesne, E. & Everall, I.P. (2010) Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biological psychiatry*, **68**, 368-376.

- Morita, I., Schindler, M., Regier, M.K., Otto, J.C., Hori, T., DeWitt, D.L. & Smith, W.L. (1995) Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *The Journal of biological chemistry*, **270**, 10902-10908.
- Moy, S.S., Nadler, J.J., Young, N.B., Perez, A., Holloway, L.P., Barbaro, R.P., Barbaro, J.R., Wilson, L.M., Threadgill, D.W., Lauder, J.M., Magnuson, T.R. & Crawley, J.N. (2007) Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behavioural brain research*, **176**, 4-20.
- Muller, N., Myint, A.M. & Schwarz, M.J. (2012) Inflammation in schizophrenia. *Advances in protein chemistry and structural biology*, **88**, 49-68.
- Muller, N. & Schwarz, M.J. (2008) COX-2 inhibition in schizophrenia and major depression. *Current pharmaceutical design*, **14**, 1452-1465.
- Murphy, D.D. & Segal, M. (1996) Regulation of dendritic spine density in cultured rat hippocampal neurons by steroid hormones. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **16**, 4059-4068.
- Nadeem, A., Ahmad, S.F., Al-Harbi, N.O., Attia, S.M., Alshammari, M.A., Alzahrani, K.S. & Bakheet, S.A. (2019) Increased oxidative stress in the cerebellum and peripheral immune cells leads to exaggerated autism-like repetitive behavior due to deficiency of antioxidant response in BTBR T+tf/J mice. *Progress in neuro-psychopharmacology & biological psychiatry*, **89**, 245-253.
- Nagiah, S., Phulukdaree, A., Naidoo, D., Ramcharan, K., Naidoo, R.N., Moodley, D. & Chuturgoon, A. (2015) Oxidative stress and air pollution exposure during pregnancy: A molecular assessment. *Human & experimental toxicology*, **34**, 838-847.
- Neri, M., Bello, S., Turillazzi, E. & Riezzo, I. (2015) Drugs of abuse in pregnancy, poor neonatal development, and future neurodegeneration. Is oxidative stress the culprit? *Current pharmaceutical design*, **21**, 1358-1368.
- Neta, G., Goldman, L.R., Barr, D., Sjodin, A., Apelberg, B.J., Witter, F.R. & Halden, R.U. (2010) Distribution and determinants of pesticide mixtures in cord serum using principal component analysis. *Environmental science & technology*, **44**, 5641-5648.
- Nicolini, C. & Fahnestock, M. (2018) The valproic acid-induced rodent model of autism. *Experimental neurology*, **299**, 217-227.

- Nimchinsky, E.A., Oberlander, A.M. & Svoboda, K. (2001) Abnormal development of dendritic spines in FMR1 knock-out mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **21**, 5139-5146.
- Ning, L.F., Yu, Y.Q., GuoJi, E.T., Kou, C.G., Wu, Y.H., Shi, J.P., Ai, L.Z. & Yu, Q. (2015) Meta-analysis of differentially expressed genes in autism based on gene expression data. *Genetics and molecular research : GMR*, **14**, 2146-2155.
- Nishihara, I., Minami, T., Watanabe, Y., Ito, S. & Hayaishi, O. (1995) Prostaglandin E2 stimulates glutamate release from synaptosomes of rat spinal cord. *Neuroscience letters*, **196**, 57-60.
- Nomiri, S., Hoshyar, R., Ambrosino, C., Tyler, C.R. & Mansouri, B. (2019) A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. *Environmental science and pollution research international*, **26**, 8459-8467.
- North, T.E., Babu, I.R., Vedder, L.M., Lord, A.M., Wishnok, J.S., Tannenbaum, S.R., Zon, L.I. & Goessling, W. (2010) PGE2-regulated wnt signaling and N-acetylcysteine are synergistically hepatoprotective in zebrafish acetaminophen injury. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 17315-17320.
- O'Neill, G.P. & Ford-Hutchinson, A.W. (1993) Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS letters*, **330**, 156-160.
- Oblak, A.L., Gibbs, T.T. & Blatt, G.J. (2010) Decreased GABA(B) receptors in the cingulate cortex and fusiform gyrus in autism. *Journal of neurochemistry*, **114**, 1414-1423.
- Ohi, K., Shimada, T., Yasuyama, T., Kimura, K., Uehara, T. & Kawasaki, Y. (2017) Spatial and temporal expression patterns of genes around nine neuroticism-associated loci. *Progress in neuro-psychopharmacology & biological psychiatry*, **77**, 164-171.
- Ohkawara, T., Katsuyama, T., Ida-Eto, M., Narita, N. & Narita, M. (2015) Maternal viral infection during pregnancy impairs development of fetal serotonergic neurons. *Brain & development*, **37**, 88-93.
- Ohno, H., Morikawa, Y. & Hirata, F. (1978) Studies on 15-hydroxyprostaglandin dehydrogenase with various prostaglandin analogues. *J Biochem*, **84**, 1485-1494.
- Ohta, K.I., Suzuki, S., Warita, K., Kaji, T., Kusaka, T. & Miki, T. (2017) Prolonged maternal separation attenuates BDNF-ERK signaling correlated with spine formation in the hippocampus during early brain development. *Journal of neurochemistry*, **141**, 179-194.

- Ohta, Y., Itoh, K., Yaoi, T., Tando, S., Fukui, K. & Fushiki, S. (2005) Spatiotemporal patterns of expression of IGSF4 in developing mouse nervous system. *Brain research. Developmental brain research*, **156**, 23-31.
- Okamoto, K., Hitora-Imamura, N., Hioki, H. & Ikegaya, Y. (2018) GABAergic malfunction in the anterior cingulate cortex underlying maternal immune activation-induced social deficits. *Journal of neuroimmunology*, **321**, 92-96.
- Okerlund, N.D. & Cheyette, B.N. (2011) Synaptic Wnt signaling-a contributor to major psychiatric disorders? *J Neurodev Disord*, **3**, 162-174.
- Oliveira, M.S., Furian, A.F., Royes, L.F., Figuera, M.R., Fiorenza, N.G., Castelli, M., Machado, P., Bohrer, D., Veiga, M., Ferreira, J., Cavaleiro, E.A. & Mello, C.F. (2008) Cyclooxygenase-2/PGE2 pathway facilitates pentylentetrazol-induced seizures. *Epilepsy research*, **79**, 14-21.
- Onay, H., Kacamak, D., Kavasoglu, A.N., Akgun, B., Yalcinli, M., Kose, S. & Ozbaran, B. (2016) Mutation analysis of the NRXN1 gene in autism spectrum disorders. *Balkan journal of medical genetics : BJMG*, **19**, 17-22.
- Ongono, J.S., Beranger, R., Baghdadli, A. & Mortamais, M. (2020) Pesticides used in Europe and autism spectrum disorder risk: can novel exposure hypotheses be formulated beyond organophosphates, organochlorines, pyrethroids and carbamates? - A systematic review. *Environmental research*, **187**, 109646.
- Ornoy, A. (2009) Valproic acid in pregnancy: how much are we endangering the embryo and fetus? *Reproductive toxicology*, **28**, 1-10.
- Oshodi, Y., Ojewunmi, O., Oshodi, T.A., Ijarogbe, G.T., Ogun, O.C., Aina, O.F. & Lesi, F. (2017) Oxidative stress markers and genetic polymorphisms of glutathione S-transferase T1, M1, and P1 in a subset of children with autism spectrum disorder in Lagos, Nigeria. *Nigerian journal of clinical practice*, **20**, 1161-1167.
- Osredkar, J., Gosar, D., Macek, J., Kumer, K., Fabjan, T., Finderle, P., Sterpin, S., Zupan, M. & Jekovec Vrhovsek, M. (2019) Urinary Markers of Oxidative Stress in Children with Autism Spectrum Disorder (ASD). *Antioxidants*, **8**.
- Ostrea, E.M., Jr., Bielawski, D.M., Posecion, N.C., Jr., Corrion, M., Villanueva-Uy, E., Bernardo, R.C., Jin, Y., Janisse, J.J. & Ager, J.W. (2009) Combined analysis of prenatal (maternal hair and blood) and neonatal (infant hair, cord blood and meconium) matrices

- to detect fetal exposure to environmental pesticides. *Environmental research*, **109**, 116-122.
- Oudin, A., Frondelius, K., Haglund, N., Kallen, K., Forsberg, B., Gustafsson, P. & Malmqvist, E. (2019) Prenatal exposure to air pollution as a potential risk factor for autism and ADHD. *Environment international*, **133**, 105149.
- Ouimet, C.C., Katona, I., Allen, P., Freund, T.F. & Greengard, P. (2004) Cellular and subcellular distribution of spinophilin, a PP1 regulatory protein that bundles F-actin in dendritic spines. *The Journal of comparative neurology*, **479**, 374-388.
- Pace, S., Rossi, A., Krauth, V., Dehm, F., Troisi, F., Bilancia, R., Weinigel, C., Rummler, S., Werz, O. & Sautebin, L. (2017) Sex differences in prostaglandin biosynthesis in neutrophils during acute inflammation. *Sci Rep*, **7**, 3759.
- Pagalan, L., Bickford, C., Weikum, W., Lanphear, B., Brauer, M., Lanphear, N., Hanley, G.E., Oberlander, T.F. & Winters, M. (2019) Association of Prenatal Exposure to Air Pollution With Autism Spectrum Disorder. *JAMA pediatrics*, **173**, 86-92.
- Palumbo, S. & Bosetti, F. (2013) Alterations of brain eicosanoid synthetic pathway in multiple sclerosis and in animal models of demyelination: role of cyclooxygenase-2. *Prostaglandins, leukotrienes, and essential fatty acids*, **89**, 273-278.
- Pang, Y., Dai, X., Roller, A., Carter, K., Paul, I., Bhatt, A.J., Lin, R.C. & Fan, L.W. (2016) Early Postnatal Lipopolysaccharide Exposure Leads to Enhanced Neurogenesis and Impaired Communicative Functions in Rats. *PLoS One*, **11**, e0164403.
- Panksepp, J.B., Jochman, K.A., Kim, J.U., Koy, J.J., Wilson, E.D., Chen, Q., Wilson, C.R. & Lahvis, G.P. (2007) Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. *PLoS One*, **2**, e351.
- Paolicelli, R.C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T.A., Guiducci, E., Dumas, L., Ragozzino, D. & Gross, C.T. (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science*, **333**, 1456-1458.
- Parellada, M., Llorente, C., Calvo, R., Gutierrez, S., Lazaro, L., Graell, M., Guisasola, M., Dorado, M.L., Boada, L., Romo, J., Dulin, E., Sanz, I., Arango, C. & Moreno, C. (2017) Randomized trial of omega-3 for autism spectrum disorders: Effect on cell membrane composition and behavior. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*, **27**, 1319-1330.

- Park, J.Y., Pillinger, M.H. & Abramson, S.B. (2006) Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. *Clinical immunology*, **119**, 229-240.
- Parker-Athill, E.C. & Tan, J. (2010) Maternal immune activation and autism spectrum disorder: interleukin-6 signaling as a key mechanistic pathway. *Neurosignals*, **18**, 113-128.
- Pastuszak, A.L., Schuler, L., Speck-Martins, C.E., Coelho, K.E., Cordello, S.M., Vargas, F., Brunoni, D., Schwarz, I.V., Larrandaburu, M., Safattle, H., Meloni, V.F. & Koren, G. (1998) Use of misoprostol during pregnancy and Mobius' syndrome in infants. *The New England journal of medicine*, **338**, 1881-1885.
- Patelia, E.M., Thakur, R. & Patel, J. (2013) Sex determination using polymerase chain reaction. *Scientific Reports*, **2**, 1-4.
- Peier, A.M., McIlwain, K.L., Kenneson, A., Warren, S.T., Paylor, R. & Nelson, D.L. (2000) (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. *Human molecular genetics*, **9**, 1145-1159.
- Perera, F., Vishnevetsky, J., Herbstman, J.B., Calafat, A.M., Xiong, W., Rauh, V. & Wang, S. (2012) Prenatal bisphenol a exposure and child behavior in an inner-city cohort. *Environmental health perspectives*, **120**, 1190-1194.
- Peretti, S., Mariano, M., Mazzocchetti, C., Mazza, M., Pino, M.C., Verrotti Di Pianella, A. & Valenti, M. (2019) Diet: the keystone of autism spectrum disorder? *Nutritional neuroscience*, **22**, 825-839.
- Perez-Pouchoulen, M., Yu, S.J., Roby, C.R., Bonsavage, N. & McCarthy, M.M. (2019) Regulatory Control of Microglial Phagocytosis by Estradiol and Prostaglandin E2 in the Developing Rat Cerebellum. *Cerebellum*, **18**, 882-895.
- Peter, S., Ten Brinke, M.M., Stedehouder, J., Reinelt, C.M., Wu, B., Zhou, H., Zhou, K., Boele, H.J., Kushner, S.A., Lee, M.G., Schmeisser, M.J., Boeckers, T.M., Schonewille, M., Hoebeek, F.E. & De Zeeuw, C.I. (2016) Dysfunctional cerebellar Purkinje cells contribute to autism-like behaviour in Shank2-deficient mice. *Nature communications*, **7**, 12627.
- Phiel, C.J., Zhang, F., Huang, E.Y., Guenther, M.G., Lazar, M.A. & Klein, P.S. (2001) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *The Journal of biological chemistry*, **276**, 36734-36741.

- Philippat, C., Barkoski, J., Tancredi, D.J., Elms, B., Barr, D.B., Ozonoff, S., Bennett, D.H. & Hertz-Picciotto, I. (2018) Prenatal exposure to organophosphate pesticides and risk of autism spectrum disorders and other non-typical development at 3 years in a high-risk cohort. *International journal of hygiene and environmental health*, **221**, 548-555.
- Philippot, G., Gordh, T., Fredriksson, A. & Viberg, H. (2017) Adult neurobehavioral alterations in male and female mice following developmental exposure to paracetamol (acetaminophen): characterization of a critical period. *Journal of applied toxicology : JAT*.
- Phillips, M. & Pozzo-Miller, L. (2015) Dendritic spine dysgenesis in autism related disorders. *Neuroscience letters*, **601**, 30-40.
- Pietropaolo, S., Guilleminot, A., Martin, B., D'Amato, F.R. & Crusio, W.E. (2011) Genetic-background modulation of core and variable autistic-like symptoms in Fmr1 knock-out mice. *PLoS One*, **6**, e17073.
- Pinzon-Parra, C., Vidal-Jimenez, B., Camacho-Abrego, I., Flores-Gomez, A.A., Rodriguez-Moreno, A. & Flores, G. (2019) Juvenile stress causes reduced locomotor behavior and dendritic spine density in the prefrontal cortex and basolateral amygdala in Sprague-Dawley rats. *Synapse*, **73**, e22066.
- Pohl, A., Jones, W.R., Marrus, N., Zhang, Y., Klin, A. & Constantino, J.N. (2019) Behavioral predictors of autism recurrence are genetically independent and influence social reciprocity: evidence that polygenic ASD risk is mediated by separable elements of developmental liability. *Translational psychiatry*, **9**, 202.
- Polioudakis, D., de la Torre-Ubieta, L., Langerman, J., Elkins, A.G., Shi, X., Stein, J.L., Vuong, C.K., Nichterwitz, S., Gevorgian, M., Opland, C.K., Lu, D., Connell, W., Ruzzo, E.K., Lowe, J.K., Hadzic, T., Hinz, F.I., Sabri, S., Lowry, W.E., Gerstein, M.B., Plath, K. & Geschwind, D.H. (2019) A Single-Cell Transcriptomic Atlas of Human Neocortical Development during Mid-gestation. *Neuron*, **103**, 785-801 e788.
- Poorani, R., Bhatt, A.N., Dwarakanath, B.S. & Das, U.N. (2016) COX-2, aspirin and metabolism of arachidonic, eicosapentaenoic and docosahexaenoic acids and their physiological and clinical significance. *European journal of pharmacology*, **785**, 116-132.
- Qasem, H., Al-Ayadhi, L., Al Dera, H. & El-Ansary, A. (2017) Increase of cytosolic phospholipase A2 as hydrolytic enzyme of phospholipids and autism cognitive, social and sensory dysfunction severity. *Lipids in health and disease*, **16**, 117.

- Qasem, H., Al-Ayadhi, L., Bjorklund, G., Chirumbolo, S. & El-Ansary, A. (2018) Impaired lipid metabolism markers to assess the risk of neuroinflammation in autism spectrum disorder. *Metabolic brain disease*, **33**, 1141-1153.
- Qin, W., Ho, L., Pompl, P.N., Peng, Y., Zhao, Z., Xiang, Z., Robakis, N.K., Shioi, J., Suh, J. & Pasinetti, G.M. (2003) Cyclooxygenase (COX)-2 and COX-1 potentiate beta-amyloid peptide generation through mechanisms that involve gamma-secretase activity. *The Journal of biological chemistry*, **278**, 50970-50977.
- Qinglin, L., Xin, W., Zhong, L., Fang, L., Cao, G. & Huang, P. (2017) A study on the anti-tumor mechanism of total flavonoids from Radix Tetrastigmae against additional cell line based on COX-2-mediated Wnt/beta-catenin signaling pathway. *Oncotarget*, **8**, 54304-54319.
- Qiu, J., Li, Q., Bell, K.A., Yao, X., Du, Y., Zhang, E., Yu, J.J., Yu, Y., Shi, Z. & Jiang, J. (2019a) Small-molecule inhibition of prostaglandin E receptor 2 impairs cyclooxygenase-associated malignant glioma growth. *British journal of pharmacology*, **176**, 1680-1699.
- Qiu, Z., Wang, Y. & Chen, J. (2019b) Perinatal exposure to nonylphenol induces microglia-mediated nitric oxide and prostaglandin E2 production in offspring hippocampus. *Toxicology letters*, **301**, 114-124.
- Quan, Y., Jiang, J. & Dingledine, R. (2013) EP2 receptor signaling pathways regulate classical activation of microglia. *The Journal of biological chemistry*, **288**, 9293-9302.
- Quilliam, R., Quilliam, S., Turnbull, M., Parkinson, S. & Oligbu, G. (2020) Catatonia as a presentation of autism in a child: a case report. *AIMS neuroscience*, **7**, 327-332.
- Rabaneda, L.G., Robles-Lanuza, E., Nieto-Gonzalez, J.L. & Scholl, F.G. (2014) Neurexin dysfunction in adult neurons results in autistic-like behavior in mice. *Cell reports*, **8**, 338-346.
- Rahbar, M.H., Samms-Vaughan, M., Pitcher, M.R., Bressler, J., Hessabi, M., Loveland, K.A., Christian, M.A., Grove, M.L., Shakespeare-Pellington, S., Beecher, C., McLaughlin, W. & Boerwinkle, E. (2016) Role of Metabolic Genes in Blood Aluminum Concentrations of Jamaican Children with and without Autism Spectrum Disorder. *International journal of environmental research and public health*, **13**.
- Rai-Bhagal, R., Ahmad, E., Li, H. & Crawford, D.A. (2018a) Microarray analysis of gene expression in the cyclooxygenase knockout mice - a connection to autism spectrum disorder. *The European journal of neuroscience*, **47**, 750-766.

- Rai-Bhagal, R., Wong, C., Kissoondoyal, A., Davidson, J., Li, H. & Crawford, D.A. (2018b) Maternal exposure to prostaglandin E2 modifies expression of Wnt genes in mouse brain - An autism connection. *Biochemistry and biophysics reports*, **14**, 43-53.
- Rai, D., Lee, B.K., Dalman, C., Golding, J., Lewis, G. & Magnusson, C. (2013) Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: population based case-control study. *Bmj*, **346**, f2059.
- Rakic, P. (2009) Evolution of the neocortex: a perspective from developmental biology. *Nature reviews*, **10**, 724-735.
- Ramos-Prats, A., Kolldorfer, J., Paolo, E., Zeidler, M., Schmid, G. & Ferraguti, F. (2019) An Appraisal of the Influence of the Metabotropic Glutamate 5 (mGlu5) Receptor on Sociability and Anxiety. *Frontiers in molecular neuroscience*, **12**, 30.
- Rangarajan, S., Rezonzew, G., Chumley, P., Fatima, H., Golovko, M., Feng, W., Hua, P. & Jaimes, E.A. (2019) COX-2 derived prostaglandins as mediators of the deleterious effects of nicotine in chronic kidney disease. *American journal of physiology. Renal physiology*.
- Rasalam, A.D., Hailey, H., Williams, J.H., Moore, S.J., Turnpenny, P.D., Lloyd, D.J. & Dean, J.C. (2005) Characteristics of fetal anticonvulsant syndrome associated autistic disorder. *Developmental medicine and child neurology*, **47**, 551-555.
- Rawal, N., Castelo-Branco, G., Sousa, K.M., Kele, J., Kobayashi, K., Okano, H. & Arenas, E. (2006) Dynamic temporal and cell type-specific expression of Wnt signaling components in the developing midbrain. *Experimental cell research*, **312**, 1626-1636.
- Ray, B., Long, J.M., Sokol, D.K. & Lahiri, D.K. (2011) Increased secreted amyloid precursor protein-alpha (sAPPalpha) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. *PLoS One*, **6**, e20405.
- Reemst, K., Noctor, S.C., Lucassen, P.J. & Hol, E.M. (2016) The Indispensable Roles of Microglia and Astrocytes during Brain Development. *Frontiers in human neuroscience*, **10**, 566.
- Reese, J., Paria, B.C., Brown, N., Zhao, X., Morrow, J.D. & Dey, S.K. (2000) Coordinated regulation of fetal and maternal prostaglandins directs successful birth and postnatal adaptation in the mouse. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 9759-9764.

