
Erin Gardiner
B. Biomed Sci (Hons I)

Doctor of Philosophy
School of Biomedical Sciences, Faculty of Health
The University of Newcastle

May 2013

Supervisors: Assoc Prof Paul Tooney & Dr Murray Cairns
DECLARATION

This PhD thesis is the original work of Erin Gardiner and does not contain any work previously submitted to the University of Newcastle or other tertiary institution, and to the best of my knowledge contains no material previously written or published by another person, except where referenced within the text. I hereby certify this thesis is in the form of a series of published journal articles of which I am a joint author. Written statements from each contributing author, endorsed by the Deputy Head of Faculty (Research and Research Training) are included as evidence of my contribution to these publications and of my collaboration with these researchers and institutions. I consent to depositing a copy of this thesis in the University Library to be made publicly available for loan and photocopying within the guidelines of the Copyright Act 1968.

Erin J. Gardiner
ACKNOWLEDGEMENTS

A most sincere Thank you to my supervisors Associate Professor Paul Tooney and Dr Murray Cairns for your guidance throughout my honours degree and for enabling my continued study in your lab for my PhD along with all the opportunities I’ve received in that time. Thanks also to my lab colleagues for camaraderie and support. We are privileged to have had the opportunity to be studying psychiatric genetics and neurobiology: the brain is truly the last great mystery.

I’m also thankful to The University of Newcastle and the Schizophrenia Research Institute for providing me with funding and support throughout my candidature.

A personal Thanks for the support of family, friends and Jim throughout this marathon. I don’t know who is more relieved that it is finished? We all need a drink.

Lastly, I’d like to dedicate this research to my Grandmother, a strong and loving woman who battled with schizophrenia whilst supporting a family in rural Australia. God Bless.
CONTENTS

DECLARATION 1

ACKNOWLEDGEMENTS 2

CONTENTS 3

MANUSCRIPTS 7

CONFERENCES 8

ABBREVIATIONS 9

ABSTRACT 11

CHAPTER 1: LITERATURE REVIEW 14

1.1 The nature of Schizophrenia: a heterogeneous disorder as part of a “spectrum of psychosis” 14

1.2 The brain in schizophrenia 15
  1.2.1 Structural and functional deficits 15
  1.2.2 Altered Neurotransmission 17

1.3 The development of schizophrenia 19

1.4 Genetics of Schizophrenia 21
  1.4.1 Linkage and Candidate Gene Studies 23
  1.4.2 Genome-Wide Association Studies and Copy Number Variations (CNVs) 25

1.5 Genes and the environment 29

1.6 Gene expression profiling in post-mortem brain in schizophrenia 30

1.7 Post-transcriptional gene silencing (PTGS) 32
  1.7.1 miRNA Biogenesis 32
  1.7.2 PTGS: Mechanism of Action 35

1.8 miRNA in neurodevelopment and brain function 38

1.9 miRNA in schizophrenia 40

1.10 Expression profiling in peripheral tissue in schizophrenia 44
1.11 The Australian Schizophrenia Research Bank (ASRB); beyond the blood versus brain debate and towards a biological signature

RATIONALE

AIMS AND HYPOTHESES

CHAPTER 2
Dysregulation of miRNA 181b in the temporal cortex in schizophrenia

CHAPTER 3
Schizophrenia is associated with an increase in cortical miRNA biogenesis

CHAPTER 4
Imprinted DLK1-DIO3 region of 14q32 defines a schizophrenia-associated miRNA signature in peripheral blood mononuclear cells

CHAPTER 5
Gene Expression Analysis Reveals Schizophrenia-Associated Dysregulation of Immune Pathways in Peripheral Blood Mononuclear Cells

CHAPTER 6
Gene expression profiling in treatment naïve schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication

CHAPTER 7
Antipsychotic drug-associated gene-miRNA interaction in T-lymphocytes

CHAPTER 8: GENERAL DISCUSSION
8.1 Expression in the Brain
8.2 Expression in Blood
8.3 Antipsychotic Influence
8.4 Limitations for Consideration
8.5 Understanding Transcriptional Regulation in Schizophrenia
8.6 The Future of Blood-Based Biomarker Discovery in Schizophrenia

Conclusion

REFERENCES
APPENDIX I: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 2 201
Supplementary Table 1 miRNA expressed in STG using DNA microarrays 201
Supplementary Table 2 miR-181b targets - Down-regulated in the STG (Bowden et al 2007) 203