- Rice, D. & Barone, S., Jr. (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environmental health perspectives*, **108 Suppl 3**, 511-533.
- Risher, W.C., Ustunkaya, T., Singh Alvarado, J. & Eroglu, C. (2014) Rapid Golgi analysis method for efficient and unbiased classification of dendritic spines. *PLoS One*, **9**, e107591.
- Riva, D. & Giorgi, C. (2000) The cerebellum contributes to higher functions during development: evidence from a series of children surgically treated for posterior fossa tumours. *Brain : a journal of neurology*, **123 (Pt 5)**, 1051-1061.
- RK, C.Y., Merico, D., Bookman, M., J, L.H., Thiruvahindrapuram, B., Patel, R.V., Whitney, J., Deflaux, N., Bingham, J., Wang, Z., Pellecchia, G., Buchanan, J.A., Walker, S., Marshall, C.R., Uddin, M., Zarrei, M., Deneault, E., D'Abate, L., Chan, A.J., Koyanagi, S., Paton, T., Pereira, S.L., Hoang, N., Engchuan, W., Higginbotham, E.J., Ho, K., Lamoureux, S., Li, W., MacDonald, J.R., Nalpathamkalam, T., Sung, W.W., Tsoi, F.J., Wei, J., Xu, L., Tasse, A.M., Kirby, E., Van Etten, W., Twigger, S., Roberts, W., Drmic, I., Jilderda, S., Modi, B.M., Kellam, B., Szego, M., Cytrynbaum, C., Weksberg, R., Zwaigenbaum, L., Woodbury-Smith, M., Brian, J., Senman, L., Iaboni, A., Doyle-Thomas, K., Thompson, A., Chrysler, C., Leef, J., Savion-Lemieux, T., Smith, I.M., Liu, X., Nicolson, R., Seifer, V., Fedele, A., Cook, E.H., Dager, S., Estes, A., Gallagher, L., Malow, B.A., Parr, J.R., Spence, S.J., Vorstman, J., Frey, B.J., Robinson, J.T., Strug, L.J., Fernandez, B.A., Elsabbagh, M., Carter, M.T., Hallmayer, J., Knoppers, B.M., Anagnostou, E., Szatmari, P., Ring, R.H., Glazer, D., Pletcher, M.T. & Scherer, S.W. (2017) Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nature neuroscience*, **20**, 602-611.
- Roberts, A.L., Lyall, K., Hart, J.E., Laden, F., Just, A.C., Bobb, J.F., Koenen, K.C., Ascherio, A. & Weisskopf, M.G. (2013) Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants. *Environmental health perspectives*, **121**, 978-984.
- Roberts, E.M., English, P.B., Grether, J.K., Windham, G.C., Somberg, L. & Wolff, C. (2007) Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley. *Environmental health perspectives*, **115**, 1482-1489.
- Robertson, C.E., Ratai, E.M. & Kanwisher, N. (2016) Reduced GABAergic Action in the Autistic Brain. *Current biology : CB*, **26**, 80-85.

- Rochester, J.R. (2013) Bisphenol A and human health: a review of the literature. *Reproductive toxicology*, **42**, 132-155.
- Rodier, P.M. (1980) Chronology of neuron development: animal studies and their clinical implications. *Developmental medicine and child neurology*, **22**, 525-545.
- Rodriguez-Dozal, S., Riojas Rodriguez, H., Hernandez-Avila, M., Van Oostdam, J., Weber, J.P., Needham, L.L. & Trip, L. (2012) Persistent organic pollutant concentrations in first birth mothers across Mexico. *Journal of exposure science & environmental epidemiology*, **22**, 60-69.
- Rodriguez, M., Domingo, E., Municio, C., Alvarez, Y., Hugo, E., Fernandez, N. & Sanchez Crespo, M. (2014) Polarization of the innate immune response by prostaglandin E2: a puzzle of receptors and signals. *Molecular pharmacology*, **85**, 187-197.
- Rohlman, D.S., Arcury, T.A., Quandt, S.A., Lasarev, M., Rothlein, J., Travers, R., Tamulinas, A., Scherer, J., Early, J., Marin, A., Phillips, J. & McCauley, L. (2005) Neurobehavioral performance in preschool children from agricultural and non-agricultural communities in Oregon and North Carolina. *Neurotoxicology*, **26**, 589-598.
- Romano, C., van den Pol, A.N. & O'Malley, K.L. (1996) Enhanced early developmental expression of the metabotropic glutamate receptor mGluR5 in rat brain: protein, mRNA splice variants, and regional distribution. *The Journal of comparative neurology*, **367**, 403-412.
- Ronald, A. & Hoekstra, R. (2014) Progress in Understanding the Causes of Autism Spectrum Disorders and Autistic Traits: Twin Studies from 1977 to the Present Day. In Rhee, S.H., Ronald, A. (eds) *Behavior Genetics of Psychopathology*. Springer New York, pp. 33-65.
- Ros-Demarize, R., Bradley, C., Kanne, S.M., Warren, Z., Boan, A., Lajonchere, C., Park, J. & Carpenter, L.A. (2020) ASD symptoms in toddlers and preschoolers: An examination of sex differences. *Autism Res*, **13**, 157-166.
- Rose, J.W., Hill, K.E., Watt, H.E. & Carlson, N.G. (2004) Inflammatory cell expression of cyclooxygenase-2 in the multiple sclerosis lesion. *Journal of neuroimmunology*, **149**, 40-49.
- Rosenberg, R.E., Law, J.K., Yenokyan, G., McGready, J., Kaufmann, W.E. & Law, P.A. (2009) Characteristics and concordance of autism spectrum disorders among 277 twin pairs. *Archives of pediatrics & adolescent medicine*, **163**, 907-914.

- Roullet, F.I., Wollaston, L., Decatanzaro, D. & Foster, J.A. (2010) Behavioral and molecular changes in the mouse in response to prenatal exposure to the anti-epileptic drug valproic acid. *Neuroscience*, **170**, 514-522.
- Roumier, A., Bechade, C., Poncer, J.C., Smalla, K.H., Tomasello, E., Vivier, E., Gundelfinger, E.D., Triller, A. & Bessis, A. (2004) Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **24**, 11421-11428.
- Roumier, A., Pascual, O., Bechade, C., Wakselman, S., Poncer, J.C., Real, E., Triller, A. & Bessis, A. (2008) Prenatal activation of microglia induces delayed impairment of glutamatergic synaptic function. *PLoS One*, **3**, e2595.
- Rudelli, R.D., Brown, W.T., Wisniewski, K., Jenkins, E.C., Laure-Kamionowska, M., Connell, F. & Wisniewski, H.M. (1985) Adult fragile X syndrome. Clinico-neuropathologic findings. *Acta neuropathologica*, **67**, 289-295.
- Russell, G., Steer, C. & Golding, J. (2011) Social and demographic factors that influence the diagnosis of autistic spectrum disorders. *Social psychiatry and psychiatric epidemiology*, **46**, 1283-1293.
- Ruzzo, E.K., Perez-Cano, L., Jung, J.Y., Wang, L.K., Kashef-Haghighi, D., Hartl, C., Singh, C., Xu, J., Hoekstra, J.N., Leventhal, O., Leppa, V.M., Gandal, M.J., Paskov, K., Stockham, N., Polioudakis, D., Lowe, J.K., Prober, D.A., Geschwind, D.H. & Wall, D.P. (2019) Inherited and De Novo Genetic Risk for Autism Impacts Shared Networks. *Cell*, **178**, 850-866 e826.
- Sagiv, S.K., Harris, M.H., Gunier, R.B., Kogut, K.R., Harley, K.G., Deardorff, J., Bradman, A., Holland, N. & Eskenazi, B. (2018) Prenatal Organophosphate Pesticide Exposure and Traits Related to Autism Spectrum Disorders in a Population Living in Proximity to Agriculture. *Environmental health perspectives*, **126**, 047012.
- Saint-Dizier, M., Guyader-Joly, C., Charpigny, G., Grimard, B., Humblot, P. & Ponter, A.A. (2011) Expression of enzymes involved in the synthesis of prostaglandin E2 in bovine in vitro-produced embryos. *Zygote (Cambridge, England)*, **19**, 277-283.
- Sala, C., Vicidomini, C., Bigi, I., Mossa, A. & Verpelli, C. (2015) Shank synaptic scaffold proteins: keys to understanding the pathogenesis of autism and other synaptic disorders. *Journal of neurochemistry*, **135**, 849-858.

- Salomons, G.S., van Dooren, S.J., Verhoeven, N.M., Marsden, D., Schwartz, C., Cecil, K.M., DeGrauw, T.J. & Jakobs, C. (2003) X-linked creatine transporter defect: an overview. *Journal of inherited metabolic disease*, **26**, 309-318.
- Sanchez-Camacho, C., Rodriguez, J., Ruiz, J.M., Trousse, F. & Bovolenta, P. (2005) Morphogens as growth cone signalling molecules. *Brain research. Brain research reviews*, **49**, 242-252.
- Sang, N. & Chen, C. (2006) Lipid signaling and synaptic plasticity. *Neuroscientist*, **12**, 425-434.
- Sang, N., Zhang, J., Marcheselli, V., Bazan, N.G. & Chen, C. (2005) Postsynaptically synthesized prostaglandin E2 (PGE2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **25**, 9858-9870.
- Sankoorikal, G.M., Kaercher, K.A., Boon, C.J., Lee, J.K. & Brodtkin, E.S. (2006) A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. *Biological psychiatry*, **59**, 415-423.
- Sare, R.M., Figueroa, C., Lemons, A., Loutaev, I. & Beebe Smith, C. (2019) Comparative Behavioral Phenotypes of Fmr1 KO, Fxr2 Het, and Fmr1 KO/Fxr2 Het Mice. *Brain sciences*, **9**.
- Sare, R.M., Harkless, L., Levine, M., Torossian, A., Sheeler, C.A. & Smith, C.B. (2017) Deficient Sleep in Mouse Models of Fragile X Syndrome. *Frontiers in molecular neuroscience*, **10**, 280.
- Sato, D., Lionel, A.C., Leblond, C.S., Prasad, A., Pinto, D., Walker, S., O'Connor, I., Russell, C., Drmic, I.E., Hamdan, F.F., Michaud, J.L., Endris, V., Roeth, R., Delorme, R., Huguet, G., Leboyer, M., Rastam, M., Gillberg, C., Lathrop, M., Stavropoulos, D.J., Anagnostou, E., Weksberg, R., Fombonne, E., Zwaigenbaum, L., Fernandez, B.A., Roberts, W., Rappold, G.A., Marshall, C.R., Bourgeron, T., Szatmari, P. & Scherer, S.W. (2012) SHANK1 Deletions in Males with Autism Spectrum Disorder. *American journal of human genetics*, **90**, 879-887.
- Satoh, A., Nakanishi, H., Obaishi, H., Wada, M., Takahashi, K., Satoh, K., Hirao, K., Nishioka, H., Hata, Y., Mizoguchi, A. & Takai, Y. (1998) Neurabin-II/spinophilin. An actin filament-binding protein with one pdz domain localized at cadherin-based cell-cell adhesion sites. *The Journal of biological chemistry*, **273**, 3470-3475.

- Satterstrom, F.K., Kosmicki, J.A., Wang, J., Breen, M.S., De Rubeis, S., An, J.Y., Peng, M., Collins, R., Grove, J., Klei, L., Stevens, C., Reichert, J., Mulhern, M.S., Artomov, M., Gerges, S., Sheppard, B., Xu, X., Bhaduri, A., Norman, U., Brand, H., Schwartz, G., Nguyen, R., Guerrero, E.E., Dias, C., Autism Sequencing, C., i, P.-B.C., Betancur, C., Cook, E.H., Gallagher, L., Gill, M., Sutcliffe, J.S., Thurm, A., Zwick, M.E., Borglum, A.D., State, M.W., Cicek, A.E., Talkowski, M.E., Cutler, D.J., Devlin, B., Sanders, S.J., Roeder, K., Daly, M.J. & Buxbaum, J.D. (2020) Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. *Cell*, **180**, 568-584 e523.
- Scattoni, M.L., Gandhi, S.U., Ricceri, L. & Crawley, J.N. (2008) Unusual repertoire of vocalizations in the BTBR T+tf/J mouse model of autism. *PLoS One*, **3**, e3067.
- Scattoni, M.L., Ricceri, L. & Crawley, J.N. (2011) Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes, brain, and behavior*, **10**, 44-56.
- Schaafsma, S.M., Gagnidze, K., Reyes, A., Norstedt, N., Mansson, K., Francis, K. & Pfaff, D.W. (2017) Sex-specific gene-environment interactions underlying ASD-like behaviors. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 1383-1388.
- Schafer, D.P., Lehrman, E.K., Kautzman, A.G., Koyama, R., Mardinly, A.R., Yamasaki, R., Ransohoff, R.M., Greenberg, M.E., Barres, B.A. & Stevens, B. (2012) Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*, **74**, 691-705.
- Schafer, D.P., Lehrman, E.K. & Stevens, B. (2013) The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS. *Glia*, **61**, 24-36.
- Schmahmann, J.D. (2019) The cerebellum and cognition. *Neuroscience letters*, **688**, 62-75.
- Schmeisser, M.J., Ey, E., Wegener, S., Bockmann, J., Stempel, A.V., Kuebler, A., Janssen, A.L., Udvardi, P.T., Shibani, E., Spilker, C., Balschun, D., Skryabin, B.V., Dieck, S., Smalla, K.H., Montag, D., Leblond, C.S., Faure, P., Torquet, N., Le Sourd, A.M., Toro, R., Grubbrucker, A.M., Shoichet, S.A., Schmitz, D., Kreutz, M.R., Bourgeron, T., Gundelfinger, E.D. & Boeckers, T.M. (2012) Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. *Nature*, **486**, 256-260.
- Schmidt, A.F., Kannan, P.S., Chougnet, C.A., Danzer, S.C., Miller, L.A., Jobe, A.H. & Kallapur, S.G. (2016) Intra-amniotic LPS causes acute neuroinflammation in preterm rhesus macaques. *Journal of neuroinflammation*, **13**, 238.

- Schultz, S.T. & Gould, G.G. (2016) Acetaminophen Use for Fever in Children Associated with Autism Spectrum Disorder. *Autism-open access*, **6**.
- Schultz, S.T., Klonoff-Cohen, H.S., Wingard, D.L., Akshoomoff, N.A., Macera, C.A., Ji, M. & Bacher, C. (2006) Breastfeeding, infant formula supplementation, and Autistic Disorder: the results of a parent survey. *International breastfeeding journal*, **1**, 16.
- Schwartzter, J.J., Careaga, M., Onore, C.E., Rushakoff, J.A., Berman, R.F. & Ashwood, P. (2013) Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. *Translational psychiatry*, **3**, e240.
- Scott, R.B., Stoodley, C.J., Anslow, P., Paul, C., Stein, J.F., Sugden, E.M. & Mitchell, C.D. (2001) Lateralized cognitive deficits in children following cerebellar lesions. *Developmental medicine and child neurology*, **43**, 685-691.
- Sealey, L.A., Hughes, B.W., Sriskanda, A.N., Guest, J.R., Gibson, A.D., Johnson-Williams, L., Pace, D.G. & Bagasra, O. (2016) Environmental factors in the development of autism spectrum disorders. *Environment international*, **88**, 288-298.
- Seeds, N.W. & Vatter, A.E. (1971) Synaptogenesis in reaggregating brain cell culture. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 3219-3222.
- Selevan, S.G., Kimmel, C.A. & Mendola, P. (2000) Identifying critical windows of exposure for children's health. *Environmental health perspectives*, **108 Suppl 3**, 451-455.
- Selimbeyoglu, A., Kim, C.K., Inoue, M., Lee, S.Y., Hong, A.S.O., Kauvar, I., Ramakrishnan, C., Fenno, L.E., Davidson, T.J., Wright, M. & Deisseroth, K. (2017) Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. *Science translational medicine*, **9**.
- Semple, B.D., Blomgren, K., Gimlin, K., Ferriero, D.M. & Noble-Haeusslein, L.J. (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Progress in neurobiology*, **106-107**, 1-16.
- Sengupta, P. (2013) The Laboratory Rat: Relating Its Age With Human's. *International journal of preventive medicine*, **4**, 624-630.

- Sgaier, S.K., Millet, S., Villanueva, M.P., Berenshteyn, F., Song, C. & Joyner, A.L. (2005) Morphogenetic and cellular movements that shape the mouse cerebellum; insights from genetic fate mapping. *Neuron*, **45**, 27-40.
- Sharma, S.R., Gonda, X. & Tarazi, F.I. (2018) Autism Spectrum Disorder: Classification, diagnosis and therapy. *Pharmacology & therapeutics*, **190**, 91-104.
- Shaw, K.N., Commins, S. & O'Mara, S.M. (2003) Deficits in spatial learning and synaptic plasticity induced by the rapid and competitive broad-spectrum cyclooxygenase inhibitor ibuprofen are reversed by increasing endogenous brain-derived neurotrophic factor. *The European journal of neuroscience*, **17**, 2438-2446.
- Shepherd, G.M. (1996) The dendritic spine: a multifunctional integrative unit. *J Neurophysiol*, **75**, 2197-2210.
- Shi, J., Johansson, J., Woodling, N.S., Wang, Q., Montine, T.J. & Andreasson, K. (2010) The prostaglandin E2 E-prostanoid 4 receptor exerts anti-inflammatory effects in brain innate immunity. *Journal of immunology*, **184**, 7207-7218.
- Shi, J., Wang, Q., Johansson, J.U., Liang, X., Woodling, N.S., Priyam, P., Loui, T.M., Merchant, M., Breyer, R.M., Montine, T.J. & Andreasson, K. (2012) Inflammatory prostaglandin E2 signaling in a mouse model of Alzheimer disease. *Annals of neurology*, **72**, 788-798.
- Shi, L., Smith, S.E., Malkova, N., Tse, D., Su, Y. & Patterson, P.H. (2009) Activation of the maternal immune system alters cerebellar development in the offspring. *Brain, behavior, and immunity*, **23**, 116-123.
- Shibayama, A., Cook, E.H., Jr., Feng, J., Glanzmann, C., Yan, J., Craddock, N., Jones, I.R., Goldman, D., Heston, L.L. & Sommer, S.S. (2004) MECP2 structural and 3'-UTR variants in schizophrenia, autism and other psychiatric diseases: a possible association with autism. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, **128B**, 50-53.
- Shie, F.S., Breyer, R.M. & Montine, T.J. (2005) Microglia lacking E Prostanoid Receptor subtype 2 have enhanced Abeta phagocytosis yet lack Abeta-activated neurotoxicity. *The American journal of pathology*, **166**, 1163-1172.
- Shimizu, E., Yazu, H., Satake, Y., Fukagawa, K., Aketa, N., Murat, D., Okada, N. & Fujishima, H. (2020) The Effect of Cytokine-Stimulation and Pharmacologic Intervention on PGE2 Production in Primary Human Conjunctival and Corneal Cells. *Ocular immunology and inflammation*, **28**, 384-390.

- Shiow, L.R., Favrais, G., Schirmer, L., Schang, A.L., Cipriani, S., Andres, C., Wright, J.N., Nobuta, H., Fleiss, B., Gressens, P. & Rowitch, D.H. (2017) Reactive astrocyte COX2-PGE2 production inhibits oligodendrocyte maturation in neonatal white matter injury. *Glia*, **65**, 2024-2037.
- Shpileva, S., Melnyk, S., Pavliv, O., Pogribny, I. & Jill James, S. (2018) Overexpression of LINE-1 Retrotransposons in Autism Brain. *Molecular neurobiology*, **55**, 1740-1749.
- Shukuri, M., Takashima-Hirano, M., Tokuda, K., Takashima, T., Matsumura, K., Inoue, O., Doi, H., Suzuki, M., Watanabe, Y. & Onoe, H. (2011) In vivo expression of cyclooxygenase-1 in activated microglia and macrophages during neuroinflammation visualized by PET with ¹¹C-ketoprofen methyl ester. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, **52**, 1094-1101.
- Sidhu, H., Dansie, L.E., Hickmott, P.W., Ethell, D.W. & Ethell, I.M. (2014) Genetic removal of matrix metalloproteinase 9 rescues the symptoms of fragile x syndrome in a mouse model. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **34**, 9867-9879.
- Sillitoe, R.V. & Joyner, A.L. (2007) Morphology, molecular codes, and circuitry produce the three-dimensional complexity of the cerebellum. *Annual review of cell and developmental biology*, **23**, 549-577.
- Silver, M.K., Shao, J., Chen, M., Xia, Y., Lozoff, B. & Meeker, J.D. (2015) Distribution and Predictors of Pesticides in the Umbilical Cord Blood of Chinese Newborns. *International journal of environmental research and public health*, **13**.
- Silverman, J.L., Tolu, S.S., Barkan, C.L. & Crawley, J.N. (2010) Repetitive self-grooming behavior in the BTBR mouse model of autism is blocked by the mGluR5 antagonist MPEP. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, **35**, 976-989.
- Silverman, J.L., Turner, S.M., Barkan, C.L., Tolu, S.S., Saxena, R., Hung, A.Y., Sheng, M. & Crawley, J.N. (2011) Sociability and motor functions in Shank1 mutant mice. *Brain research*, **1380**, 120-137.
- Simerly, R.B. (1998) Organization and regulation of sexually dimorphic neuroendocrine pathways. *Behavioural brain research*, **92**, 195-203.

- Skafidas, E., Testa, R., Zantomio, D., Chana, G., Everall, I.P. & Pantelis, C. (2014) Predicting the diagnosis of autism spectrum disorder using gene pathway analysis. *Molecular psychiatry*, **19**, 504-510.
- Slawinski, B.L., Talge, N., Ingersoll, B., Smith, A., Glazier, A., Kerver, J., Paneth, N. & Racicot, K. (2018) Maternal cytomegalovirus sero-positivity and autism symptoms in children. *American journal of reproductive immunology*, **79**, e12840.
- Smith, C.C., Smith, L.A., Bredemann, T.M. & McMahon, L.L. (2016) 17beta estradiol recruits GluN2B-containing NMDARs and ERK during induction of long-term potentiation at temporoammonic-CA1 synapses. *Hippocampus*, **26**, 110-117.
- Sokol, D.K., Chen, D., Farlow, M.R., Dunn, D.W., Maloney, B., Zimmer, J.A. & Lahiri, D.K. (2006) High levels of Alzheimer beta-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. *Journal of child neurology*, **21**, 444-449.
- Song, L., Zheng, J., Li, H., Jia, N., Suo, Z., Cai, Q., Bai, Z., Cheng, D. & Zhu, Z. (2009) Prenatal stress causes oxidative damage to mitochondrial DNA in hippocampus of offspring rats. *Neurochemical research*, **34**, 739-745.
- Soorya, L., Kolevzon, A., Zweifach, J., Lim, T., Dobry, Y., Schwartz, L., Frank, Y., Wang, A.T., Cai, G., Parkhomenko, E., Halpern, D., Grodberg, D., Angarita, B., Willner, J.P., Yang, A., Canitano, R., Chaplin, W., Betancur, C. & Buxbaum, J.D. (2013) Prospective investigation of autism and genotype-phenotype correlations in 22q13 deletion syndrome and SHANK3 deficiency. *Molecular autism*, **4**, 18.
- Sousa, K.M., Villaescusa, J.C., Cajanek, L., Ondr, J.K., Castelo-Branco, G., Hofstra, W., Bryja, V., Palmberg, C., Bergman, T., Wainwright, B., Lang, R.A. & Arenas, E. (2010) Wnt2 regulates progenitor proliferation in the developing ventral midbrain. *The Journal of biological chemistry*, **285**, 7246-7253.
- Spaan, S., Pronk, A., Koch, H.M., Jusko, T.A., Jaddoe, V.W., Shaw, P.A., Tiemeier, H.M., Hofman, A., Pierik, F.H. & Longnecker, M.P. (2015) Reliability of concentrations of organophosphate pesticide metabolites in serial urine specimens from pregnancy in the Generation R Study. *Journal of exposure science & environmental epidemiology*, **25**, 286-294.
- Spencer, C.M., Alekseyenko, O., Serysheva, E., Yuva-Paylor, L.A. & Paylor, R. (2005) Altered anxiety-related and social behaviors in the Fmr1 knockout mouse model of fragile X syndrome. *Genes, brain, and behavior*, **4**, 420-430.