APPENDIX II: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 3 207
Supplementary Table 1 Demographic information for STG and DLPFC post-mortem tissue. 207
Supplementary Table 2 Differentially Expressed miRNA by microarray as determined by SAM analysis 211
Supplementary Table 3 Total RNA analysis 213
Supplementary Table 4 Over-represented KEGG pathways predicted to be regulated by miR-107 and the miR-15 family 214
Supplementary Table 5 Investigating miRNA/target gene relationships by luciferase reporter gene assay 216

APPENDIX III: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 4 217
Supplementary Table 1 Details of demographic and clinical variables of ASRB cohort used in miRNA microarray expression profiling 217
Supplementary Table 2 Expression levels and variance for differentially expressed microRNA 222
Supplementary Table 3 Oligonucleotide Sequences 224
Supplementary Table 4 Functional Categories and Biological Function Annotation 241
Supplementary Table 5 Over-represented KEGG pathways predicted to be regulated by miR-107 and the miR-15 family 214
Supplementary Table 5 Investigating miRNA/target gene relationships by luciferase reporter gene assay 216

APPENDIX IV: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 5 228
Supplementary Table 1 Full Cohort Demographics and Clinical Variables 228
Supplementary Table 2 Full list of differentially expressed genes/probes 233
Supplementary Table 3 Primer Sequences 240
Supplementary Table 4 Functional Categories and Biological Function Annotation 241
Supplementary Table 5 Top networks Enriched with Differentially Expressed Genes in Schizophrenia using IPA 245
Supplementary Table 6 Top canonical pathways enriched with significantly differentially expressed mRNA 246
Supplementary Figure 1 Network 1 247
Supplementary Table 7 Predicted miRNA-mRNA pairs (inverse expression) 248

APPENDIX V: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 6 255
Supplementary Table S1: Primer sequences and TaqMan Assay details for qPCR 255
Supplementary Table S2: Differentially expressed genes in PBMCs from schizophrenia patients prior to antipsychotic drug treatment (CTL v SZ BT) 256
Supplementary Table S3: Differentially expressed genes in PBMCs from schizophrenia patients after antipsychotic drug treatment (CTL v SZ AT) 266
Supplementary Table S4: Differentially expressed genes in PBMCs from schizophrenia patients that did not change with antipsychotic drug treatment 268
Supplementary Table S5: Top ranked biological functions overrepresented by genes dysregulated in schizophrenia before and after antipsychotic drug treatment 270
APPENDIX VI: SUPPLEMENTARY DATA ACCOMPANYING CHAPTER 7

Supplementary Table S1 Significantly differentially expressed genes in Jurkat cells after acute chlorpromazine exposure compared to controls

Supplementary Table S2 Significantly differentially expressed genes in Jurkat cells after acute clozapine exposure compared to controls

Supplementary Table S3 Significantly differentially expressed genes in Jurkat cells after acute haloperidol exposure compared to controls

Supplementary Table S4 Significantly differentially expressed genes in Jurkat cells after subacute chlorpromazine exposure compared to controls

Supplementary Table S5 Significantly differentially expressed genes in Jurkat cells after subacute clozapine exposure compared to controls

Supplementary Table S6 Significantly differentially expressed genes in Jurkat cells after subacute haloperidol exposure compared to controls

Supplementary Table S7 Primer sequences for miRNA and gene expression Q-PCR validation

Supplementary Figure S1

Supplementary Table S8 Inter-treatment comparison of differentially expressed genes in Jurkat T-lymphocytes after acute APD exposure

Supplementary Table S9 Differentially expressed genes in Jurkat T-lymphocytes after acute and subacute exposure to haloperidol and clozapine

Supplementary Table S10 Neurological Disease: top category in Diseases & Disorders for genes commonly differentially expressed after acute and subacute clozapine and haloperidol treated Jurkat T-lymphocytes

Supplementary Table S11 All biological functions

Supplementary Table S12 Canonical Pathways for genes commonly differentially expressed after APD exposure in Jurkat T-lymphocytes

Supplementary Table S13 mRNA:miRNA interaction

Supplementary Table S14 Top ranked functional categories of differentially expressed mRNA:miRNA pairs after acute haloperidol exposure

Supplementary Figure S2 Comparison of genes differentially expressed after APD-treatment in T-lymphocytes and in PBMCs from patients with schizophrenia

Supplementary Table S15 Genes differentially expressed in PBMCs from patients with schizophrenia and in APD-treated Jurkat T-lymphocytes

Supplementary Table S16 Genes differentially expressed in Jurkat T-lymphocytes and responding to medication in PBMCs from patients with schizophrenia
MANUSCRIPTS

Manuscripts arising from this thesis describe miRNA and gene expression profiles from peripheral blood mononuclear cells collected from subjects with schizophrenia and in an antipsychotic-treated lymphocyte cell line. Additionally, technical support contributed toward the content of two other manuscripts based on the function of miRNA and in identifying their targets within the context of schizophrenia.