- Sram, R.J., Veleminsky, M., Jr., Veleminsky, M., Sr. & Stejskalova, J. (2017) The impact of air pollution to central nervous system in children and adults. *Neuro endocrinology letters*, **38**, 389-396.
- Sreeramkumar, V., Hons, M., Punzon, C., Stein, J.V., Sancho, D., Fresno, M. & Cuesta, N. (2016) Efficient T-cell priming and activation requires signaling through prostaglandin E2 (EP) receptors. *Immunology and cell biology*, **94**, 39-51.
- Stamova, B., Green, P.G., Tian, Y., Hertz-Picciotto, I., Pessah, I.N., Hansen, R., Yang, X., Teng, J., Gregg, J.P., Ashwood, P., Van de Water, J. & Sharp, F.R. (2011) Correlations between gene expression and mercury levels in blood of boys with and without autism. *Neurotoxicity research*, **19**, 31-48.
- Stein, T.P., Schluter, M.D., Steer, R.A., Guo, L. & Ming, X. (2015) Bisphenol A Exposure in Children With Autism Spectrum Disorders. *Autism Res*, **8**, 272-283.
- Stephenson, J.R., Wang, X., Perfitt, T.L., Parrish, W.P., Shonesy, B.C., Marks, C.R., Mortlock, D.P., Nakagawa, T., Sutcliffe, J.S. & Colbran, R.J. (2017) A Novel Human CAMK2A Mutation Disrupts Dendritic Morphology and Synaptic Transmission, and Causes ASD-Related Behaviors. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **37**, 2216-2233.
- Steward, J.P., Ornellas, E.P., Beernink, K.D. & Northway, W.H. (1968) Errors in the technique of intraperitoneal injection of mice. *Applied microbiology*, **16**, 1418-1419.
- Stromland, K., Nordin, V., Miller, M., Akerstrom, B. & Gillberg, C. (1994) Autism in thalidomide embryopathy: a population study. *Developmental medicine and child neurology*, **36**, 351-356.
- Sturza, J., Silver, M.K., Xu, L., Li, M., Mai, X., Xia, Y., Shao, J., Lozoff, B. & Meeker, J. (2016) Prenatal exposure to multiple pesticides is associated with auditory brainstem response at 9months in a cohort study of Chinese infants. *Environment international*, **92-93**, 478-485.
- Su, T., Fan, H.X., Jiang, T., Sun, W.W., Den, W.Y., Gao, M.M., Chen, S.Q., Zhao, Q.H. & Yi, Y.H. (2011) Early continuous inhibition of group 1 mGlu signaling partially rescues dendritic spine abnormalities in the Fmr1 knockout mouse model for fragile X syndrome. *Psychopharmacology*, **215**, 291-300.
- Sun, G.Y., Xu, J., Jensen, M.D. & Simonyi, A. (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. *Journal of lipid research*, **45**, 205-213.

- Sungur, A.O., Vorckel, K.J., Schwarting, R.K. & Wohr, M. (2014) Repetitive behaviors in the Shank1 knockout mouse model for autism spectrum disorder: developmental aspects and effects of social context. *Journal of neuroscience methods*, **234**, 92-100.
- Svennerholm, L. (1968) Distribution and fatty acid composition of phosphoglycerides in normal human brain. *Journal of lipid research*, **9**, 570-579.
- Sweeten, T.L., Posey, D.J. & McDougle, C.J. (2004) Brief report: autistic disorder in three children with cytomegalovirus infection. *Journal of autism and developmental disorders*, **34**, 583-586.
- Szklarczyk, A., Oyler, G., McKay, R., Gerfen, C. & Conant, K. (2007) Cleavage of neuronal synaptosomal-associated protein of 25 kDa by exogenous matrix metalloproteinase-7. *Journal of neurochemistry*, **102**, 1256-1263.
- Tai, C., Chang, C.W., Yu, G.Q., Lopez, I., Yu, X., Wang, X., Guo, W. & Mucke, L. (2020) Tau Reduction Prevents Key Features of Autism in Mouse Models. *Neuron*, **106**, 421-437 e411.
- Talkowski, M.E., Rosenfeld, J.A., Blumenthal, I., Pillalamarri, V., Chiang, C., Heilbut, A., Ernst, C., Hanscom, C., Rossin, E., Lindgren, A.M., Pereira, S., Ruderfer, D., Kirby, A., Ripke, S., Harris, D.J., Lee, J.H., Ha, K., Kim, H.G., Solomon, B.D., Gropman, A.L., Lucente, D., Sims, K., Ohsumi, T.K., Borowsky, M.L., Loranger, S., Quade, B., Lage, K., Miles, J., Wu, B.L., Shen, Y., Neale, B., Shaffer, L.G., Daly, M.J., Morton, C.C. & Gusella, J.F. (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell*, **149**, 525-537.
- Tamiji, J. & Crawford, D.A. (2010a) Misoprostol elevates intracellular calcium in Neuro-2a cells via protein kinase A. *Biochem Biophys Res Commun*, **399**, 565-570.
- Tamiji, J. & Crawford, D.A. (2010b) The neurobiology of lipid metabolism in autism spectrum disorders. *Neurosignals*, **18**, 98-112.
- Tamiji, J. & Crawford, D.A. (2010c) Prostaglandin E(2) and misoprostol induce neurite retraction in Neuro-2a cells. *Biochem Biophys Res Commun*, **398**, 450-456.
- Tang, B., Capitao, C., Dean, B. & Thomas, E.A. (2012) Differential age- and disease-related effects on the expression of genes related to the arachidonic acid signaling pathway in schizophrenia. *Psychiatry research*, **196**, 201-206.

- Tang, C.H., Yang, R.S. & Fu, W.M. (2005) Prostaglandin E2 stimulates fibronectin expression through EP1 receptor, phospholipase C, protein kinase Calpha, and c-Src pathway in primary cultured rat osteoblasts. *The Journal of biological chemistry*, **280**, 22907-22916.
- Taurin, S., Sandbo, N., Qin, Y., Browning, D. & Dulin, N.O. (2006) Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase. *The Journal of biological chemistry*, **281**, 9971-9976.
- Taurin, S., Sandbo, N., Yau, D.M., Sethakorn, N. & Dulin, N.O. (2008) Phosphorylation of beta-catenin by PKA promotes ATP-induced proliferation of vascular smooth muscle cells. *American journal of physiology. Cell physiology*, **294**, C1169-1174.
- Tavano, A., Grasso, R., Gagliardi, C., Triulzi, F., Bresolin, N., Fabbro, F. & Borgatti, R. (2007) Disorders of cognitive and affective development in cerebellar malformations. *Brain : a journal of neurology*, **130**, 2646-2660.
- Terbeck, S., Akkus, F., Chesterman, L.P. & Hasler, G. (2015) The role of metabotropic glutamate receptor 5 in the pathogenesis of mood disorders and addiction: combining preclinical evidence with human Positron Emission Tomography (PET) studies. *Frontiers in neuroscience*, **9**, 86.
- Tessner, T.G., Muhale, F., Riehl, T.E., Anant, S. & Stenson, W.F. (2004) Prostaglandin E2 reduces radiation-induced epithelial apoptosis through a mechanism involving AKT activation and bax translocation. *The Journal of clinical investigation*, **114**, 1676-1685.
- Tetreault, N.A., Hakeem, A.Y., Jiang, S., Williams, B.A., Allman, E., Wold, B.J. & Allman, J.M. (2012) Microglia in the cerebral cortex in autism. *Journal of autism and developmental disorders*, **42**, 2569-2584.
- Thabtah, F. & Peebles, D. (2019) Early Autism Screening: A Comprehensive Review. *International journal of environmental research and public health*, **16**.
- Tian, J., Kim, S.F., Hester, L. & Snyder, S.H. (2008) S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10537-10540.
- Tian, Y., Green, P.G., Stamova, B., Hertz-Picciotto, I., Pessah, I.N., Hansen, R., Yang, X., Gregg, J.P., Ashwood, P., Jickling, G., Van de Water, J. & Sharp, F.R. (2011) Correlations of gene expression with blood lead levels in children with autism compared to typically developing controls. *Neurotoxicity research*, **19**, 1-13.

- Toledo, M.A., Wen, T.H., Binder, D.K., Ethell, I.M. & Razak, K.A. (2019) Reversal of ultrasonic vocalization deficits in a mouse model of Fragile X Syndrome with minocycline treatment or genetic reduction of MMP-9. *Behavioural brain research*, **372**, 112068.
- Tomson, T., Battino, D. & Perucca, E. (2016) Valproic acid after five decades of use in epilepsy: time to reconsider the indications of a time-honoured drug. *The Lancet. Neurology*, **15**, 210-218.
- Tordjman, S., Somogyi, E., Coulon, N., Kermarrec, S., Cohen, D., Bronsard, G., Bonnot, O., Weismann-Arcache, C., Botbol, M., Lauth, B., Ginchat, V., Roubertoux, P., Barbueroth, M., Kovess, V., Geoffray, M.M. & Xavier, J. (2014) Gene x Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Frontiers in psychiatry*, **5**, 53.
- Torres, M.D., Garcia, O., Tang, C. & Busciglio, J. (2018) Dendritic spine pathology and thrombospondin-1 deficits in Down syndrome. *Free radical biology & medicine*, **114**, 10-14.
- Toscano, C.D., Prabhu, V.V., Langenbach, R., Becker, K.G. & Bosetti, F. (2007) Differential gene expression patterns in cyclooxygenase-1 and cyclooxygenase-2 deficient mouse brain. *Genome biology*, **8**, R14.
- Tostes, M.H., Polonini, H.C., Mendes, R., Brandao, M.A., Gattaz, W.F. & Raposo, N.R. (2013) Fatty acid and phospholipase A2 plasma levels in children with autism. *Trends in psychiatry and psychotherapy*, **35**, 76-80.
- Tsutsui, K., Sakamoto, H. & Ukena, K. (2003) Biosynthesis and action of neurosteroids in the cerebellar Purkinje neuron. *The Journal of steroid biochemistry and molecular biology*, **85**, 311-321.
- Tu, B. & Bazan, N.G. (2003) Hippocampal kindling epileptogenesis upregulates neuronal cyclooxygenase-2 expression in neocortex. *Experimental neurology*, **179**, 167-175.
- Tyzio, R., Nardou, R., Ferrari, D.C., Tsintsadze, T., Shahrokhi, A., Eftekhari, S., Khalilov, I., Tsintsadze, V., Bouchoud, C., Chazal, G., Lemonnier, E., Lozovaya, N., Burnashev, N. & Ben-Ari, Y. (2014) Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science*, **343**, 675-679.
- Udobi, K.C., Kokenge, A.N., Hautman, E.R., Ullio, G., Coene, J., Williams, M.T., Vorhees, C.V., Mabondzo, A. & Skelton, M.R. (2018) Cognitive deficits and increases in creatine

- precursors in a brain-specific knockout of the creatine transporter gene *Slc6a8*. *Genes, brain, and behavior*, **17**, e12461.
- Ushikubi, F., Segi, E., Sugimoto, Y., Murata, T., Matsuoka, T., Kobayashi, T., Hizaki, H., Tuboi, K., Katsuyama, M., Ichikawa, A., Tanaka, T., Yoshida, N. & Narumiya, S. (1998) Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature*, **395**, 281-284.
- Uzunova, G., Pallanti, S. & Hollander, E. (2016) Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, **17**, 174-186.
- Vafeiadi, M., Vrijheid, M., Fthenou, E., Chalkiadaki, G., Rantakokko, P., Kiviranta, H., Kyrtopoulos, S.A., Chatzi, L. & Kogevinas, M. (2014) Persistent organic pollutants exposure during pregnancy, maternal gestational weight gain, and birth outcomes in the mother-child cohort in Crete, Greece (RHEA study). *Environment international*, **64**, 116-123.
- Valayi, S., Eftekharian, M.M., Taheri, M. & Alikhani, M.Y. (2017) Evaluation of antibodies to cytomegalovirus and Epstein-Barr virus in patients with autism spectrum disorder. *Human antibodies*, **26**, 165-169.
- van den Dries, M.A., Guxens, M., Pronk, A., Spaan, S., El Marroun, H., Jusko, T.A., Longnecker, M.P., Ferguson, K.K. & Tiemeier, H. (2019) Organophosphate pesticide metabolite concentrations in urine during pregnancy and offspring attention-deficit hyperactivity disorder and autistic traits. *Environment international*, **131**, 105002.
- van den Dries, M.A., Pronk, A., Guxens, M., Spaan, S., Voortman, T., Jaddoe, V.W., Jusko, T.A., Longnecker, M.P. & Tiemeier, H. (2018) Determinants of organophosphate pesticide exposure in pregnant women: A population-based cohort study in the Netherlands. *International journal of hygiene and environmental health*, **221**, 489-501.
- Van Driesche, S.J. & Martin, K.C. (2018) New frontiers in RNA transport and local translation in neurons. *Developmental neurobiology*, **78**, 331-339.
- Vancassel, S., Durand, G., Barthelemy, C., Lejeune, B., Martineau, J., Guilloteau, D., Andres, C. & Chalon, S. (2001) Plasma fatty acid levels in autistic children. *Prostaglandins, leukotrienes, and essential fatty acids*, **65**, 1-7.

- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. & Speleman, F. (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome biology*, **3**, 1-12.
- Varcin, K.J., Alvares, G.A., Uljarevic, M. & Whitehouse, A.J.O. (2017) Prenatal maternal stress events and phenotypic outcomes in Autism Spectrum Disorder. *Autism Res*, **10**, 1866-1877.
- Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W. & Pardo, C.A. (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of neurology*, **57**, 67-81.
- Vasilache, A.M., Andersson, J. & Nilsberth, C. (2007) Expression of PGE2 EP3 receptor subtypes in the mouse preoptic region. *Neuroscience letters*, **423**, 179-183.
- Vazquez-Tello, A., Fan, L., Hou, X., Joyal, J.S., Mancini, J.A., Quiniou, C., Clyman, R.I., Gobeil, F., Jr., Varma, D.R. & Chemtob, S. (2004) Intracellular-specific colocalization of prostaglandin E2 synthases and cyclooxygenases in the brain. *Am J Physiol Regul Integr Comp Physiol*, **287**, R1155-1163.
- Vecil, G.G., Larsen, P.H., Corley, S.M., Herx, L.M., Besson, A., Goodyer, C.G. & Yong, V.W. (2000) Interleukin-1 is a key regulator of matrix metalloproteinase-9 expression in human neurons in culture and following mouse brain trauma in vivo. *Journal of neuroscience research*, **61**, 212-224.
- Verheyen, E.M. & Gottardi, C.J. (2010) Regulation of Wnt/beta-catenin signaling by protein kinases. *Developmental dynamics : an official publication of the American Association of Anatomists*, **239**, 34-44.
- Verhoeven, J.S., De Cock, P., Lagae, L. & Sunaert, S. (2010) Neuroimaging of autism. *Neuroradiology*, **52**, 3-14.
- Veyhe, A.S., Nost, T.H., Sandanger, T.M., Hansen, S., Odland, J.O. & Nieboer, E. (2013) Is meconium useful to predict fetal exposure to organochlorines and hydroxylated PCBs? *Environmental science. Processes & impacts*, **15**, 1490-1500.
- Vierk, R., Brandt, N. & Rune, G.M. (2014) Hippocampal estradiol synthesis and its significance for hippocampal synaptic stability in male and female animals. *Neuroscience*, **274**, 24-32.
- Vizcaino, E., Grimalt, J.O., Lopez-Espinosa, M.J., Llop, S., Rebagliato, M. & Ballester, F. (2010) Maternal origin and other determinants of cord serum organochlorine compound

- concentrations in infants from the general population. *Environmental science & technology*, **44**, 6488-6495.
- Vo, H.T., Phillips, M.L., Herskowitz, J.H. & King, G.D. (2019) Klotho deficiency affects the spine morphology and network synchronization of neurons. *Molecular and cellular neurosciences*, **98**, 1-11.
- von Bredow, D.C., Cress, A.E., Howard, E.W., Bowden, G.T. & Nagle, R.B. (1998) Activation of gelatinase-tissue-inhibitors-of-metalloproteinase complexes by matrilysin. *The Biochemical journal*, **331** (Pt 3), 965-972.
- von Ehrenstein, O.S., Ling, C., Cui, X., Cockburn, M., Park, A.S., Yu, F., Wu, J. & Ritz, B. (2019) Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: population based case-control study. *Bmj*, **364**, 1962.
- Vonkeman, H.E. & van de Laar, M.A. (2010) Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. *Seminars in arthritis and rheumatism*, **39**, 294-312.
- Wahlsten, D., Metten, P. & Crabbe, J.C. (2003) Survey of 21 inbred mouse strains in two laboratories reveals that BTBR T/+ tf/tf has severely reduced hippocampal commissure and absent corpus callosum. *Brain research*, **971**, 47-54.
- Wan, H., Zhang, C., Li, H., Luan, S. & Liu, C. (2018) Association of maternal diabetes with autism spectrum disorders in offspring: A systemic review and meta-analysis. *Medicine*, **97**, e9438.
- Wang, B., Yi, D., Jin, L., Li, Z., Liu, J., Zhang, Y., Qiu, X., Liu, W., Tao, S. & Ren, A. (2014a) Organochlorine pesticide levels in maternal serum and risk of neural tube defects in offspring in Shanxi Province, China: a case-control study. *The Science of the total environment*, **490**, 1037-1043.
- Wang, D. (2008) Discrepancy between mRNA and protein abundance: insight from information retrieval process in computers. *Computational biology and chemistry*, **32**, 462-468.
- Wang, F., Reierstad, S. & Fishman, D.A. (2006) Matrilysin over-expression in MCF-7 cells enhances cellular invasiveness and pro-gelatinase activation. *Cancer letters*, **236**, 292-301.
- Wang, J., Lou, S.S., Wang, T., Wu, R.J., Li, G., Zhao, M., Lu, B., Li, Y.Y., Zhang, J., Cheng, X., Shen, Y., Wang, X., Zhu, Z.C., Li, M.J., Takumi, T., Yang, H., Yu, X., Liao, L. & Xiong, Z.Q. (2019) UBE3A-mediated PTPA ubiquitination and degradation regulate PP2A

- activity and dendritic spine morphology. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 12500-12505.
- Wang, M.T., Honn, K.V. & Nie, D. (2007) Cyclooxygenases, prostanoids, and tumor progression. *Cancer metastasis reviews*, **26**, 525-534.
- Wang, P., Zhao, D., Lachman, H.M. & Zheng, D. (2018) Enriched expression of genes associated with autism spectrum disorders in human inhibitory neurons. *Translational psychiatry*, **8**, 13.
- Wang, S.S., Kloth, A.D. & Badura, A. (2014b) The cerebellum, sensitive periods, and autism. *Neuron*, **83**, 518-532.
- Wang, Y., Tang, S., Xu, S., Weng, S. & Liu, Z. (2016) Maternal Body Mass Index and Risk of Autism Spectrum Disorders in Offspring: A Meta-analysis. *Sci Rep*, **6**, 34248.
- Wang, Y., Zhao, A.M. & Lin, Q.D. (2010) Role of cyclooxygenase-2 signaling pathway dysfunction in unexplained recurrent spontaneous abortion. *Chin Med J (Engl)*, **123**, 1543-1547.
- Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D. & Morris, Q. (2010) The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res*, **38**, W214-220.
- Wassink, T.H., Piven, J., Vieland, V.J., Huang, J., Swiderski, R.E., Pietila, J., Braun, T., Beck, G., Folstein, S.E., Haines, J.L. & Sheffield, V.C. (2001) Evidence supporting WNT2 as an autism susceptibility gene. *Am J Med Genet*, **105**, 406-413.
- Watanabe, Y., Murakami, T., Kawashima, M., Hasegawa-Baba, Y., Mizukami, S., Imatanaka, N., Akahori, Y., Yoshida, T. & Shibutani, M. (2017) Maternal Exposure to Valproic Acid Primarily Targets Interneurons Followed by Late Effects on Neurogenesis in the Hippocampal Dentate Gyrus in Rat Offspring. *Neurotoxicity research*, **31**, 46-62.
- Watts, T.J. (2008) The pathogenesis of autism. *Clinical medicine. Pathology*, **1**, 99-103.
- Wegiel, J., Frackowiak, J., Mazur-Kolecka, B., Schanen, N.C., Cook, E.H., Jr., Sigman, M., Brown, W.T., Kuchna, I., Wegiel, J., Nowicki, K., Imaki, H., Ma, S.Y., Chauhan, A., Chauhan, V., Miller, D.L., Mehta, P.D., Flory, M., Cohen, I.L., London, E., Reisberg, B., de Leon, M.J. & Wisniewski, T. (2012) Abnormal intracellular accumulation and

- extracellular Abeta deposition in idiopathic and Dup15q11.2-q13 autism spectrum disorders. *PLoS One*, **7**, e35414.
- Wei, H., Liang, F., Meng, G., Nie, Z., Zhou, R., Cheng, W., Wu, X., Feng, Y. & Wang, Y. (2016a) Redox/methylation mediated abnormal DNA methylation as regulators of ambient fine particulate matter-induced neurodevelopment related impairment in human neuronal cells. *Sci Rep*, **6**, 33402.
- Wei, H., Ma, Y., Ding, C., Jin, G., Liu, J., Chang, Q., Hu, F. & Yu, L. (2016b) Reduced Glutamate Release in Adult BTBR Mouse Model of Autism Spectrum Disorder. *Neurochemical research*, **41**, 3129-3137.
- Weinstein-Fudim, L., Ergaz, Z., Turgeman, G., Yanai, J., Szyf, M. & Ornoy, A. (2019) Gender Related Changes in Gene Expression Induced by Valproic Acid in A Mouse Model of Autism and the Correction by S-adenosyl Methionine. Does It Explain the Gender Differences in Autistic Like Behavior? *International journal of molecular sciences*, **20**.
- Weisskopf, M.G., Anderson, H.A., Hanrahan, L.P., Kanarek, M.S., Falk, C.M., Steenport, D.M., Draheim, L.A. & Great Lakes, C. (2005) Maternal exposure to Great Lakes sport-caught fish and dichlorodiphenyl dichloroethylene, but not polychlorinated biphenyls, is associated with reduced birth weight. *Environmental research*, **97**, 149-162.
- Wiegand, H.L., Lu, S. & Cullen, B.R. (2003) Exon junction complexes mediate the enhancing effect of splicing on mRNA expression. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 11327-11332.
- Wiest, M.M., German, J.B., Harvey, D.J., Watkins, S.M. & Hertz-Picciotto, I. (2009) Plasma fatty acid profiles in autism: a case-control study. *Prostaglandins, leukotrienes, and essential fatty acids*, **80**, 221-227.
- Wiggins, L.D., Rubenstein, E., Daniels, J., DiGuseppi, C., Yeargin-Allsopp, M., Schieve, L.A., Tian, L.H., Sabourin, K., Moody, E., Pinto-Martin, J., Reyes, N. & Levy, S.E. (2019) A Phenotype of Childhood Autism Is Associated with Preexisting Maternal Anxiety and Depression. *Journal of abnormal child psychology*, **47**, 731-740.
- Wilkinson, B., Grepo, N., Thompson, B.L., Kim, J., Wang, K., Evgrafov, O.V., Lu, W., Knowles, J.A. & Campbell, D.B. (2015) The autism-associated gene chromodomain helicase DNA-binding protein 8 (CHD8) regulates noncoding RNAs and autism-related genes. *Translational psychiatry*, **5**, e568.

- Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B. & Hersh, J.H. (2001) Fetal valproate syndrome and autism: additional evidence of an association. *Developmental medicine and child neurology*, **43**, 202-206.
- Windham, G.C., Zhang, L., Gunier, R., Croen, L.A. & Grether, J.K. (2006) Autism spectrum disorders in relation to distribution of hazardous air pollutants in the san francisco bay area. *Environmental health perspectives*, **114**, 1438-1444.
- Wisniowiecka-Kowalnik, B., Nesteruk, M., Peters, S.U., Xia, Z., Cooper, M.L., Savage, S., Amato, R.S., Bader, P., Browning, M.F., Haun, C.L., Duda, A.W., 3rd, Cheung, S.W. & Stankiewicz, P. (2010) Intragenic rearrangements in NRXN1 in three families with autism spectrum disorder, developmental delay, and speech delay. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*, **153B**, 983-993.
- Wisniowiecka-Kowalnik, B. & Nowakowska, B.A. (2019) Genetics and epigenetics of autism spectrum disorder-current evidence in the field. *Journal of applied genetics*, **60**, 37-47.
- Wohr, M. (2014) Ultrasonic vocalizations in Shank mouse models for autism spectrum disorders: detailed spectrographic analyses and developmental profiles. *Neuroscience and biobehavioral reviews*, **43**, 199-212.
- Wohr, M., Rouillet, F.I. & Crawley, J.N. (2011a) Reduced scent marking and ultrasonic vocalizations in the BTBR T+tf/J mouse model of autism. *Genes, brain, and behavior*, **10**, 35-43.
- Wohr, M., Rouillet, F.I., Hung, A.Y., Sheng, M. & Crawley, J.N. (2011b) Communication impairments in mice lacking Shank1: reduced levels of ultrasonic vocalizations and scent marking behavior. *PLoS One*, **6**, e20631.
- Wolff, M.S., Engel, S., Berkowitz, G., Teitelbaum, S., Siskind, J., Barr, D.B. & Wetmur, J. (2007) Prenatal pesticide and PCB exposures and birth outcomes. *Pediatric research*, **61**, 243-250.
- Wong, C.T., Ahmad, E., Li, H. & Crawford, D.A. (2014a) Prostaglandin E2 alters Wnt-dependent migration and proliferation in neuroectodermal stem cells: implications for autism spectrum disorders. *Cell communication and signaling : CCS*, **12**, 19.
- Wong, C.T., Ahmad, E., Li, H. & Crawford, D.A. (2014b) Prostaglandin E2 alters Wnt-dependent migration and proliferation in neuroectodermal stem cells: implications for autism spectrum disorders. *Cell Commun Signal*, **12**, 19.

- Wong, C.T., Bestard-Lorigados, I. & Crawford, D.A. (2019) Autism-related behaviors in the cyclooxygenase-2-deficient mouse model. *Genes, brain, and behavior*, **18**, e12506.
- Wong, C.T., Ussyshkin, N., Ahmad, E., Rai-Bhogal, R., Li, H. & Crawford, D.A. (2016) Prostaglandin E2 promotes neural proliferation and differentiation and regulates Wnt target gene expression. *Journal of neuroscience research*, **94**, 759-775.
- Wong, C.T., Wais, J. & Crawford, D.A. (2015a) Prenatal exposure to common environmental factors affects brain lipids and increases risk of developing Autism Spectrum Disorders. *Eur J Neurosci*.
- Wong, C.T., Wais, J. & Crawford, D.A. (2015b) Prenatal exposure to common environmental factors affects brain lipids and increases risk of developing autism spectrum disorders. *The European journal of neuroscience*, **42**, 2742-2760.
- Wong, M. (2005) Modulation of dendritic spines in epilepsy: cellular mechanisms and functional implications. *Epilepsy & behavior : E&B*, **7**, 569-577.
- Woodling, N.S., Colas, D., Wang, Q., Minhas, P., Panchal, M., Liang, X., Mhatre, S.D., Brown, H., Ko, N., Zagol-Ikapitte, I., van der Hart, M., Khroyan, T.V., Chuluun, B., Priyam, P.G., Milne, G.L., Rassoulpour, A., Boutaud, O., Manning-Bog, A.B., Heller, H.C. & Andreasson, K.I. (2016) Cyclooxygenase inhibition targets neurons to prevent early behavioural decline in Alzheimer's disease model mice. *Brain : a journal of neurology*, **139**, 2063-2081.
- Woskie, S., Kongtip, P., Thanasanpaiboon, W., Kiatdamrong, N., Charoonrungsirikul, N., Nankongnab, N., Surach, A. & Phamonphon, A. (2017) A pilot study of maternal exposure to organophosphate pesticides and newborn neurodevelopment in Thailand. *International journal of occupational and environmental health*, **23**, 193-201.
- Wright, C.L., Hoffman, J.H. & McCarthy, M.M. (2019) Evidence that inflammation promotes estradiol synthesis in human cerebellum during early childhood. *Translational psychiatry*, **9**, 58.
- Wright, C.L. & McCarthy, M.M. (2009) Prostaglandin E2-induced masculinization of brain and behavior requires protein kinase A, AMPA/kainate, and metabotropic glutamate receptor signaling. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **29**, 13274-13282.

- Wu, G. & He, X. (2006) Threonine 41 in beta-catenin serves as a key phosphorylation relay residue in beta-catenin degradation. *Biochemistry*, **45**, 5319-5323.
- Wu, H.F., Chen, P.S., Chen, Y.J., Lee, C.W., Chen, I.T. & Lin, H.C. (2016) Alleviation of N-Methyl-D-Aspartate Receptor-Dependent Long-Term Depression via Regulation of the Glycogen Synthase Kinase-3 β Pathway in the Amygdala of a Valproic Acid-Induced Animal Model of Autism. *Molecular neurobiology*.
- Wu, M., Guan, J., Li, C., Gunter, S., Nusrat, L., Ng, S., Dhand, K., Morshead, C., Kim, A. & Das, S. (2017a) Aberrantly activated Cox-2 and Wnt signaling interact to maintain cancer stem cells in glioblastoma. *Oncotarget*, **8**, 82217-82230.
- Wu, S., Wu, F., Ding, Y., Hou, J., Bi, J. & Zhang, Z. (2017b) Advanced parental age and autism risk in children: a systematic review and meta-analysis. *Acta psychiatrica Scandinavica*, **135**, 29-41.
- Wyatt, G.L., Crump, L.S., Young, C.M., Wessells, V.M., McQueen, C.M., Wall, S.W., Gustafson, T.L., Fan, Y.Y., Chapkin, R.S., Porter, W.W. & Lyons, T.R. (2019) Cross-talk between SIM2s and NF κ B regulates cyclooxygenase 2 expression in breast cancer. *Breast cancer research : BCR*, **21**, 131.
- Xia, W., Liu, Y. & Jiao, J. (2015) GRM7 regulates embryonic neurogenesis via CREB and YAP. *Stem cell reports*, **4**, 795-810.
- Xiang, A.H., Wang, X., Martinez, M.P., Walthall, J.C., Curry, E.S., Page, K., Buchanan, T.A., Coleman, K.J. & Getahun, D. (2015) Association of maternal diabetes with autism in offspring. *Jama*, **313**, 1425-1434.
- Xu, C., Kim, N.G. & Gumbiner, B.M. (2009) Regulation of protein stability by GSK3 mediated phosphorylation. *Cell cycle*, **8**, 4032-4039.
- Xu, D., Liu, A., Wang, X., Zhang, M., Zhang, Z., Tan, Z. & Qiu, M. (2018) Identifying suitable reference genes for developing and injured mouse CNS tissues. *Developmental neurobiology*, **78**, 39-50.
- Xu, X., Miller, E.C. & Pozzo-Miller, L. (2014) Dendritic spine dysgenesis in Rett syndrome. *Frontiers in neuroanatomy*, **8**, 97.
- Yamagata, K., Andreasson, K.I., Kaufmann, W.E., Barnes, C.A. & Worley, P.F. (1993) Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron*, **11**, 371-386.

- Yamashita, Y., Fujimoto, C., Nakajima, E., Isagai, T. & Matsuishi, T. (2003) Possible association between congenital cytomegalovirus infection and autistic disorder. *Journal of autism and developmental disorders*, **33**, 455-459.
- Yan, E., Castillo-Melendez, M., Nicholls, T., Hirst, J. & Walker, D. (2004) Cerebrovascular responses in the fetal sheep brain to low-dose endotoxin. *Pediatric research*, **55**, 855-863.
- Yan, J., Oliveira, G., Coutinho, A., Yang, C., Feng, J., Katz, C., Sram, J., Bockholt, A., Jones, I.R., Craddock, N., Cook, E.H., Jr., Vicente, A. & Sommer, S.S. (2005) Analysis of the neuroligin 3 and 4 genes in autism and other neuropsychiatric patients. *Molecular psychiatry*, **10**, 329-332.
- Yang, M., Zhodzishsky, V. & Crawley, J.N. (2007) Social deficits in BTBR T+tf/J mice are unchanged by cross-fostering with C57BL/6J mothers. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*, **25**, 515-521.
- Yang, X.T., Bi, Y.Y., Chen, E.T. & Feng, D.F. (2014) Overexpression of Wnt3a facilitates the proliferation and neural differentiation of neural stem cells in vitro and after transplantation into an injured rat retina. *Journal of neuroscience research*, **92**, 148-161.
- Yangngam, S., Plong-On, O., Sripo, T., Roongpraiwan, R., Hansakunachai, T., Wirojanan, J., Sombuntham, T., Ruangdaraganon, N. & Limprasert, P. (2014) Mutation screening of the neurexin 1 gene in thai patients with intellectual disability and autism spectrum disorder. *Genetic testing and molecular biomarkers*, **18**, 510-515.
- Yao, W., Huang, J. & He, H. (2019) Over-expressed LOC101927196 suppressed oxidative stress levels and neuron cell proliferation in a rat model of autism through disrupting the Wnt signaling pathway by targeting FZD3. *Cellular signalling*, **62**, 109328.
- Ye, X., Pierik, F.H., Angerer, J., Meltzer, H.M., Jaddoe, V.W., Tiemeier, H., Hoppin, J.A. & Longnecker, M.P. (2009) Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). *International journal of hygiene and environmental health*, **212**, 481-491.
- Yee, N., Schwarting, R.K., Fuchs, E. & Wöhr, M. (2012) Increased affective ultrasonic communication during fear learning in adult male rats exposed to maternal immune activation. *Journal of psychiatric research*, **46**, 1199-1205.

- Yin, Z.S., Zhang, H., Wang, W., Hua, X.Y., Hu, Y., Zhang, S.Q. & Li, G.W. (2007) Wnt-3a protein promote neuronal differentiation of neural stem cells derived from adult mouse spinal cord. *Neurological research*, **29**, 847-854.
- Yochum, C.L., Dowling, P., Reuhl, K.R., Wagner, G.C. & Ming, X. (2008) VPA-induced apoptosis and behavioral deficits in neonatal mice. *Brain research*, **1203**, 126-132.
- Yokota, O., Terada, S., Ishihara, T., Nakashima, H., Kugo, A., Ujike, H., Tsuchiya, K., Ikeda, K., Saito, Y., Murayama, S., Ishizu, H. & Kuroda, S. (2004) Neuronal expression of cyclooxygenase-2, a pro-inflammatory protein, in the hippocampus of patients with schizophrenia. *Progress in neuro-psychopharmacology & biological psychiatry*, **28**, 715-721.
- Yoo, H.J., Cho, I.H., Park, M., Cho, E., Cho, S.C., Kim, B.N., Kim, J.W. & Kim, S.A. (2008) Association between PTGS2 polymorphism and autism spectrum disorders in Korean trios. *Neurosci Res*, **62**, 66-69.
- Yoshida, G.J., Saya, H. & Zouboulis, C.C. (2013) Three-dimensional culture of sebaceous gland cells revealing the role of prostaglandin E2-induced activation of canonical Wnt signaling. *Biochem Biophys Res Commun*, **438**, 640-646.
- Yu, S., Hou, Q., Sun, H., Liu, J. & Li, J. (2015) Upregulation of C-C chemokine receptor type 7 expression by membrane-associated prostaglandin E synthase-1/prostaglandin E2 requires glycogen synthase kinase 3 β -mediated signal transduction in colon cancer cells. *Molecular medicine reports*, **12**, 7169-7175.
- Yu, Y., Fan, J., Chen, X.S., Wang, D., Klein-Szanto, A.J., Campbell, R.L., FitzGerald, G.A. & Funk, C.D. (2006) Genetic model of selective COX2 inhibition reveals novel heterodimer signaling. *Nature medicine*, **12**, 699-704.
- Yui, K., Imataka, G., Kawasak, Y. & Yamada, H. (2016a) Increased omega-3 polyunsaturated fatty acid/arachidonic acid ratios and upregulation of signaling mediator in individuals with autism spectrum disorders. *Life sciences*, **145**, 205-212.
- Yui, K., Imataka, G., Kawasaki, Y. & Yamada, H. (2016b) Down-regulation of a signaling mediator in association with lowered plasma arachidonic acid levels in individuals with autism spectrum disorders. *Neuroscience letters*, **610**, 223-228.
- Zaqout, S. & Kaindl, A.M. (2016) Golgi-Cox Staining Step by Step. *Frontiers in neuroanatomy*, **10**, 38.

- Zatkova, M., Bakos, J., Hodosy, J. & Ostatnikova, D. (2016) Synapse alterations in autism: Review of animal model findings. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia*, **160**, 201-210.
- Zeng, F., Lu, J.J., Zhou, X.F. & Wang, Y.J. (2011) Roles of p75NTR in the pathogenesis of Alzheimer's disease: a novel therapeutic target. *Biochemical pharmacology*, **82**, 1500-1509.
- Zhang, B., Gokce, O., Hale, W.D., Brose, N. & Sudhof, T.C. (2018) Autism-associated neuroligin-4 mutation selectively impairs glycinergic synaptic transmission in mouse brainstem synapses. *The Journal of experimental medicine*, **215**, 1543-1553.
- Zhang, H.J., Sun, R.P., Lei, G.F., Yang, L. & Liu, C.X. (2008) Cyclooxygenase-2 inhibitor inhibits hippocampal synaptic reorganization in pilocarpine-induced status epilepticus rats. *Journal of Zhejiang University. Science. B*, **9**, 903-915.
- Zhang, Q., Wu, H., Zou, M., Li, L., Li, Q., Sun, C., Xia, W., Cao, Y. & Wu, L. (2019) Folic acid improves abnormal behavior via mitigation of oxidative stress, inflammation, and ferroptosis in the BTBR T+ tf/J mouse model of autism. *The Journal of nutritional biochemistry*, **71**, 98-109.
- Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O'Keefe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., Deng, S., Liddelow, S.A., Zhang, C., Daneman, R., Maniatis, T., Barres, B.A. & Wu, J.Q. (2014a) An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, **34**, 11929-11947.
- Zhang, Y., Yuan, X., Wang, Z. & Li, R. (2014b) The canonical Wnt signaling pathway in autism. *CNS Neurol Disord Drug Targets*, **13**, 765-770.
- Zhao, H., Wang, Q., Yan, T., Zhang, Y., Xu, H.J., Yu, H.P., Tu, Z., Guo, X., Jiang, Y.H., Li, X.J., Zhou, H. & Zhang, Y.Q. (2019) Maternal valproic acid exposure leads to neurogenesis defects and autism-like behaviors in non-human primates. *Translational psychiatry*, **9**, 267.
- Zhao, J., Yue, W., Zhu, M.J., Sreejayan, N. & Du, M. (2010) AMP-activated protein kinase (AMPK) cross-talks with canonical Wnt signaling via phosphorylation of beta-catenin at Ser 552. *Biochem Biophys Res Commun*, **395**, 146-151.

- Zhen, Y. & Li, W. (2015) Impairment of autophagosome-lysosome fusion in the buff mutant mice with the VPS33A(D251E) mutation. *Autophagy*, **11**, 1608-1622.
- Zheng, C., Qu, Y.X., Wang, B., Shen, P.F., Xu, J.D. & Chen, Y.X. (2019) COX-2/PGE2 facilitates fracture healing by activating the Wnt/beta-catenin signaling pathway. *European review for medical and pharmacological sciences*, **23**, 9721-9728.
- Zheng, Z., Zheng, P. & Zou, X. (2018) Association between schizophrenia and autism spectrum disorder: A systematic review and meta-analysis. *Autism Res*, **11**, 1110-1119.
- Zhu, H., Xiang, H.C., Li, H.P., Lin, L.X., Hu, X.F., Zhang, H., Meng, W.Y., Liu, L., Chen, C., Shu, Y., Zhang, R.Y., Zhang, P., Si, J.Q. & Li, M. (2019a) Inhibition of GABAergic Neurons and Excitation of Glutamatergic Neurons in the Ventrolateral Periaqueductal Gray Participate in Electroacupuncture Analgesia Mediated by Cannabinoid Receptor. *Frontiers in neuroscience*, **13**, 484.
- Zhu, Y., Shi, C., Zeng, L., Liu, G., Jiang, W., Zhang, X., Chen, S., Guo, J., Jian, X., Ouyang, J., Xia, J., Kuang, C., Fan, S., Wu, X., Wu, Y., Zhou, W. & Guan, Y. (2019b) High COX-2 expression in cancer-associated fibroblasts contributes to poor survival and promotes migration and invasiveness in nasopharyngeal carcinoma. *Molecular carcinogenesis*.
- Zhu, Z., Li, X., Chen, W., Zhao, Y., Li, H., Qing, C., Jia, N., Bai, Z. & Liu, J. (2004) Prenatal stress causes gender-dependent neuronal loss and oxidative stress in rat hippocampus. *Journal of neuroscience research*, **78**, 837-844.
- Zuloaga, K.L., O'Connor, D.T., Handa, R.J. & Gonzales, R.J. (2012) Estrogen receptor beta dependent attenuation of cytokine-induced cyclooxygenase-2 by androgens in human brain vascular smooth muscle cells and rat mesenteric arteries. *Steroids*, **77**, 835-844.

APPENDICES

Appendix A. Effect of 1 μ M PGE₂ on Neuroectodermal (NE-4C) Stem Cells

As was established in Chapter 3, maternal PGE₂ exposure at the onset of neurogenesis resulted in abnormal gene and protein expression of critical genes in the offspring. Previously, Wong et al (Wong *et al.*, 2014b; Wong *et al.*, 2016) (2014; 2016), showed that PGE₂ can modify the proliferation, migration and differentiation of NE-4C stem cells. In particular, PGE₂ caused the NE-4C cells to differentiate earlier, than serum-free media (SFM) alone. Therefore, we wanted to investigate whether there is an effect of the growth of neurite extensions of the differentiated cells. We tested a 1 μ M PGE₂ treatment on days 8, 9, 10, 11, and 12, following induction of differentiation (As per methods in chapter 2). We were able to acquire neurite length measurements from days 8-10, after which the neurite extensions were so abundant and intertwined, that they were impossible to trace. There was no significant difference ($p = 0.7$) in neurite length between SFM ($24.8 \pm 3.3\mu\text{m}$) and PGE₂ ($26.5 \pm 2.0\mu\text{m}$) on days 8, and 9 (SFM = $45.7 \pm 3.3\mu\text{m}$; PGE₂ = $54.6 \pm 2.0\mu\text{m}$; $p = 0.08$) (Figure C-1). On day 10 following differentiation, the neurite extensions were significantly longer ($p = 0.01$) in the PGE₂-treated NE-4C cells ($72.3 \pm 2.0\mu\text{m}$) compared to SFM only ($53.0 \pm 4.3\mu\text{m}$) (Figure C-1). Therefore, we can conclude that 1 μ M PGE₂ treatment in neuronal stem cells accelerates neurite outgrowth which may potentially lead to abnormal connections or overgrowth in the brain

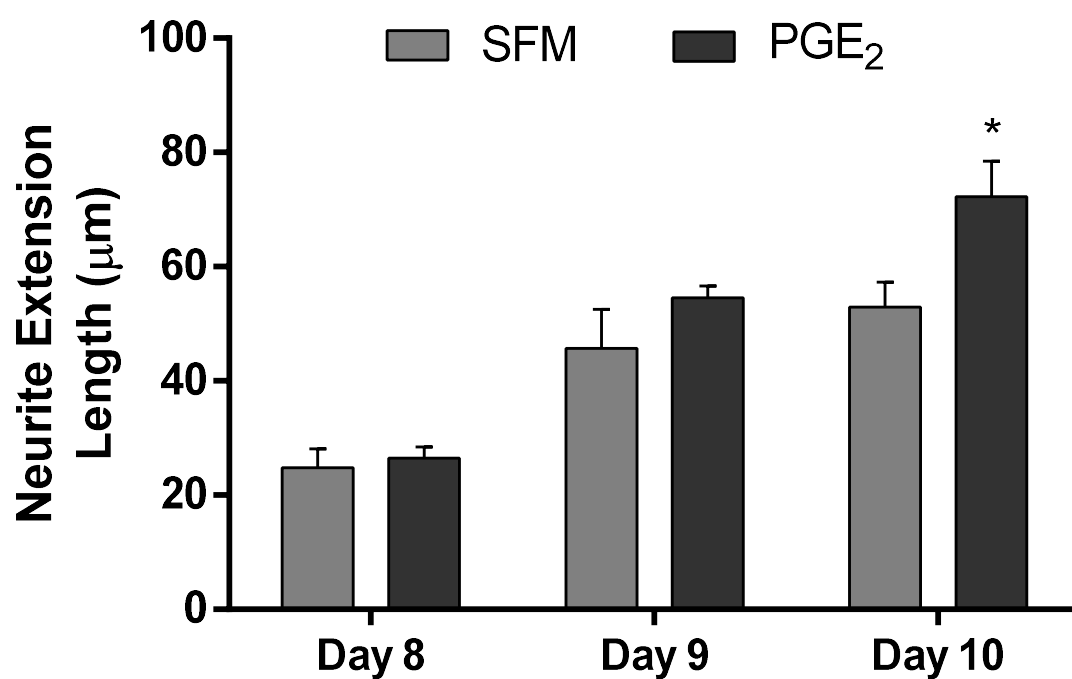


Figure A-1. The effect of 1μM PGE₂ treatment on the length of neurite extensions of differentiated NE-4C cells. On day 10 following differentiation, there was a significant increase in length of neurite extensions following PGE₂ treatment. * $p < 0.05$

Appendix B. Supplementary Tables

B1. Microarray Data – Differential Expression of All Genes in the COX-1 and -2 KO Male Mouse Brain

Supplementary Table 1 – Differential expression of all genes detected by whole genome analysis (Mouse WG-6 V2 BeadChip (Illumina) microarray) in the male COX-1 and -2 KO mouse brain at embryonic days 16 (E16) and 19 (E19), compared to male WT mouse. Fold-change values from each developmental stage are mean representations from three male offspring (n=3)

Embryonic Day 16 (E16)						Embryonic Day 19 (E19)					
COX-1-KO			COX-2-KO			COX-1-KO			COX-2-KO		
Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value
<i>Upregulated genes</i>											
Hbb-y	4.34	5.46E-08	Hbb-b2	10.9	5.46E-08	Hbb-b2	11.2	5.46E-08	Hbb-b2	12.8	5.46E-08
SOX11	3.39	8.78E-06	Hbb-y	5.15	5.46E-08	C030014C1 2Rik	3.91	6.86E-03	Paip1	3.74	1.77E-04
slc35f1	2.09	2.37E-02	Xlr4a	3.99	2.97E-02	SOX11	3.17	8.78E-06	PBX1	3.68	1.05E-03
Lyplal1	2.03	6.04E-05	SOX11	3.30	8.78E-06	LOC10004 5240	2.89	1.00E-04	Ankrd37	3.01	1.76E-02
TMEM87A	2.02	4.57E-02	Paip1	3.21	1.77E-04	Ankrd37	2.75	1.76E-02	SOX11	2.73	8.78E-06
RBM45	2.01	8.26E-03	0710001D0 7Rik	2.42	4.97E-03	CAP1	2.66	5.05E-03	Sfrp1	2.59	1.12E-04
sdcbp	1.97	3.86E-02	ABCC10	2.36	1.95E-05	Paip1	2.58	1.77E-04	GLTPD1	2.49	2.06E-05
LOC10004 4314	1.92	3.10E-03	emp1	2.34	1.90E-03	GLTPD1	2.46	2.06E-05	trim2	2.25	3.09E-02
0710001D0 7Rik	1.89	4.97E-03	LUM	2.32	5.45E-03	Prkd3	2.45	1.12E-02	Serpina3n	2.22	3.17E-03
DCN	1.89	3.90E-02	trim2	2.22	3.09E-02	Egr1	2.42	6.45E-06	DAAM1	2.12	3.34E-03
Rnase4	1.88	4.04E-02	LOC10004 4314	2.17	3.10E-03	TMEM87A	2.35	4.57E-02	C030014C12 Rik	2.10	6.86E-03
Hba-x	1.88	3.67E-04	Lyplal1	2.09	6.04E-05	LOC10004 4314	2.18	3.10E-03	0710001D07 Rik	2.08	4.97E-03
Snape3	1.86	2.35E-02	COL3A1	2.05	8.30E-03	trim2	2.13	3.09E-02	LOC1000443 14	1.98	3.10E-03
CAP1	1.86	5.05E-03	slc35f1	2.01	2.37E-02	FOS	2.13	3.53E-05	ENSMUSG0 0000068790	1.93	1.66E-02
Paip1	1.85	1.77E-04	GLO1	2.01	4.16E-03	Fezf1	2.11	3.45E-02	4930455C21 Rik	1.92	8.44E-03
NFIB	1.84	5.91E-04	gjb2	2.00	2.09E-02	RBM45	1.97	8.26E-03	GLO1	1.90	4.16E-03
ITGB3BP	1.84	6.91E-03	GLTPD1	1.96	2.06E-05	Sfrp1	1.96	1.12E-04	NEK3	1.89	1.57E-03
LUM	1.82	5.45E-03	DCN	1.96	3.90E-02	Lyplal1	1.96	6.04E-05	Lyplal1	1.87	6.04E-05
DTD1	1.81	2.45E-02	Hba-x	1.91	3.67E-04	C530036F0 5Rik	1.88	4.23E-04	Prkd3	1.83	1.12E-02
IL18	1.79	1.34E-03	sdcbp	1.88	3.86E-02	GRLF1	1.84	4.11E-03	WARS2	1.82	8.92E-03
2810417H1 3Rik	1.76	9.32E-04	Slc13a4	1.88	8.44E-03	TXNIP	1.83	2.12E-03	Ttc15	1.81	4.33E-05
SCP2	1.76	3.21E-03	Sfrp1	1.84	1.12E-04	6330415B2 1Rik	1.82	2.92E-02	GRLF1	1.80	4.11E-03
ABCC10	1.74	1.95E-05	IL22RA1	1.83	5.67E-04	DAAM1	1.81	3.34E-03	ADI1	1.78	7.46E-04
ADI1	1.74	7.46E-04	GNG10	1.79	4.05E-02	mro	1.80	1.34E-02	4833414E09 Rik	1.77	1.07E-02
LOC10004 5240	1.72	1.00E-04	2810417H1 3Rik	1.79	9.32E-04	2900011L1 8Rik	1.80	1.92E-02	pitx2	1.74	1.29E-02
vps33a	1.68	9.95E-05	Hexb	1.79	3.76E-03	NRXN2	1.79	2.15E-02	LOC1000460 25	1.72	5.91E-03
nosip	1.68	3.21E-03	NUBP2	1.79	3.54E-04	pitx2	1.78	1.29E-02	LOC1000452 40	1.72	1.00E-04
Rpa3	1.65	7.64E-04	Rpa3	1.78	7.64E-04	Ttc15	1.78	4.33E-05	lhx1	1.69	6.04E-03
Hbb-bh1	1.65	2.07E-05	NDUFB10	1.78	1.48E-02	Nrxn3	1.76	1.18E-02	SSBP4	1.68	2.15E-03
Hexb	1.65	3.76E-03	HEBP1	1.76	4.91E-04	2310016E0 2Rik	1.76	1.37E-02	LYPD1	1.68	1.45E-04
CUEDC1	1.64	1.18E-02	PFDN4	1.76	5.73E-03	ADI1	1.73	7.46E-04	cdkal1	1.67	1.21E-02

emp1	1.63	1.90E-03	MRPL47	1.75	6.76E-04	GPM6B	1.71	2.34E-02	Arcn1	1.67	1.03E-02
ZBTB8OS	1.63	1.01E-02	TMEM47	1.74	6.07E-03	3830612M24	1.69	1.01E-03	ahnak	1.65	2.34E-02
itgb1bp1	1.62	3.16E-04	Col6a3	1.74	8.80E-03	0710001D07Rik	1.69	4.97E-03	2310016E02Rik	1.65	1.37E-02
SSBP4	1.62	2.15E-03	Gpx8	1.73	1.21E-02	ITGB3BP	1.69	6.91E-03	UNC80	1.62	1.93E-03
fign	1.62	5.26E-03	Arcn1	1.73	1.03E-02	FANK1	1.69	4.92E-04	GNB4	1.61	9.64E-04
LYPD1	1.61	1.45E-04	Cln4-2	1.71	3.46E-03	Klf4	1.68	2.98E-03	Psg23	1.61	2.01E-02
Asnsd1	1.61	3.50E-04	TUBB6	1.71	7.89E-04	LYPD1	1.68	1.45E-04	ATP10D	1.61	1.82E-02
LOC280487	1.61	2.98E-03	sfrp2	1.70	4.16E-03	Nnat	1.67	4.16E-03	Gins4	1.61	9.25E-03
Cln4-2	1.61	3.46E-03	LOC100046025	1.69	5.91E-03	9930031P18Rik	1.63	7.27E-03	TMEM66	1.61	5.01E-03
GTF3C2	1.60	2.45E-02	TSPAN12	1.68	1.40E-02	SCP2	1.62	3.21E-03	pdia6	1.60	2.71E-03
Col5a2	1.60	3.72E-04	OGN	1.68	4.20E-02	Hexb	1.61	3.76E-03	C530036F05Rik	1.60	4.23E-04
PFDN4	1.60	5.73E-03	2310016E02Rik	1.67	1.37E-02	SSBP4	1.61	2.15E-03	MPP7	1.60	6.14E-03
Ttc15	1.59	4.33E-05	SEPP1	1.67	3.59E-02	Hist1h1c	1.59	5.71E-03	COL3A1	1.59	8.30E-03
TUBB6	1.59	7.89E-04	ADI1	1.66	7.46E-04	Nkx6-2	1.59	2.10E-02	ST18	1.59	2.06E-03
AURKAIP1	1.58	4.12E-02	SSBP4	1.66	2.15E-03	nkx2-1	1.58	1.24E-03	gjb2	1.58	2.09E-02
Col6a3	1.57	8.80E-03	CETN2	1.66	4.63E-02	ST18	1.57	2.06E-03	emp1	1.57	1.90E-03
TRAPPC2	1.57	2.14E-02	Col5a2	1.65	3.72E-04	ZWINT	1.56	3.53E-04	NUBP2	1.57	3.54E-04
SH3BGRL	1.57	1.18E-02	DAAM1	1.64	3.34E-03	Uevld	1.54	6.76E-04	myo6	1.56	1.03E-02
Egr1	1.55	6.45E-06	mgst1	1.64	2.65E-02	Phkb	1.54	3.08E-03	GMCL1	1.56	3.09E-02
TSPAN12	1.55	1.40E-02	ahnak	1.64	2.34E-02	ZFYVE27	1.54	3.38E-02	SORL1	1.55	1.68E-03
Gpx8	1.55	1.21E-02	2410076I21Rik	1.63	3.14E-04	ZBTB33	1.54	2.96E-02	FANK1	1.55	4.92E-04
Prpf40a	1.54	1.47E-03	Asnsd1	1.63	3.50E-04	CEBPB	1.53	5.51E-03	2900011L18Rik	1.55	1.92E-02
TFAM	1.54	2.27E-03	E130114P18Rik	1.62	7.35E-04	ANGPT2	1.53	3.39E-04	CHCHD8	1.55	2.24E-02
2310016E02Rik	1.54	1.37E-02	p2ry12	1.62	5.21E-03	vps33a	1.53	9.95E-05	Zfp286	1.54	1.93E-03
E130114P18Rik	1.54	7.35E-04	MLF1	1.62	2.71E-02	NEK3	1.52	1.57E-03	Hexb	1.54	3.76E-03
HIF1A	1.54	4.82E-02	ST18	1.61	2.06E-03	Ppp1ca	1.51	3.78E-02	esd	1.52	2.18E-04
GNG10	1.54	4.05E-02	COMMD1	1.61	1.43E-03	Col5a2	1.51	3.72E-04	Phkb	1.52	3.08E-03
mrps10	1.53	2.00E-02	Jam3	1.61	1.59E-02	Arcn1	1.50	1.03E-02	Col6a3	1.50	8.80E-03
ARIH1	1.53	4.06E-02	Tm9sf2	1.61	2.43E-02	UNC80	1.50	1.93E-03			
GMPS	1.52	5.08E-03	pdia6	1.61	2.71E-03						
STAG2	1.52	5.44E-03	DTD1	1.60	2.45E-02						
spc25	1.51	2.29E-05	KPNA2	1.60	1.58E-02						
RECK	1.51	2.55E-02	ppiC	1.60	4.60E-02						
RBM8A	1.50	3.64E-04	NFIB	1.60	5.91E-04						
2810008M24Rik	1.50	3.68E-02	EBF3	1.59	9.32E-04						
			TRAPPC2	1.58	2.14E-02						
			Tmed2	1.58	2.30E-02						
			GMPS	1.58	5.08E-03						
			DLK1	1.58	2.98E-04						
			FKBP7	1.58	3.09E-03						
			TPBG	1.57	2.56E-03						
			spc25	1.57	4.08E-05						
			Zfp606	1.57	1.49E-03						
			ENSMUSG00000068790	1.57	1.66E-02						
			Ttc15	1.57	4.33E-05						
			fundc1	1.56	5.29E-03						
			fign	1.56	5.26E-03						
			calml4	1.56	7.25E-03						
			SH3BGRL	1.56	1.18E-02						
			RSAD2	1.55	6.13E-04						
			USP14	1.55	3.67E-02						
			ESCO2	1.55	3.79E-06						

			LOC280487	1.55	2.98E-03						
			Tspan6	1.55	1.93E-02						
			ccng1	1.55	2.20E-02						
			tpmT	1.55	2.05E-03						
			UBE2N	1.55	2.01E-02						
			cdkal1	1.54	1.21E-02						
			HIST1H2BK	1.54	1.22E-03						
			HIF1A	1.54	4.82E-02						
			tmod3	1.54	1.72E-02						
			KCNN1	1.54	2.87E-02						
			Tgfbr2	1.54	3.25E-03						
			HNRNPA2B1	1.54	1.88E-02						
			Phkb	1.54	3.08E-03						
			RPGR	1.54	3.28E-02						
			Zfp286	1.54	1.93E-03						
			Pcp411	1.54	1.37E-04						
			Prpf40a	1.54	1.47E-03						
			LYPD1	1.54	1.45E-04						
			THBD	1.53	7.47E-03						
			Rrm1	1.53	1.94E-03						
			3830406C13Rik	1.53	1.43E-02						
			1810035L17Rik	1.53	2.62E-02						
			UBE2A	1.52	4.29E-02						
			STAG2	1.51	5.44E-03						
			frmd3	1.51	2.14E-02						
			FOXC2	1.51	4.65E-02						
			GTF3C2	1.51	2.45E-02						
			srp9	1.51	2.43E-02						
			Hbb-bh1	1.51	2.07E-05						
			2610036L11Rik	1.50	2.25E-03						
			RBM8A	1.50	3.64E-04						
			3110052M02Rik	1.50	1.06E-04						
Embryonic Day 16 (E16)						Embryonic Day 19 (E19)					
COX-1-KO			COX-2-KO			COX-1-KO			COX-2-KO		
Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value
Downregulated Genes											
SPARC	-8.05	3.25E-06	EPM2AIP1	-7.39	3.18E-07	Cox7a2l	-8.06	1.37E-07	EPM2AIP1	-10.02	3.18E-07
Zfp68	-6.10	6.45E-06	UBE2I	-5.38	5.83E-05	Zfp68	-5.73	6.45E-06	TMEM25	-7.27	5.46E-08
ACTL6B	-5.79	6.45E-06	TM7SF3	-5.17	6.19E-04	MCM6	-5.68	1.63E-06	NDUFB10	-6.53	3.34E-06
MYT1L	-5.48	1.88E-02	Bach2	-4.99	1.13E-02	SPARC	-5.35	3.25E-06	Cops8	-5.01	1.40E-07
Cops8	-5.34	1.40E-07	Cops8	-4.76	1.40E-07	ACTL6B	-5.29	6.45E-06	UBE2I	-4.98	5.83E-05
CCNG2	-5.32	6.08E-03	SPARC	-4.72	3.25E-06	Cops8	-5.22	1.40E-07	Gpr137b-ps	-4.92	1.25E-04
TM7SF3	-4.27	6.19E-04	TMEM25	-4.69	5.46E-08	MYT1L	-4.05	1.88E-02	TM7SF3	-4.84	6.19E-04
Bach2	-4.20	1.13E-02	wdr82	-4.47	2.76E-02	Rn18s	-3.90	3.39E-03	Rn18s	-4.52	3.39E-03
MCM6	-4.15	1.63E-06	rpl29	-4.36	1.19E-02	Hbb-b1	-3.89	8.78E-06	rpl29	-4.44	1.19E-02
exadr	-4.14	1.85E-05	Mtap2	-3.73	2.18E-02	TM7SF3	-3.68	6.19E-04	wdr82	-3.76	2.76E-02
PRKAG2	-3.97	4.10E-02	Gpr137b-ps	-3.44	1.25E-04	ACTB	-3.12	1.78E-03	EMB	-3.74	6.92E-07
Cox7a2l	-3.84	1.37E-07	GRIA2	-3.14	1.34E-02	Akr1c19	-2.91	2.43E-04	SPARC	-3.32	3.25E-06
ACTB	-3.51	1.78E-03	Rn18s	-3.12	3.39E-03	B3galt6	-2.85	1.37E-03	Slc25a18	-3.19	3.35E-02
ARV1	-3.31	1.00E-02	Dpysl3	-3.12	2.02E-03	arl5a	-2.78	1.20E-03	FCER1G	-3.12	2.06E-05
Akr1c19	-3.16	2.43E-04	EMB	-3.07	6.92E-07	Gpr137b-ps	-2.64	1.25E-04	MCM6	-3.05	1.63E-06
Mtap2	-3.15	1.48E-02	5330423I11Rik	-3.07	5.94E-03	PUSL1	-2.59	7.10E-04	FGFR1OP2	-2.69	1.68E-04
Mboat2	-3.03	3.90E-05	2900003A17Rik	-2.97	1.70E-07	Mboat2	-2.55	3.90E-05	Mboat2	-2.66	3.90E-05
2900003A17Rik	-3.01	1.70E-07	MEF2C	-2.96	3.57E-02	2900003A17Rik	-2.48	1.70E-07	Hbb-b1	-2.51	8.78E-06

twistnb	-2.99	2.32E-03	Mboat2	-2.95	3.90E-05	CAPN2	-2.36	1.88E-02	2900003A17 Rik	-2.44	1.70E-07
arl5a	-2.95	1.20E-03	ODZ4	-2.90	8.85E-03	twistnb	-2.19	2.32E-03	mgst1	-2.44	2.84E-05
MEF2C	-2.85	3.57E-02	Man2b1	-2.88	1.93E-02	SFRS16	-2.18	1.94E-03	CAPN2	-2.40	1.88E-02
Grm5	-2.81	1.56E-02	PCDH7	-2.83	4.13E-03	PKIG	-2.15	7.86E-03	C030014I23R ik	-2.37	3.59E-05
TAF6	-2.80	6.85E-05	CNTN2	-2.83	3.37E-03	homer2	-2.13	4.33E-05	arrdc3	-2.32	1.78E-03
Hbb-b1	-2.78	8.78E-06	Grm5	-2.83	1.56E-02	hpcal1	-2.09	5.35E-03	SFRS16	-2.31	1.94E-03
Man2b1	-2.75	1.93E-02	AGPAT5	-2.77	1.43E-03	CNTN2	-2.09	3.37E-03	SV2A	-2.27	2.27E-03
Zmiz1	-2.75	3.56E-03	FGFR1OP2	-2.76	1.68E-04	DHRX	-2.09	1.10E-03	6330418B08 Rik	-2.25	7.11E-04
CNTN2	-2.74	3.37E-03	CCNG2	-2.71	6.08E-03	FGFR1OP2	-2.04	1.68E-04	Nudt6	-2.25	3.25E-06
B3galt6	-2.70	1.37E-03	PRDX2	-2.68	3.73E-02	PKM2	-2.02	1.19E-02	TAGAP	-2.23	2.46E-04
melA	-2.63	2.29E-03	Zmiz1	-2.68	3.56E-03	Cd200	-2.02	1.70E-02	Defb11	-2.20	3.71E-03
PCDH17	-2.63	3.20E-03	SV2A	-2.65	2.27E-03	melA	-2.01	2.29E-03	ATP8A1	-2.20	7.07E-04
Dpys13	-2.58	2.02E-03	PCDH17	-2.60	3.20E-03	TAF6	-2.00	6.85E-05	B3galt6	-2.19	1.37E-03
FGFR1OP2	-2.58	1.68E-04	Bach1	-2.59	1.43E-02	6330418B08 Rik	-1.99	7.11E-04	NT5C3	-2.14	3.57E-03
ALG9	-2.57	1.12E-04	ARV1	-2.51	1.00E-02	ALG9	-1.97	1.12E-04	melA	-2.11	2.29E-03
PKM2	-2.57	1.19E-02	D4Ert681e	-2.50	1.22E-02	Stk25	-1.96	1.10E-02	Stk25	-2.10	1.10E-02
AGPAT5	-2.54	1.43E-03	BRD2	-2.47	3.24E-02	Trappc5	-1.94	1.00E-02	PUSL1	-2.10	7.10E-04
1500035N22 Rik	-2.53	1.82E-02	ZDHHC21	-2.46	3.47E-02	GIN1	-1.90	5.48E-05	4833420G17 Rik	-2.06	1.41E-02
ZDHHC21	-2.52	3.47E-02	melA	-2.43	2.29E-03	RpL30	-1.89	7.74E-05	CNTN2	-2.04	3.37E-03
PCDH7	-2.43	4.13E-03	RIOK1	-2.38	7.00E-03	APOE	-1.88	5.55E-05	A1316807	-1.99	3.14E-04
Rn18s	-2.40	5.41E-03	DENND1C	-2.34	1.33E-04	AGPAT5	-1.87	1.43E-03	GIN1	-1.98	5.48E-05
DENND1C	-2.36	1.33E-04	1200016E24 Rik	-2.33	3.99E-02	Tia1	-1.87	1.35E-03	ppplr14c	-1.96	2.52E-03
GRIA2	-2.36	1.34E-02	LOC100047888	-2.32	1.61E-03	Znhit1	-1.86	1.18E-02	PHTF1	-1.91	1.74E-04
hpcal1	-2.35	5.35E-03	NECAB1	-2.32	6.04E-05	MRPS27	-1.85	1.13E-04	rps3a	-1.91	1.10E-02
PRDX2	-2.34	3.73E-02	CSNK1E	-2.27	5.72E-03	Rec8	-1.84	1.37E-02	Cox7a2l	-1.90	1.37E-07
BRD2	-2.28	3.24E-02	BCL11B	-2.25	1.40E-02	STXBP2	-1.83	8.68E-03	ahcY	-1.90	1.82E-02
4833414E09 Rik	-2.27	1.07E-02	Gcap14	-2.24	7.64E-03	TAGAP	-1.81	2.46E-04	D830030K20 Rik	-1.88	2.63E-02
Bach1	-2.24	1.43E-02	4933407C03 Rik	-2.19	3.89E-02	lancl1	-1.81	1.82E-02	CCRN4L	-1.88	2.11E-02
THAP4	-2.24	3.57E-04	POU6F1	-2.18	4.24E-02	TMEM25	-1.78	5.46E-08	Map4k5	-1.87	1.60E-03
NECAB1	-2.15	6.04E-05	KLF7	-2.18	1.83E-02	Defb11	-1.78	3.71E-03	Lars2	-1.86	8.04E-03
Rec8	-2.09	1.37E-02	6330418B08 Rik	-2.17	7.11E-04	Mrpl55	-1.76	7.68E-03	TSC2	-1.83	1.91E-03
SLC4A4	-2.05	1.12E-02	LOC100048372	-2.17	1.33E-02	ahcY	-1.75	1.82E-02	rps2	-1.82	3.33E-03
DHRX	-2.02	1.10E-03	H2afj	-2.13	1.98E-03	pts	-1.72	7.51E-03	UAP1	-1.80	5.73E-04
RIOK1	-2.01	7.00E-03	Stk25	-2.10	1.10E-02	ZBTB45	-1.71	1.22E-02	Tmed4	-1.80	3.94E-02
Tia1	-2.01	1.35E-03	FCER1G	-2.10	2.06E-05	THAP4	-1.70	3.57E-04	EME2	-1.78	2.10E-04
MRPS27	-2.00	1.13E-04	TSC2	-2.07	1.91E-03	LASSA	-1.68	7.43E-03	STXBP2	-1.78	8.68E-03
fam168a	-2.00	3.75E-02	Ppp1r1c	-2.06	3.64E-02	Fbxo46	-1.67	3.54E-03	Ipo9	-1.77	1.78E-02
KIF1B	-1.97	2.10E-02	ACTB	-2.06	1.78E-03	iars2	-1.62	4.72E-03	SRPR	-1.76	8.54E-05
DHCR24	-1.95	3.47E-02	ATP8A1	-2.05	7.07E-04	Insig2	-1.62	3.12E-03	Mrpl55	-1.76	7.68E-03
SIN3A	-1.92	1.30E-04	Fbxo41	-2.05	1.57E-03	abcd3	-1.62	5.38E-03	THAP4	-1.74	3.57E-04
PBX1	-1.91	1.05E-03	Hook3	-2.04	2.85E-02	Gpr17	-1.61	4.34E-04	RpL30	-1.71	7.74E-05
homer2	-1.91	4.33E-05	THAP4	-2.03	3.57E-04	SEMA5A	-1.60	1.36E-03	AGPAT5	-1.68	1.43E-03
6330418B08 Rik	-1.91	7.11E-04	9330164J24 Rik	-2.02	1.06E-02	CHRNA3	-1.59	5.07E-03	Fbxo46	-1.67	3.54E-03
Stk25	-1.90	1.10E-02	SRPR	-2.00	8.54E-05	ANAPC5	-1.59	2.00E-04	mthfd1	-1.66	5.47E-04
Hook3	-1.90	2.85E-02	TAGAP	-1.99	2.46E-04	KCNF1	-1.58	3.90E-03	Spsb3	-1.63	5.47E-03
LOC100047888	-1.89	1.61E-03	NRXN1	-1.98	5.65E-03	2900060N12 Rik	-1.57	5.27E-05	PEX11B	-1.63	2.01E-04
AHDC1	-1.89	4.81E-02	TMEM66	-1.97	1.31E-03	camk4	-1.56	5.55E-05	FLYWCH2	-1.61	8.74E-03
gtpbp2	-1.86	1.58E-03	ppplr14c	-1.96	2.52E-03	9030624G23 Rik	-1.56	2.72E-03	klhl21	-1.61	2.15E-02
PKIG	-1.86	7.86E-03	mmp16	-1.96	4.63E-03	pygb	-1.56	1.13E-02	1700029J07R ik	-1.60	8.09E-03
PUSL1	-1.85	7.10E-04	igsf3	-1.96	2.39E-02	ttc17	-1.55	5.34E-04	Cirbp	-1.60	3.30E-02
Gcap14	-1.85	7.64E-03	C77370	-1.95	4.07E-02	SERINC3	-1.55	3.29E-03	ACTL6B	-1.59	6.45E-06

Cd200	-1.85	1.70E-02	PCDHA7	-1.92	1.74E-04	9930105H17Rik	-1.54	2.78E-02	2900060N12Rik	-1.57	5.27E-05
mmp16	-1.85	4.63E-03	2700089E24Rik	-1.92	1.08E-04	Gstm6	-1.53	1.13E-02	BDH1	-1.57	2.51E-03
ODZ4	-1.84	8.85E-03	GLG1	-1.92	3.51E-04	AI316807	-1.53	3.14E-04	Zfp160	-1.57	5.73E-03
D430041D05Rik	-1.84	1.53E-02	CASKIN1	-1.91	1.87E-04	TMEM158	-1.51	1.94E-03	DMWD	-1.56	6.83E-03
H2afj	-1.82	1.98E-03	Gnai1	-1.91	3.55E-02	Phlda3	-1.51	1.37E-02	Znhit1	-1.56	1.18E-02
MDGA2	-1.81	9.20E-03	6330415B21Rik	-1.90	2.92E-02				MED23	-1.55	9.36E-04
PCDHA7	-1.79	1.74E-04	D430007A19Rik	-1.90	1.46E-02				CDKN1B	-1.55	3.89E-02
Epb4.112	-1.79	6.17E-03	hpcal1	-1.89	5.35E-03				NLRX1	-1.55	1.45E-04
Gpr137b-ps	-1.78	1.25E-04	PKM2	-1.89	1.19E-02				H2afj	-1.55	1.98E-03
UBE2I	-1.78	5.83E-05	gtpbp2	-1.89	1.58E-03				SEMA5A	-1.55	1.36E-03
igsf3	-1.78	2.39E-02	Epb4.112	-1.89	6.17E-03				3110021A11Rik	-1.54	4.94E-03
AFAP1	-1.76	2.57E-02	nrp	-1.89	1.44E-02				lanc11	-1.53	1.82E-02
Zeb2	-1.75	4.80E-02	B3galt6	-1.88	1.37E-03				iars2	-1.53	4.72E-03
RBBP9	-1.75	6.72E-03	RAI1	-1.88	3.64E-02				fut10	-1.53	1.64E-04
SYT4	-1.75	2.75E-03	MDGA2	-1.87	9.20E-03				Zfp39	-1.53	5.89E-03
palm	-1.75	3.67E-02	napB	-1.86	4.38E-03				DHRX	-1.52	1.10E-03
camk4	-1.75	5.55E-05	Map4k5	-1.86	1.60E-03				Ccdc117	-1.51	2.09E-02
Fbxo41	-1.74	1.57E-03	SNX27	-1.84	2.55E-03				OLFML3	-1.51	3.37E-02
SERINC3	-1.73	3.29E-03	Zeb2	-1.84	4.80E-02				LOC674427	-1.51	2.36E-03
CAMKV	-1.72	1.13E-04	gpsm1	-1.83	6.35E-03				Trappc5	-1.51	1.00E-02
9330164J24Rik	-1.72	1.06E-02	irs2	-1.83	1.94E-03						
LOC100048372	-1.72	1.33E-02	prpf40b	-1.82	4.55E-02						
GLTPD1	-1.72	4.43E-02	Clasp1	-1.82	1.99E-02						
CDH8	-1.72	4.43E-02	TNIK	-1.81	4.89E-02						
Adam17	-1.71	1.15E-02	SOX5	-1.81	1.82E-02						
Fbxl18	-1.71	4.32E-02	keap1	-1.81	1.30E-04						
ATM	-1.69	3.12E-03	SLC4A3	-1.80	1.21E-03						
C130045I22Rik	-1.69	2.50E-05	Mll1	-1.79	1.18E-02						
NRXN1	-1.68	5.65E-03	TRIO	-1.77	5.22E-03						
SLC4A3	-1.67	3.54E-03	ALG9	-1.77	1.12E-04						
irs2	-1.67	1.94E-03	Adrbk2	-1.76	6.24E-03						
KCND2	-1.67	2.78E-02	DAG1	-1.76	3.90E-02						
GLG1	-1.66	3.51E-04	CAMKV	-1.76	1.13E-04						
KLF7	-1.66	1.83E-02	C030014I23Rik	-1.75	3.59E-05						
SEMA5A	-1.66	1.36E-03	Tssc8	-1.75	1.19E-03						
esd	-1.65	2.18E-04	Scn1a	-1.74	6.65E-03						
gpsm1	-1.65	6.35E-03	Gpd2	-1.73	6.05E-03						
Acsf6	-1.64	4.48E-02	RBM28	-1.73	5.65E-03						
DAG1	-1.64	3.90E-02	rps2	-1.73	3.33E-03						
napB	-1.64	4.38E-03	H13	-1.73	7.28E-03						
CSNK1E	-1.63	5.72E-03	PHTF1	-1.73	1.74E-04						
abcd3	-1.63	5.38E-03	SLC4A4	-1.73	1.12E-02						
Hps1	-1.63	4.64E-05	GSK3B	-1.72	1.64E-03						
SERPINE2	-1.63	1.94E-03	lsm12	-1.72	4.89E-03						
SNX27	-1.63	2.55E-03	ahcY	-1.72	1.82E-02						
ppp3ca	-1.62	1.36E-02	SFRS16	-1.72	1.94E-03						
Sen1a	-1.61	6.65E-03	3110021A11Rik	-1.72	4.94E-03						
2900060N12Rik	-1.60	5.27E-05	KCND2	-1.71	2.78E-02						
rpe	-1.60	3.83E-02	DHCR24	-1.71	3.47E-02						
H13	-1.60	7.28E-03	palm	-1.71	3.67E-02						
myh10	-1.60	3.95E-02	4833414E09Rik	-1.70	1.07E-02						
Insig2	-1.60	3.12E-03	KIF1B	-1.70	2.10E-02						
AQP4	-1.60	1.02E-04	Rec8	-1.70	1.37E-02						

ttc17	-1.60	5.34E-04	CDH8	-1.69	4.43E-02						
Epb4.111	-1.59	3.11E-02	AFAP1	-1.69	2.57E-02						
pts	-1.59	7.51E-03	dnajc5	-1.69	4.81E-02						
dnajc5	-1.58	4.81E-02	A2bp1	-1.69	1.76E-02						
nrp	-1.58	1.44E-02	MRPS27	-1.68	1.13E-04						
6430548M08Rik	-1.58	4.63E-02	arrdc3	-1.68	1.78E-03						
Adrbk2	-1.58	6.24E-03	MCM6	-1.67	1.63E-06						
BCL11B	-1.57	1.40E-02	D11Bwg0517e	-1.67	3.57E-03						
prpf40b	-1.57	4.55E-02	Nudt6	-1.67	3.25E-06						
camkk2	-1.57	1.06E-02	NAV1	-1.67	9.04E-03						
Syp	-1.57	9.74E-04	6430548M08Rik	-1.67	4.63E-02						
Ptprd	-1.57	4.13E-02	E130307A14Rik	-1.66	4.47E-02						
UBTF	-1.56	3.06E-02	LOC100046343	-1.66	1.31E-02						
CASKIN1	-1.56	2.93E-03	C130045I22Rik	-1.66	2.50E-05						
THY1	-1.56	3.25E-06	AI316807	-1.65	3.14E-04						
Znhit1	-1.56	1.18E-02	ppp3ca	-1.65	1.36E-02						
6430411K18Rik	-1.56	3.77E-02	usp48	-1.65	2.68E-02						
Ppp1r1c	-1.55	9.62E-03	2900060N12Rik	-1.64	5.27E-05						
TAGAP	-1.55	2.46E-04	Ptprd	-1.64	4.13E-02						
SGSM1	-1.55	2.07E-02	ZCCHC6	-1.64	3.91E-03						
keap1	-1.55	1.30E-04	SETBP1	-1.64	5.98E-03						
DCTN4	-1.55	2.71E-02	mapt	-1.64	2.12E-03						
RAPGEFL1	-1.54	7.40E-05	GRIP1	-1.64	1.83E-03						
ANAPC5	-1.54	2.00E-04	SEZ6	-1.63	4.73E-02						
mapt	-1.53	2.12E-03	Epb4.111	-1.63	4.38E-03						
Zfp236	-1.53	3.60E-02	CLCN7	-1.63	1.22E-02						
ZBTB45	-1.53	1.22E-02	AHDC1	-1.63	4.81E-02						
Cops5	-1.52	1.35E-02	D830030K20Rik	-1.63	2.63E-02						
BAI2	-1.52	6.07E-03	RNF182	-1.63	2.89E-02						
DGKH	-1.52	1.31E-02	them4	-1.62	2.85E-03						
Pbx4	-1.52	6.42E-03	LDB2	-1.62	5.31E-03						
Camk2b	-1.52	1.83E-04	A130054J05Rik	-1.61	3.12E-02						
GUCY1A3	-1.52	6.76E-04	D430041D05Rik	-1.61	1.53E-02						
EXOC6B	-1.52	1.61E-03	Zfp131	-1.61	1.19E-02						
CHRNA3	-1.52	5.07E-03	Syt1	-1.61	1.08E-03						
KCNF1	-1.51	3.90E-03	myh10	-1.59	3.95E-02						
A2bp1	-1.51	1.76E-02	EME2	-1.59	2.10E-04						
cspg5	-1.50	7.06E-06	LOC100045679	-1.59	3.99E-02						
Hmg20a	-1.50	4.87E-02	SERPINE2	-1.58	1.94E-03						
Ncdn	-1.50	2.06E-03	Snapc3	-1.58	4.91E-02						
GIN1	-1.50	5.48E-05	BAI2	-1.57	6.07E-03						
			ANKMY2	-1.57	1.18E-02						
			GIN1	-1.57	5.48E-05						
			Fbxl18	-1.57	4.32E-02						
			Tia1	-1.57	1.35E-03						
			Spag9	-1.57	1.75E-02						
			Pbx4	-1.57	6.42E-03						
			APIB1	-1.56	1.48E-02						
			KLC4	-1.56	1.13E-02						
			PUSL1	-1.56	7.10E-04						
			Car11	-1.56	2.90E-03						
			EXOSC10	-1.55	4.82E-02						
			Spsb3	-1.55	8.65E-03						

			LOC100046746	-1.55	1.54E-02						
			Plxna2	-1.55	2.60E-02						
			Sox6	-1.55	1.58E-03						
			1700029J07Rik	-1.55	8.09E-03						
			Zfp281	-1.55	3.68E-03						
			LPHN1	-1.55	1.04E-02						
			PIP5K1A	-1.54	5.38E-03						
			ZBTB17	-1.54	3.14E-03						
			LIMCH1	-1.54	4.08E-02						
			vldlr	-1.54	2.04E-02						
			homer2	-1.54	4.33E-05						
			CHD5	-1.54	1.65E-02						
			CDC42BPB	-1.54	3.80E-04						
			GUCY1A3	-1.54	6.76E-04						
			RAD23B	-1.53	2.24E-02						
			GABARAPL1	-1.53	1.56E-02						
			Defb11	-1.53	3.71E-03						
			CCND2	-1.53	2.37E-02						
			MYT1L	-1.53	2.07E-02						
			DHRX	-1.53	1.10E-03						
			D930015E06Rik	-1.52	3.82E-02						
			Ncdn	-1.52	2.06E-03						
			6430590A07Rik	-1.52	3.29E-02						
			zmynd11	-1.52	1.99E-02						
			srebf2	-1.52	3.00E-02						
			C030048H21Rik	-1.52	8.03E-05						
			cspg5	-1.51	7.06E-06						
			Sf1	-1.51	3.29E-02						
			TNRC6C	-1.51	3.64E-02						
			Ngef	-1.51	1.02E-05						
			SPATA7	-1.51	1.85E-04						
			Cfl2	-1.51	2.60E-02						
			MED23	-1.51	9.36E-04						

B2. Microarray Data – Differential Expression of All Genes in the COX-2 KO Female Mouse Brain

Supplementary Table 2 – Differential expression of all genes detected by whole genome analysis (Mouse Genome 2.0ST GeneChip® (Affymetrix) microarray) in the female COX-2-KO mouse brain at embryonic days 16 (E16) and 19 (E19), compared to female WT mouse. Fold-change values from each developmental stage are mean representations from three female offspring (n=3)

Embryonic Day 16 (E16)			Embryonic Day 19 (E19)			Embryonic Day 16 (E16)			Embryonic Day 19 (E19)		
Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value	Gene	FC	P-value
<i>Upregulated Genes</i>						<i>Downregulated Genes</i>					
Zfp125	5.28	6.50E-04	Zfp125	4.75	6.50E-04	Gm9855 Tdg	-3.67	6.50E-04	Gm9855 Tdg	-2.83	6.50E-04
Gm3579	4.51	0.008825	Gm26110	4.22	0.00492	Rpl3 Rpl3-ps1	-2.98	0.002236	Pisd-ps3	-2.59	0.029539
Gm42035 Gm3579	4.51	0.008825	Rnu1b6	4.22	0.00492	Snora44	-2.79	0.014618	Tmem181b-ps	-2.41	0.012715
Gm3579	4.51	0.008825	Rnu1b1 Rnu1b2	4.22	0.00492	Hist4h4	-2.79	0.009614	Tpm3	-2.37	0.021029
Gm42035 Gm3579	4.51	0.008825	Rnu1b6	4.22	0.00492	Rps2 Snora64	-2.56	0.010323	Rpl3 Rpl3-ps1	-2.27	0.002236
Gm17167 Gm29964 Gm21075 Gm13301	3.86	0.001954	Gm25890 Gm22614	4.22	0.00492	Hist2h2aa1 Hist2h2aa2	-2.44	0.01304	Rps3a3 Rps3a1	-2.23	0.00648
LOC100041599	3.52	0.003575	Rnu1b1 Rnu1b2	4.22	0.00492	Hist2h2aa2 Hist2h2aa1	-2.44	0.01304	Gm8356 Gm3591 Gm3629	-2.22	0.026558
Gm13303	2.84	0.004804	Rnu1b6	4.22	0.00492	Snord73a	-2.38	0.033468	Gm3500 Gm3373 Gm10406	-2.11	0.038484
Gm3893 Gm21093 Gm3883 Gm7819	2.60	0.00492	Gm25890 Gm22614	4.22	0.00492	Gm25989	-2.30	0.006272	Mir139	-2.10	0.033193
Gm29804 Gm5859 Gm29801 Gm21075 Gm13301	2.56	0.004307	Gm22042	4.22	0.00492	Snora69	-2.30	0.007962	Gabra2	-2.09	0.010712
4833420G17Rik	2.54	0.007962	Gm3579	4.20	0.008825	Gm25394	-2.27	0.012494	Glo1	-2.09	0.009656
LOC100041536 Gm13303	2.50	0.00492	Gm42035 Gm3579	4.20	0.008825	Ptp4a1	-2.25	0.017239	Pcdhb6	-1.97	0.021286
LOC102637418 LOC102637312 LOC100041516 Gm3893	2.49	0.004177	Gm3579	4.20	0.008825	Snord15a	-2.25	0.013557	Serpina3n	-1.97	0.001954
Tmem254b Tmem254c Tmem254a	2.37	0.00492	Gm42035 Gm3579	4.20	0.008825	Tmem181b-ps	-2.24	0.012715	Pla2g4e	-1.94	0.020521
4933409K07Rik Gm21093 Gm3883	2.33	0.004416	Gm24888	3.72	0.011299	Gm23136	-2.21	0.009132	Abhd4	-1.92	0.00785
4933409K07Rik Gm21093 Gm3883	2.33	0.004416	mt-Tf	3.69	0.03081	Gm25395	-2.18	0.009362	Gm3435	-1.90	0.001501

Tmem254b Tmem254c Tmem254a	2.31	0.00521	Gm23245	3.45	0.024271	Rprl3	-2.14	0.011413	Gm5784	-1.83	0.009812
Gm3893 G m7819	2.25	0.01304	Gm22270	3.38	0.012513	Mir667	-2.13	0.013189	Arv1	-1.83	0.012087
Cyb5r3	2.23	0.013189	Rnu73b	3.26	0.020497	Gm22303	-2.12	0.014469	Gm3194 G m3500 Gm 5796	-1.81	0.039153
Tmem254b Tmem254c Tmem254a	2.20	0.006022	Scarna6	3.20	0.009362	Snord11	-2.10	0.039153	Gm3264 G m5796	-1.80	0.041299
Gm3893 G m2163 Gm 7819	2.09	0.020839	Gm26225	3.06	0.045115	Snord70	-2.10	0.038545	Nrbp2	-1.74	0.026945
Ide	1.92	0.001954	Ptcd3	2.84	0.013096	Gm22358	-2.09	0.014698	Mir1931	-1.74	0.007456
Gm17167 Gm29964	1.91	0.014239	Gm24046	2.79	0.007962	Gm22973	-2.08	0.01545	Wdfy1	-1.73	6.50E-04
Rps3a2	1.91	0.023954	Snora62	2.71	0.018355	Snord118	-2.07	0.018355	1500035N2 2Rik	-1.72	0.009256
Hist1h2bc	1.87	0.007392	Gm25395	2.71	0.009362	1500012F0 1Rik	-2.06	0.03552	Mir1906- 1 Mir1906- 2	-1.71	0.036116
Zfp781	1.87	0.014555	Snora69	2.65	0.007962	Gm22767	-2.06	0.008686	Gm21811	-1.70	0.017781
Zfp157	1.83	0.016291	Gm23344	2.64	0.032147	Wdfy1	-2.05	6.50E-04	Gm4983	-1.70	0.026111
Gm21158 Gm2192	1.83	0.012715	Snord14e H spa8	2.59	0.019136	Rmst	-2.03	0.006997	Leprot11	-1.66	0.017893
Gm21158 Gm2192	1.83	0.012715	Snora16a	2.57	0.012494	Scarna6	-2.01	0.009362	Gm3020 G m10409	-1.65	0.04958
Rgs4	1.80	0.037017	Snora28	2.53	0.038765	Snord49a	-2.01	0.038258	Gm3020 G m10409 G m3696	-1.65	0.04958
Zfp40	1.79	0.010717	Snord15a	2.52	0.013557	Snora3 Rpl 27a	-2.01	0.009798	Clcn6	-1.64	0.009212
B3galt2	1.75	0.012715	n-R5s28	2.51	0.016775	Ptcd3	-2.01	0.013096	Tmem230	-1.64	0.004804
Gm9615	1.75	0.004128	Gm25873	2.49	0.03605	Snord1b	-2.00	0.037829	1500004A1 3Rik	-1.62	0.017537
Ptgds	1.74	0.004998	Gm23136	2.44	0.009132	AF357399	-2.00	0.004346	Esco1	-1.61	0.00504
Dctd Gm39 174	1.72	0.006272	Snord72	2.41	0.017127	Gm23344	-1.99	0.032147	Sike1	-1.60	0.036192
Cbr1	1.70	0.016242	Snord118	2.41	0.018355	n-R5s28	-1.98	0.016775	Tfap2b	-1.60	0.024239
Hjrup 6430 706D22Rik A730008H 23Rik	1.69	0.001954	Gm25835	2.39	0.012715	Snora28	-1.96	0.038765	Slc6a8	-1.60	0.010703
Apoa1bp	1.69	0.00492	Snord11	2.38	0.039153	Snord14a R ps13	-1.96	0.017917	Camk2a	-1.58	0.013772
Sdhc	1.68	0.030889	Mir1983	2.37	0.035905	Gm24888	-1.95	0.011299	Tmem181a	-1.58	0.034871
LOC10086 2043	1.67	0.016021	Snora31	2.36	0.023325	Snord42b	-1.95	0.028637	Fcho1	-1.58	0.014899
Capza1	1.66	0.038646	Snord12 15 00012F01R ik	2.36	0.016242	Snord8	-1.95	0.00589	Tspan18	-1.57	0.04084
Pcp4 lgsf5	1.66	0.005038	Gm25989	2.32	0.006272	Gm24438	-1.95	0.012483	Mir5125 Sr rm2	-1.56	0.043015
Dad1	1.66	0.030119	Gm17167 Gm29964 Gm21075 Gm13301	2.30	0.001954	Rps8	-1.94	0.026189	Porcn	-1.56	0.007962
Gm6415	1.65	0.011492	AF357399	2.30	0.004346	Gm23406	-1.93	0.038484	1810037I17 Rik	-1.56	0.016217
Dynlrb1	1.63	0.009201	Gm24445	2.29	0.043994	Snord58b	-1.93	0.033521	6820431F2 0Rik	-1.55	0.027813
Arl3	1.63	0.014434	Snord49a	2.28	0.038258	Snord16a	-1.91	0.031888	Mir9- 2 C130071 C03Rik	-1.55	0.047982
Isyna1	1.63	0.008565	Gm23264	2.28	0.014684	Eif4a2	-1.90	0.00492	Gpr137b	-1.54	0.018355
Akr1e1	1.63	0.026189	Gm22422	2.27	0.017239	Snord95 Gn b211	-1.90	0.018355	Ttll1	-1.54	0.025009

Tmem150c	1.63	0.010665	LOC100041599	2.27	0.003575	Snord1c	-1.89	0.020459	Tceb2	-1.54	0.022783
Zfp458	1.62	0.020367	Gm24119	2.26	0.007329	Gm25835	-1.89	0.012715	Btbd9	-1.53	0.01304
Pisd-ps2	1.61	0.023846	Snord16a	2.24	0.031888	Snora62	-1.89	0.018355	Aldh2	-1.53	0.008886
Fabp3-ps1	1.61	0.018123	Snora73b	2.24	0.005904	Snora23	-1.88	0.005134	Gm3526 Gm3344	-1.52	0.049666
Chordc1	1.61	0.048155	Snora74a	2.24	0.018123	Pisd-ps3	-1.88	0.029539	Slc50a1	-1.52	0.029501
Snhg6	1.61	0.020064	Gm25633	2.24	0.007962	Snora15	-1.86	0.009238	Fbxw5	-1.51	0.00232
2610507101Rik	1.60	0.039614	1500012F01Rik	2.23	0.03552	Gm24494	-1.86	0.028059	Hddc3	-1.51	0.020521
Jmjd7 Gm28042	1.60	0.004416	Rps8	2.22	0.026189	Gm24253	-1.84	0.01954	Gm3095 Gm3029	-1.51	0.04958
LOC102638850 Gm5963	1.60	0.017127	Gm26175	2.21	0.02132	Gm25099	-1.84	0.004416			
Zfp131	1.58	0.029143	Gm23969	2.20	0.016704	Gm23795	-1.84	0.018601			
Zdhhc18 Gm13213	1.57	0.013189	Gm25394	2.18	0.012494	Gm24119	-1.83	0.007329			
Scpep1	1.56	0.032751	Snora15	2.16	0.009238	Rnu12	-1.81	0.005219			
Hist1h4i	1.56	0.046529	Snord61	2.15	0.016387	Gm22620	-1.81	0.015532			
LOC102637606	1.56	0.006269	Snora23	2.14	0.005134	Gm24698	-1.81	0.034973			
Eif3e	1.56	0.015039	Snord49b	2.13	0.023863	Snord61	-1.79	0.016387			
Gabrb1	1.55	0.013411	Snord69	2.12	0.044784	Gm25970	-1.78	0.021413			
Sfr1	1.55	0.008895	Gm23503	2.10	0.007962	Rpl30	-1.78	0.022757			
Cdh10	1.55	0.031051	Rnu3b1 Rnu3b3 Rnu3b2 Gm42087	2.09	0.011297	Gm24105	-1.78	0.0178			
Ranbp6	1.55	0.035537	Rnu3b1 Rnu3b3 Rnu3b2	2.09	0.011297	Rps3a3 Rps3a1	-1.77	0.00648			
Ifit27	1.54	0.021642	Rnu3b4 Rnu3b1 Rnu3b3 Rnu3b2	2.09	0.011297	Snora17 Snhg7	-1.77	0.012752			
Exosc9	1.54	0.026831	Rnu3b4 Rnu3b1 Rnu3b3 Rnu3b2	2.09	0.011297	Snord67 Ckap5	-1.77	0.017802			
Zfp518a Mir8092	1.54	0.013122	Snora34 Mir1291	2.09	0.017582	Terc	-1.77	0.014698			
Vps39	1.54	0.03399	Snord15b Rps3	2.08	0.026352	Gm25053	-1.77	0.024571			
Entpd4 Gm21685	1.54	0.014899	Rpl21	2.07	0.018388	Taf1d	-1.76	0.020064			
Gm12174 Rpl7	1.54	0.016608	Gm22303	2.07	0.014469	Mir139	-1.76	0.033193			
Got1	1.54	0.007962	Gm22358	2.06	0.014698	Snord69	-1.76	0.044784			
Gm9892	1.53	0.044334	Gm22403	2.05	0.024955	Snord49b	-1.75	0.023863			
Map9	1.53	0.015471	Snord1b	2.04	0.037829	Snord45b Rabggb	-1.75	0.01304			
Lrrtm2	1.53	0.024136	Snord58b	2.04	0.033521	Snord72	-1.75	0.017127			
Tmem126a	1.53	0.02408	Snord89	2.04	0.033193	Snord12 1500012F01Rik	-1.75	0.016242			
2610044015Rik8	1.53	0.009362	Rpl30	2.02	0.022757	Snora16a	-1.74	0.012494			
Snapc3	1.53	0.022912	Snord42b	2.01	0.028637	Gm3435	-1.74	0.001501			
Ndufa3	1.52	0.027171	1500012F01Rik	2.00	0.028957	Snora73a	-1.73	0.010917			
C1qbp	1.52	0.017893	Snora81 Eif4a2	2.00	0.008296	Mir543	-1.73	0.012461			
Zfp943	1.52	0.025009	Gm24624	2.00	0.005846	Gm22711	-1.72	0.042931			
Entpd4 Gm21685	1.52	0.016021	Gm29804 Gm5859 Gm29801 Gm21075 Gm13301	1.99	0.004307	Gm3500 Gm3373 Gm10406	-1.72	0.038484			

Lynx1	1.52	0.001501	Gm25514	1.99	0.013189	Snora81 Eif4a2	-1.72	0.008296			
Psm6	1.52	0.032147	4833420G17Rik	1.99	0.007962	1500012F01Rik	-1.72	0.028957			
Dstn	1.51	0.013189	Gm26825	1.98	0.036305	Gm25321	-1.71	0.010342			
Pak6	1.51	0.021563	Gm25039	1.97	0.030798	Snora34 Mir1291	-1.71	0.017582			
Stmn4	1.51	0.033174	LOC102642670	1.97	0.041748	Scarna2	-1.70	0.012514			
Olig1	1.51	0.00589	Gm23795	1.96	0.018601	Snora5c Tbarg4	-1.70	0.039703			
Zfp180	1.50	0.045457	Rps2 Snora64	1.94	0.010323	Gm24233	-1.69	0.017106			
Pla2g4b Gm28042	1.50	0.006062	Snord70	1.92	0.038545	Gabra2	-1.69	0.010712			
Srsf10	1.50	0.012796	Snord47	1.90	0.013833	Rpph1	-1.68	0.016242			
Cyp51	1.50	0.011727	Gm22620	1.90	0.015532	Rpl21	-1.67	0.018388			
Pdia6	1.50	0.028187	Gm22866	1.90	0.011586	Gm24616	-1.66	0.047072			
Lin7c	1.50	0.018	Snord87	1.90	0.039349	Gm25492	-1.66	0.027999			
			Gm24438	1.90	0.012483	Snora21	-1.65	0.009209			
			H2afz	1.88	0.015039	Gm23969	-1.65	0.016704			
			Rpl5	1.87	0.021563	Pla2g4e	-1.65	0.020521			
			Gm23927	1.87	0.010656	Snord57 Nopp56	-1.64	0.048261			
			Snord1c	1.87	0.020459	Gm26448	-1.64	0.013189			
			AF357355	1.87	0.03182	Mir377	-1.64	0.022602			
			Gm22638	1.86	0.018303	Gm26236	-1.64	0.009229			
			Gm26493	1.86	0.015794	Snord60 Rab26os	-1.64	0.016217			
			Taf1d	1.86	0.020064	Gm25506	-1.63	0.034038			
			Scarna10 Ncapd2	1.85	0.006807	Snord89	-1.63	0.033193			
			Scarna2	1.83	0.012514	Gm23346	-1.60	0.04294			
			Zfp40	1.83	0.010717	Gm22866	-1.60	0.011586			
			Hjurp 6430706D22Rik A730008H23Rik	1.82	0.001954	Esco1	-1.60	0.00504			
			Atp5e	1.82	0.032571	Rpl5	-1.60	0.021563			
			Rnu12	1.82	0.005219	Gm26110	-1.59	0.00492			
			Gm24920	1.82	0.030362	Rnu1b6	-1.59	0.00492			
			Gm25506	1.81	0.034038	Rnu1b1 Rnu1b2	-1.59	0.00492			
			Zfp458	1.81	0.020367	Rnu1b6	-1.59	0.00492			
			Gm24698	1.81	0.034973	Gm25890 Gm22614	-1.59	0.00492			
			Gm3893 Gm21093 Gm3883 Gm7819	1.80	0.00492	Rnu1b1 Rnu1b2	-1.59	0.00492			
			Tmem254b Tmem254c Tmem254a	1.80	0.00521	Rnu1b6	-1.59	0.00492			
			Tmem254b Tmem254c Tmem254a	1.79	0.00492	Gm25890 Gm22614	-1.59	0.00492			
			Tmem254b Tmem254c Tmem254a	1.79	0.006022	Gm22042	-1.59	0.00492			
			Gm24253	1.78	0.01954	Gm23297	-1.59	0.022134			
			Pisd-ps2	1.78	0.023846	Gnb2l1	-1.59	0.032813			
			Gm25813	1.78	0.030494	Mir487b	-1.58	0.02671			
			Nop56	1.78	0.011727	Gm25128	-1.58	0.04275			
			Snord67 Ckap5	1.77	0.017802	Snord15b Rps3	-1.58	0.026352			
			Snora21	1.77	0.009209	Scarna13 Mir3069	-1.57	0.012417			

			S100a10	1.76	0.030362	Gm26175	-1.56	0.02132			
			Gm25492	1.75	0.027999	Gm23264	-1.56	0.014684			
			Gm14567	1.74	0.013742	Gm24525	-1.55	0.020226			
			Gm26448	1.74	0.013189	9030025P2 0Rik	-1.55	0.012513			
			Gm25117	1.73	0.013189	Gm23503	-1.54	0.007962			
			Rps3a2	1.73	0.023954	Gm23639	-1.54	0.02221			
			Gm26442	1.73	0.007962	Scarna3b M ir1843a	-1.54	0.013189			
			Gm13303	1.72	0.004804	Snora33	-1.54	0.016547			
			Gm22748	1.72	0.033387	Gm24046	-1.53	0.007962			
			Ide	1.72	0.001954	Rnu3b1 Rn u3b3 Rnu3 b2 Gm4208 7	-1.53	0.011297			
			Snord22	1.72	0.014556	Rnu3b1 Rn u3b3 Rnu3 b2	-1.53	0.011297			
			Terc	1.71	0.014698	Rnu3b4 Rn u3b1 Rnu3 b3 Rnu3b2	-1.53	0.011297			
			Snora73a	1.70	0.010917	Rnu3b4 Rn u3b1 Rnu3 b3 Rnu3b2	-1.53	0.011297			
			Hist1h2an	1.70	0.008565	Gm3194 G m3500 Gm 5796	-1.53	0.039153			
			Snhg6	1.69	0.020064	Scarna10 N capd2	-1.53	0.006807			
			Gm23301	1.69	0.012603	Sec24a	-1.53	0.049039			
			Snora68	1.69	0.037024	Gm24727	-1.53	0.028059			
			Gm25128	1.68	0.04275	Gm26493	-1.52	0.015794			
			LOC10004 1536 Gm13 303	1.68	0.00492	Gm24920	-1.52	0.030362			
			Stk3	1.67	0.025205	Gm25107	-1.52	0.008895			
			Snora7a Rp l32	1.67	0.046529	Snord93	-1.51	0.038545			
			Gm25970	1.66	0.021413	Gm25776	-1.51	0.018396			
			Dctd Gm39 174	1.66	0.006272	Pcdhb6	-1.51	0.021286			
			Sec24a	1.66	0.049039	Gm25117	-1.51	0.013189			
			Gm24727	1.65	0.028059	Gm25360	-1.51	0.029539			
			Gm26202	1.65	0.018258						
			Gm24704	1.65	0.034477						
			Gm23297	1.64	0.022134						
			Snord95 Gn b2l1	1.64	0.018355						
			Rmrp	1.64	0.03763						
			Gm25107	1.64	0.008895						
			Wdyhvl	1.63	0.049222						
			Gm24105	1.63	0.0178						
			Gm14412	1.63	0.019409						
			Gm25776	1.62	0.018396						
			Snora52 Rp lp2	1.62	0.032717						
			Gm22442	1.60	0.036482						
			Eif4a2	1.60	0.00492						
			Cyp51	1.60	0.011727						
			Rnu11	1.60	0.02438						
			Apoalbp	1.60	0.00492						
			n-R5s89	1.59	0.009238						
			Gm24044	1.58	0.01954						
			Gm26236	1.58	0.009229						
			4933409K0 7Rik Gm21	1.57	0.004416						

			093 Gm388 3								
			4933409K0 7Rik Gm21 093 Gm388 3	1.57	0.004416						
			Gm14420	1.57	0.017423						
			LOC10263 7418 LOC1 02637312 L OC100041 516 Gm389 3	1.57	0.004177						
			2610044O1 5Rik8	1.57	0.009362						
			Gm23639	1.57	0.02221						
			Snord14a R ps13	1.56	0.017917						
			Gm3893 G m7819	1.56	0.01304						
			Zfp938	1.56	0.016217						
			Gm23444 Gm23472	1.55	0.018388						
			Gm24497	1.55	0.018388						
			Gm25939	1.55	0.018388						
			Gm23444 Gm23472	1.55	0.018388						
			Sord	1.54	0.024239						
			Gas5	1.54	0.012325						
			Snora3 Rpl 27a	1.54	0.009798						
			Scarna13 M ir3069	1.54	0.012417						
			Gm24233	1.54	0.017106						
			Gm9615	1.54	0.004128						
			Gm22980	1.53	0.029009						
			Snora30	1.53	0.039473						
			Mmd2	1.53	0.012513						
			Gm22009	1.53	0.040878						
			Gm17167 Gm29964	1.53	0.014239						
			Gm3893 G m2163 Gm 7819	1.52	0.020839						
			Snora33	1.52	0.016547						
			Gm6023	1.52	0.012579						
			Gm10819	1.51	0.045796						
			Snord60 Ra b26os	1.51	0.016217						
			Ndufc2	1.51	0.020941						

B3. Differential Expression of Common Genes between E16 and E19 in the COX-1 and -2 KO Male Mouse Brain

Supplementary Table 3 – Differential expression of common genes between embryonic days 16 (E16) and 19 (E19) expressed in the COX-1-KO and COX-2-KO male mouse brain.

COX-1 ^{-/-} Male Mouse Brain			COX-2 ^{-/-} Male Mouse Brain		
	E16	E19		E16	E19
Gene	FC	FC	Gene	FC	FC
SOX11	3.39	3.17	Hbb-b2	10.88	12.83
Lyplal1	2.03	1.96	SOX11	3.30	2.73
TMEM87A	2.02	2.35	Paip1	3.21	3.74
RBM45	2.01	1.97	0710001D07Rik	2.42	2.08
LOC100044314	1.92	2.18	emp1	2.34	1.57
0710001D07Rik	1.89	1.69	trim2	2.22	2.25
CAP1	1.86	2.66	LOC100044314	2.17	1.98
Paip1	1.85	2.58	Lyplal1	2.09	1.87
ITGB3BP	1.84	1.69	COL3A1	2.05	1.59
SCP2	1.76	1.62	GLO1	2.01	1.90
ADI1	1.74	1.73	gjb2	2.00	1.58
LOC100045240	1.72	2.89	GLTPD1	1.96	2.49
vps33a	1.68	1.53	Sfrp1	1.84	2.59
Hexb	1.65	1.61	Hexb	1.79	1.54
SSBP4	1.62	1.61	NUBP2	1.79	1.57
LYPD1	1.61	1.68	NDUFB10	1.78	-6.53
Col5a2	1.60	1.51	Col6a3	1.74	1.50
Ttc15	1.59	1.78	Arcn1	1.73	1.67
Egr1	1.55	2.42	LOC100046025	1.69	1.72
2310016E02Rik	1.54	1.76	2310016E02Rik	1.67	1.65
SPARC	-8.05	-5.35	ADI1	1.66	1.78
Zfp68	-6.10	-5.73	SSBP4	1.66	1.68
ACTL6B	-5.79	-5.29	DAAM1	1.64	2.12
MYT1L	-5.48	-4.05	mgst1	1.64	-2.44
Cops8	-5.34	-5.22	ahnak	1.64	1.65
TM7SF3	-4.27	-3.68	ST18	1.61	1.59
MCM6	-4.15	-5.68	pdia6	1.61	1.60
Cox7a2l	-3.84	-8.06	ENSMUSG00000068790	1.57	1.93
ACTB	-3.51	-3.12	Ttc15	1.57	1.81
Akr1c19	-3.16	-2.91	cdkal1	1.54	1.67
Mboat2	-3.03	-2.55	Phkb	1.54	1.52
2900003A17Rik	-3.01	-2.48	Zfp286	1.54	1.54
twistnb	-2.99	-2.19	LYPD1	1.54	1.68

arl5a	-2.95	-2.78	EPM2AIP1	-7.39	-10.02
TAF6	-2.80	-2.00	UBE2I	-5.38	-4.98
Hbb-b1	-2.78	-3.89	TM7SF3	-5.17	-4.84
CNTN2	-2.74	-2.09	Cops8	-4.76	-5.01
B3galt6	-2.70	-2.85	SPARC	-4.72	-3.32
melA	-2.63	-2.01	TMEM25	-4.69	-7.27
FGFR1OP2	-2.58	-2.04	wdr82	-4.47	-3.76
ALG9	-2.57	-1.97	rpl29	-4.36	-4.44
PKM2	-2.57	-2.02	Gpr137b-ps	-3.44	-4.92
AGPAT5	-2.54	-1.87	Rn18s	-3.12	-4.52
Rn18s	-2.40	-3.90	EMB	-3.07	-3.74
hpcal1	-2.35	-2.09	2900003A17Rik	-2.97	-2.44
THAP4	-2.24	-1.70	Mboat2	-2.95	-2.66
Rec8	-2.09	-1.84	CNTN2	-2.83	-2.04
DHRX	-2.02	-2.09	AGPAT5	-2.77	-1.68
Tia1	-2.01	-1.87	FGFR1OP2	-2.76	-2.69
MRPS27	-2.01	-1.87	SV2A	-2.65	-2.27
homer2	-1.91	-2.13	melA	-2.43	-2.11
6330418B08Rik	-1.91	-1.99	6330418B08Rik	-2.17	-2.25
Stk25	-1.90	-1.96	H2afj	-2.13	-1.55
PKIG	-1.86	-2.15	Stk25	-2.10	-2.10
PUSL1	-1.85	-2.59	FCER1G	-2.10	-3.12
Cd200	-1.85	-2.02	TSC2	-2.07	-1.83
Gpr137b-ps	-1.78	-2.64	ATP8A1	-2.05	-2.20
camk4	-1.75	-1.56	THAP4	-2.03	-1.74
SERINC3	-1.73	-1.55	SRPR	-2.00	-1.76
GLTPD1	-1.72	2.46	TAGAP	-1.99	-2.23
SEMA5A	-1.66	-1.60	TMEM66	-1.97	1.61
abcd3	-1.63	-1.62	ppp1r14c	-1.96	-1.96
2900060N12Rik	-1.60	-1.57	B3galt6	-1.88	-2.19
Insig2	-1.60	-1.62	Map4k5	-1.86	-1.87
ttc17	-1.60	-1.55	C030014I23Rik	-1.75	-2.37
pts	-1.59	-1.72	rps2	-1.73	-1.82
Znhit1	-1.56	-1.86	PHTF1	-1.73	-1.91
TAGAP	-1.55	-1.81	ahcY	-1.72	-1.90
ANAPC5	-1.54	-1.59	SFRS16	-1.72	-2.31
ZBTB45	-1.53	-1.71	3110021A11Rik	-1.72	-1.54
CHRNA3	-1.52	-1.59	4833414E09Rik	-1.70	-2.06
KCNF1	-1.51	-1.58	arrdc3	-1.68	-2.32
GIN1	-1.50	-1.90	MCM6	-1.67	-3.05
			Nudt6	-1.67	-2.25

			AI316807	-1.65	-1.99
			2900060N12Rik	-1.64	-1.57
			D830030K20Rik	-1.63	-1.88
			EME2	-1.59	-1.78
			GIN1	-1.57	-1.98
			PUSL1	-1.56	-2.10
			Spsb3	-1.55	-1.63
			1700029J07Rik	-1.55	-1.60
			Defb11	-1.53	-2.20
			DHR SX	-1.53	-1.52
			MED23	-1.51	-1.55

B4. Differential Expression of Common Genes between E16 and E19 in the COX-2 KO Female Mouse Brain

Supplementary Table 4 – Differential expression of common genes between embryonic days 16 (E16) and 19 (E19) expressed in the female COX-2-KO mouse brain.

COX-2 ^{-/-} Female Mouse Brain		
Gene	E16 FC	E19 FC
Zfp125	5.28	4.75
Gm3579	4.51	4.20
Gm42035 Gm3579	4.51	4.20
Gm17167 Gm29964 Gm21075 Gm13301	3.86	2.30
LOC100041599	3.52	2.27
Gm13303	2.84	1.72
Gm3893 Gm21093 Gm3883 Gm7819	2.60	1.80
Gm29804 Gm5859 Gm29801 Gm21075 Gm13301	2.56	1.99
4833420G17Rik	2.54	1.99
LOC100041536 Gm13303	2.50	1.68
LOC102637418 LOC102637312 LOC100041516 Gm3893	2.49	1.57
Tmem254b Tmem254c Tmem254a	2.37	1.80
4933409K07Rik Gm21093 Gm3883	2.33	1.57
Gm3893 Gm7819	2.25	1.56
Gm3893 Gm2163 Gm7819	2.09	1.52
Ide	1.92	1.72
Gm17167 Gm29964	1.91	1.53
Rps3a2	1.91	1.73
Zfp40	1.79	1.83
Gm9615	1.75	1.54
Dctd Gm39174	1.72	1.66
Hjurp 6430706D22Rik A730008H23Rik	1.69	1.82
Apoa1bp	1.69	1.60
Zfp458	1.62	1.81
Pisd-ps2	1.61	1.78
Snhg6	1.61	1.69
2610044O15Rik8	1.53	1.57
Cyp51	1.50	1.60
Gm9855 Tdg	-3.67	-2.83
Rpl3 Rpl3-ps1	-2.98	-2.27
Rps2 Snora64	-2.56	1.94
Gm25989	-2.30	2.32
Snora69	-2.30	2.65
Gm25394	-2.27	2.18

Gm22422	-2.25	2.27
Snord15a	-2.25	2.52
Tmem181b-ps	-2.24	-2.41
Gm23136	-2.21	
Gm25395	-2.18	-2.21
Gm22303	-2.12	-2.18
Snord11	-2.10	-2.12
Snord70	-2.10	-2.10
Gm22358	-2.09	-2.10
Snord118	-2.07	-2.09
1500012F01Rik	-2.06	-2.07
Wdfy1	-2.05	-2.06
Scarna6	-2.01	-1.73
Snord49a	-2.01	3.20
Snora3 Rpl27a	-2.01	2.28
Ptcd3	-2.01	1.54
Snord1b	-2.00	2.84
AF357399	-2.00	2.04
Gm23344	-1.99	2.30
n-R5s28	-1.98	2.64
Snora28	-1.96	2.51
Snord14a Rps13	-1.96	2.53
Gm24888	-1.95	1.56
Snord42b	-1.95	3.72
Gm24438	-1.95	2.01
Rps8	-1.94	1.90
Snord58b	-1.93	2.22
Snord16a	-1.91	2.04
Eif4a2	-1.90	2.24
Snord95 Gnb211	-1.90	1.60
Snord1c	-1.89	1.64
Gm25835	-1.89	1.87
Snora62	-1.89	2.39
Snora23	-1.88	2.71
Pisd-ps3	-1.88	2.14
Snora15	-1.86	-2.59
Gm24253	-1.84	2.16
Gm23795	-1.84	1.78
Gm24119	-1.83	1.96
Rnu12	-1.81	2.26
Gm22620	-1.81	1.82

Gm24698	-1.81	1.90
Snord61	-1.79	1.81
Gm25970	-1.78	2.15
Rpl30	-1.78	1.66
Gm24105	-1.78	2.02
Rps3a3 Rps3a1	-1.77	1.63
Snord67 Ckap5	-1.77	-2.23
Terc	-1.77	1.77
Taf1d	-1.76	1.71
Mir139	-1.76	1.86
Snord69	-1.76	-2.10
Snord49b	-1.75	2.12
Snord72	-1.75	2.13
Snord12 1500012F01Rik	-1.75	2.41
Snora16a	-1.74	2.36
Gm3435	-1.74	2.57
Snora73a	-1.73	-1.90
Gm3500 Gm3373 Gm10406	-1.72	1.70
Snora81 Eif4a2	-1.72	-2.11
Snora34 Mir1291	-1.71	2.00
Scarna2	-1.70	2.09
Gm24233	-1.69	1.83
Gabra2	-1.69	1.54
Rpl21	-1.67	-2.09
Gm25492	-1.66	2.07
Snora21	-1.65	1.75
Gm23969	-1.65	1.77
Pla2g4e	-1.65	2.20
Gm26448	-1.64	-1.94
Gm26236	-1.64	1.74
Snord60 Rab26os	-1.64	1.58
Gm25506	-1.63	1.51
Snord89	-1.63	1.81
Gm22866	-1.60	2.04
Esco1	-1.60	1.90
Rpl5	-1.60	-1.61
Gm26110	-1.59	1.87
Rnu1b6	-1.59	4.22
Rnu1b1 Rnu1b2	-1.59	4.22
Gm25890 Gm22614	-1.59	4.22
Gm22042	-1.59	4.22

Gm23297	-1.59	4.22
Gm25128	-1.58	1.64
Snord15b Rps3	-1.58	1.68
Scarna13 Mir3069	-1.57	2.08
Gm26175	-1.56	1.54
Gm23264	-1.56	2.21
Gm23503	-1.54	2.28
Gm23639	-1.54	2.10
Snora33	-1.54	1.57
Gm24046	-1.53	1.52
Rnu3b1 Rnu3b3 Rnu3b2 Gm42087	-1.53	2.79
Rnu3b1 Rnu3b3 Rnu3b2	-1.53	2.09
Rnu3b4 Rnu3b1 Rnu3b3 Rnu3b2	-1.53	2.09
Gm3194 Gm3500 Gm5796	-1.53	2.09
Scarna10 Ncapd2	-1.53	-1.81
Sec24a	-1.53	1.85
Gm24727	-1.53	1.66
Gm26493	-1.52	1.65
Gm24920	-1.52	1.86
Gm25107	-1.52	1.82
Gm25776	-1.51	1.64
Pcdhb6	-1.51	1.62
Gm25117	-1.51	-1.97



Maternal exposure to prostaglandin E₂ modifies expression of Wnt genes in mouse brain – An autism connection

Ravneet Rai-Bhogal^{a,b}, Christine Wong^{a,c}, Ashby Kissoondoyal^{a,c}, Jennilee Davidson^{a,b}, Hongyan Li^c, Dorota A. Crawford^{a,b,c,*}

^a Neuroscience Graduate Diploma Program, York University, Toronto, ON, Canada M3J 1P3

^b Department of Biology, York University, Toronto, ON, Canada M3J 1P3

^c School of Kinesiology and Health Science, York University, 4700 Keele Street, Toronto, ON, Canada M3J 1P3



ARTICLE INFO

Keywords:

Lipids
Prostaglandin E₂
Gene expression
Wnt signaling pathway
β-catenin
Autism Spectrum Disorder

ABSTRACT

Prostaglandin E₂ (PGE₂) is a lipid signaling molecule important for brain development and function. Various genetic and environmental factors can influence the level of PGE₂ and increase the risk of developing Autism Spectrum Disorder (ASD). We have previously shown that in neuronal cell lines and mouse brain, PGE₂ can interfere with the Wnt canonical pathway, which is essential during early brain development. Higher levels of PGE₂ increased Wnt-dependent motility and proliferation of neuroectodermal stem cells, and modified the expression of Wnt genes previously linked to autism disorders. We also recently established a cross-talk between these two pathways in the prenatal mouse brain lacking PGE₂ producing enzyme (COX^{-/-}). The current study complements the published data and reveals that PGE₂ signaling also converges with the Wnt canonical pathway in the developing mouse brain after maternal exposure to PGE₂ at the onset of neurogenesis. We found significant changes in the expression level of Wnt-target genes, *Mmp7*, *Wnt2*, and *Wnt3a*, during prenatal and early postnatal stages. Interestingly, we observed variability in the expression level of these genes between genetically-identical pups within the same pregnancy. Furthermore, we found that all the affected genes have been previously associated with disorders of the central nervous system, including autism. We determined that prenatal exposure to PGE₂ affects the Wnt pathway at the level of β-catenin, the major downstream regulator of Wnt-dependent gene transcription. We discuss how these results add new knowledge into the molecular mechanisms by which PGE₂ may interfere with neuronal development during critical periods.

1. Introduction

The brain contains high lipid content and its healthy development relies on the supply and function of these molecules [15]. Prostaglandin E₂ (PGE₂) is one of the major lipid metabolites released from phospholipid membranes through the action of phospholipase A₂ (PLA₂) and cyclooxygenase-1 or -2 (COX-1 or -2) [25]. PGE₂ exerts its physiological function through four G-protein coupled E-prostanoid receptors (EP1–4) [31,36,57]. Recent research shows that PGE₂ can play an important role in brain development including dendritic spine formation [14,2] and neuronal plasticity [16,34]. Increased levels of COX-2 during embryogenesis [52], and higher expression levels of EP receptors during neurogenesis [61], further strengthen the importance of PGE₂ in development.

We previously described that abnormalities in the PGE₂ signaling pathway due to genetic defects or exposure to environmental factors, during critical periods of brain development, have been associated with

the etiology of neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) [61,76]. The overwhelming evidence shows that various environmental risk factors, including deficient dietary supplementation, infections, exposure to drugs such as misoprostol (prostaglandin E analogue), air pollutants, chemicals found in food and personal care products like cosmetics, disrupt the level of PGE₂ and are independently linked to ASD [61,76]. These exogenous chemicals can be shuttled through the Blood Brain Barrier during a critical time in development and impact signaling of the PGE₂ pathway [76]. The molecular mechanisms by which changes in PGE₂ levels influence brain development are still not well known.

Thus far, in vitro research in our lab has shown that increased levels of PGE₂ can augment proliferation and migration of neuroectodermal stem cells [74], accelerate neuronal differentiation [75], retract neuronal extensions [62] and elevate calcium level in the cytosol and growth cones [22,60]. More interestingly, we showed for the first time in neuronal cell lines that PGE₂ signaling can interact with the major

* Corresponding author at: Faculty of Health, York University, 4700 Keele Street, Toronto, ON, Canada M3J 1P3.
E-mail address: dakc@yorku.ca (D.A. Crawford).

<https://doi.org/10.1016/j.bbrep.2018.03.012>

Received 19 December 2016; Received in revised form 28 March 2018; Accepted 31 March 2018

Available online 10 April 2018

2405-5808/ © 2018 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Microarray analysis of gene expression in the cyclooxygenase knockout mice – a connection to autism spectrum disorder

Ravneet Rai-Bhogal,^{1,2,*} Eizaaz Ahmad,^{1,2,*} Hongyan Li³ and Dorota A. Crawford^{1,2,3} 

¹Neuroscience Graduate Diploma Program, York University, Toronto, ON M3J 1P3, Canada

²Department of Biology, York University, Toronto, ON, Canada

³School of Kinesiology and Health Science, York University, Toronto, ON, Canada

Keywords: COX-1, COX-2, lipid signalling, prostaglandin E₂, Wnt signalling

Abstract

The cellular and molecular events that take place during brain development play an important role in governing function of the mature brain. Lipid-signalling molecules such as prostaglandin E₂ (PGE₂) play an important role in healthy brain development. Abnormalities along the COX-PGE₂ signalling pathway due to genetic or environmental causes have been linked to autism spectrum disorder (ASD). This study aims to evaluate the effect of altered COX-PGE₂ signalling on development and function of the prenatal brain using male mice lacking cyclooxygenase-1 and cyclooxygenase-2 (COX-1^{-/-} and COX-2^{-/-}) as potential model systems of ASD. Microarray analysis was used to determine global changes in gene expression during embryonic days 16 (E16) and 19 (E19). Gene Ontology: Biological Process (GO:BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were implemented to identify affected developmental genes and cellular processes. We found that in both knockouts the brain at E16 had nearly twice as many differentially expressed genes, and affected biological pathways containing various ASD-associated genes important in neuronal function. Interestingly, using GeneMANIA and Cytoscape we also show that the ASD-risk genes identified in both COX-1^{-/-} and COX-2^{-/-} models belong to protein-interaction networks important for brain development despite of different cellular localization of these enzymes. Lastly, we identified eight genes that belong to the Wnt signalling pathways exclusively in the COX-2^{-/-} mice at E16. The level of PKA-phosphorylated β -catenin (S552), a major activator of the Wnt pathway, was increased in this model, suggesting crosstalk between the COX-2-PGE₂ and Wnt pathways during early brain development. Overall, these results provide further molecular insight into the contribution of the COX-PGE₂ pathways to ASD and demonstrate that COX-1^{-/-} and COX-2^{-/-} animals might be suitable new model systems for studying the disorders.

Introduction

In the last decade, significant progress has been made in elucidating the role of the COX-PGE₂ signalling pathway in the healthy brain, including dendritic spine formation, learning and memory, as well as synaptic transmission and plasticity (Chen *et al.*, 2002; Chen & Bazan, 2005; Sang & Chen, 2006; Burks *et al.*, 2007). PGE₂ derives from arachidonic acid released from plasma membrane phospholipids through the key enzymatic activity of phospholipase A₂

(PLA₂) followed by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2) (Vazquez-Tello *et al.*, 2004; Sang & Chen, 2006). COX-PGE₂ signalling normally regulates expression of genes involved in diverse physiological responses by activation of four G-protein-coupled E-prostanoid (EP1-4) receptors (Furuyashiki & Narumiya, 2011). During embryonic development, there is an increase in PGE₂ levels and expression of all EP receptors in the brain coinciding with the peak of neurogenesis (Hatae *et al.*, 2002; Tamiji & Crawford, 2010c). Abundant expression of COX-1 and COX-2 was also found in the foetal brain, signifying its important role during brain development (Gersting *et al.*, 2009). COX-1 is constitutively expressed throughout the brain (Amateau & McCarthy, 2004; Kirkby *et al.*, 2012), mainly by microglia and can be upregulated in pathological conditions (Hoozemans *et al.*, 2001). COX-2 is induced in response to inflammatory stimuli in most tissues but in the brain it is constitutively expressed specifically in glutamatergic neurons, in regions such as the cerebral cortex, hippocampus (Yamagata *et al.*, 1993; Adams *et al.*, 1996; Kaufmann *et al.*, 1996), cerebellum and spinal cord (Hewett *et al.*, 2016).

Correspondence: Dorota A. Crawford, as above.
E-mail: dakc@yorku.ca

*R.R. and E.A. contributed equally to this work.

Received 1 February 2017, revised 11 November 2017, accepted 14 November 2017

Edited by Stephane Baudouin

Reviewed by Margaret McCarthy, Maryland University, USA; and Charles Claudianos, Monash University, Australia

The associated peer review process communications can be found in the online version of this article.

Appendix D. Copyright Permissions for Published Work

D1. Study 1



Maternal exposure to prostaglandin E2 modifies expression of Wnt genes in mouse brain – An autism connection

Author: Ravneet Rai-Bhogal, Christine Wong, Ashby Kissoondoyal, Jennilee Davidson, Hongyan Li, Dorota A. Crawford

Publication: Biochemistry and Biophysics Reports

Publisher: Elsevier

Date: July 2018

© 2018 The Authors. Published by Elsevier B.V.

Journal Author Rights

Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: <https://www.elsevier.com/about/our-business/policies/copyright#Author-rights>

[BACK](#) [CLOSE WINDOW](#)

Author rights

The below table explains the rights that authors have when they publish with Elsevier, for authors who choose to publish either open access or subscription. These apply to the corresponding author and all co-authors.

Author rights in Elsevier's proprietary journals	Published open access	Published subscription
Retain patent and trademark rights	✓	✓
Retain the rights to use their research data freely without any restriction	✓	✓
Receive proper attribution and credit for their published work	✓	✓
Re-use their own material in new works without permission or payment (with full acknowledgement of the original article): 1. Extend an article to book length 2. Include an article in a subsequent compilation of their own work 3. Re-use portions, excerpts, and their own figures or tables in other works.	✓	✓
Use and share their works for scholarly purposes (with full acknowledgement of the original article): 1. In their own classroom teaching. Electronic and physical distribution of copies is permitted 2. If an author is speaking at a conference, they can present the article and distribute copies to the attendees 3. Distribute the article, including by email, to their students and to research colleagues who they know for their personal use 4. Share and publicize the article via Share Links, which offers 50 days' free access for anyone, without signup or registration 5. Include in a thesis or dissertation (provided this is not published commercially) 6. Share copies of their article privately as part of an invitation-only work group on commercial sites with which the publisher has a hosting agreement	✓	✓

Publicly share the preprint on any website or repository at any time.	✓	✓
Publicly share the accepted manuscript on non-commercial sites	✓	✓ using a CC BY-NC-ND license and usually only after an embargo period (see Sharing Policy for more information)
Publicly share the final published article	✓ in line with the author's choice of end user license	×
Retain copyright	✓	×

D2. Study 2



Special Issue Article

Microarray analysis of gene expression in the cyclooxygenase knockout mice – a connection to autism spectrum disorder

Ravneet Rai-Bhogal, Eizaaz Ahmad, Hongyan Li, Dorota A. Crawford ✉

First published: 21 November 2017 | <https://doi.org/10.1111/ejn.13781> | Citations: 7

Reviewed by Margaret McCarthy, Maryland University, USA; and Charles Claudianos, Monash University, Australia

Edited by Stephane Baudouin

The associated peer review process communications can be found in the online version of this article.

JOHN WILEY AND SONS LICENSE TERMS AND CONDITIONS

May 04, 2021

This Agreement between York University -- Ravneet Rai-Bhogal ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

License Number	5061740988719
License date	May 04, 2021
Licensed Content Publisher	John Wiley and Sons
Licensed Content Publication	European Journal of Neuroscience
Licensed Content Title	Microarray analysis of gene expression in the cyclooxygenase knockout mice – a connection to autism spectrum disorder

Licensed Content Author	Dorota A. Crawford, Hongyan Li, Eizaaz Ahmad, et al
Licensed Content Date	Dec 15, 2017
Licensed Content Volume	47
Licensed Content Issue	6
Licensed Content Pages	17
Type of use	Dissertation/Thesis
Requestor type	Author of this Wiley article
Format	Print and electronic
Portion	Full article
Will you be translating?	No
Title	Abnormal lipid signalling in the developing mouse brain: Implications in autism spectrum disorder
Institution name	York University
Expected presentation date	May 2021
	York University 131 William Street
Requestor Location	Elmira, ON N3B 0C2 Canada Attn: York University
Publisher Tax ID	EU826007151
Total	0.00 CAD
Terms and Conditions	

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or handled on behalf of a society with which a Wiley Company has exclusive publishing rights in relation to a particular work (collectively "WILEY"). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance

Center Inc., ("CCC's Billing and Payment terms and conditions"), at the time that you opened your RightsLink account (these are available at any time at <http://myaccount.copyright.com>).

Terms and Conditions

- The materials you have requested permission to reproduce or reuse (the "Wiley Materials") are protected by copyright.
- You are hereby granted a personal, non-exclusive, non-sub licensable (on a stand-alone basis), non-transferable, worldwide, limited license to reproduce the Wiley Materials for the purpose specified in the licensing process. This license, **and any CONTENT (PDF or image file) purchased as part of your order**, is for a one-time use only and limited to any maximum distribution number specified in the license. The first instance of republication or reuse granted by this license must be completed within two years of the date of the grant of this license (although copies prepared before the end date may be distributed thereafter). The Wiley Materials shall not be used in any other manner or for any other purpose, beyond what is granted in the license. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Wiley Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Wiley Material. Any third party content is expressly excluded from this permission.
- With respect to the Wiley Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Wiley Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Wiley Materials without the prior permission of the respective copyright owner. **For STM Signatory Publishers clearing permission under the terms of the [STM Permissions Guidelines](#) only, the terms of the license are extended to include subsequent editions and for editions in other languages, provided such editions are for the work as a whole in situ and does not involve the separate exploitation of the permitted figures or extracts,** You may not alter, remove or suppress in any manner any

copyright, trademark or other notices displayed by the Wiley Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Wiley Materials on a stand-alone basis, or any of the rights granted to you hereunder to any other person.

- The Wiley Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc, the Wiley Companies, or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Wiley Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Wiley Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto
- NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.
- WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.
- You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.
- IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER

CAUSED, ARISING OUT OF OR IN CONNECTION WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

- Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.
- The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party's right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.
- This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY's prior written consent.
- Any fee required for this permission shall be non-refundable after thirty (30) days from receipt by the CCC.
- These terms and conditions together with CCC's Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall

be binding upon and inure to the benefit of the parties' successors, legal representatives, and authorized assigns.

- In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall prevail.
- WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.
- This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.
- This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state's conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

WILEY OPEN ACCESS TERMS AND CONDITIONS

Wiley Publishes Open Access Articles in fully Open Access Journals and in Subscription journals offering Online Open. Although most of the fully Open Access journals publish open access articles under the terms of the Creative Commons Attribution (CC BY) License only, the subscription journals and a few of the Open Access Journals offer a choice of Creative Commons Licenses. The license type is clearly identified on the article.

The Creative Commons Attribution License

The [Creative Commons Attribution License \(CC-BY\)](#) allows users to copy, distribute and transmit an article, adapt the article and make commercial use of the article. The CC-BY license permits commercial and non-

Creative Commons Attribution Non-Commercial License

The [Creative Commons Attribution Non-Commercial \(CC-BY-NC\)License](#) permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.(see below)

Creative Commons Attribution-Non-Commercial-NoDerivs License

The [Creative Commons Attribution Non-Commercial-NoDerivs License](#) (CC-BY-NC-ND) permits use, distribution and reproduction in any medium, provided the original work is properly cited, is not used for commercial purposes and no modifications or adaptations are made. (see below)

Use by commercial "for-profit" organizations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee.

Further details can be found on Wiley Online

Library <http://olabout.wiley.com/WileyCDA/Section/id-410895.html>

Other Terms and Conditions:

v1.10 Last updated September 2015

Questions? customercare@copyright.com or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.