Beveridge NJ, Tooney PA, Carroll AP, Gardiner E, Bowden N, Scott RJ, Tran N, Dedova I, Cairns MJ. Dysregulation of miRNA 181b in the temporal cortex in schizophrenia. Human Molecular Genetics. 2008 Apr 15;17(8):1156-68.


CONFERENCES

Material presented in this thesis has previously been presented by the author of the thesis at national and international conferences.

Oral presentations

Sept 2010 Australasian Schizophrenia Conference, Sydney, Australia - *miRNA expression profiling in peripheral blood mononuclear cells in schizophrenia.*

Poster presentations

Nov 2008 Australasian Society for Psychiatric Research, Newcastle, Australia - *Investigation of miRNA influence on RGS4 and NRG1 gene expression in schizophrenia.*

Oct 2010 World Congress on Psychiatric Genetics, Athens, Greece - *Investigation of miRNA expression in peripheral blood mononuclear cells in schizophrenia.*

Nov 2011 Biological Psychiatry Australia, Melbourne, Australia - *Immune-related mRNA expression profile in peripheral blood mononuclear cells in schizophrenia.*

Dec 2011 Priority Research Centre for Brain and Mental Health, Postgraduate and Postdoctoral Conference, Newcastle, Australia - *Immune-related mRNA expression profile in peripheral blood mononuclear cells in schizophrenia.*

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'-UTR</td>
<td>3 prime untranslated region</td>
</tr>
<tr>
<td>5'-UTR</td>
<td>5 prime untranslated region</td>
</tr>
<tr>
<td>AKT1</td>
<td>v-akt murine thymoma viral oncogene homolog 1</td>
</tr>
<tr>
<td>APD</td>
<td>antipsychotic</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>cRNA</td>
<td>complementary RNA</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CNV</td>
<td>copy number variant</td>
</tr>
<tr>
<td>DGCR8</td>
<td>DiGeorge critical region 8</td>
</tr>
<tr>
<td>DISC1</td>
<td>disrupted in schizophrenia 1</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>dsRNA</td>
<td>double stranded RNA</td>
</tr>
<tr>
<td>FDR</td>
<td>false discovery rate</td>
</tr>
<tr>
<td>GRIA2</td>
<td>glutamate receptor, ionotropic, AMPA 2</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association study</td>
</tr>
<tr>
<td>MCS</td>
<td>multiple cloning site</td>
</tr>
<tr>
<td>miRNA</td>
<td>microRNA</td>
</tr>
<tr>
<td>MRE</td>
<td>miRNA recognition element</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartic acid</td>
</tr>
<tr>
<td>NRG1</td>
<td>neuregulin 1</td>
</tr>
<tr>
<td>p-bodies</td>
<td>processing bodies</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>Pre-miRNA</td>
<td>precursor miRNA</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Pri-miRNA</td>
<td>primary miRNA</td>
</tr>
<tr>
<td>PTGS</td>
<td>post transcriptional gene silencing</td>
</tr>
<tr>
<td>QPCR</td>
<td>quantitative real time PCR</td>
</tr>
<tr>
<td>RGS4</td>
<td>regulator of G-protein signalling 4</td>
</tr>
<tr>
<td>RISC</td>
<td>RNA induced silencing complex</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA interference</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription PCR</td>
</tr>
<tr>
<td>siRNA</td>
<td>short interfering RNA</td>
</tr>
<tr>
<td>snoRNA</td>
<td>small nucleolar RNA</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>STG</td>
<td>superior temporal gyrus</td>
</tr>
<tr>
<td>VSNL1</td>
<td>visinin-like 1</td>
</tr>
</tbody>
</table>
ABSTRACT

Schizophrenia is a complex and heterogeneous disorder that has strong genetic links. While no single gene of large effect size has been identified, it has been hypothesised that dysregulation of many genes could be involved in the underlying molecular biology.

The expression of microRNA (miRNA), master regulators of gene expression at the post-transcriptional level, was analysed in post-mortem superior temporal gyrus (STG) and dorsolateral prefrontal cortex (DLPFC) from patients with schizophrenia and healthy controls using high-throughput microarray technology. miR-181b was up-regulated in the STG of patients with schizophrenia, and indeed a global up-regulation of miRNA was observed in both the STG and the DLPFC along with an up-regulation of components of the machinery involved in miRNA biogenesis. Luciferase reporter expression was then analysed to validate regulatory relationships between miRNA and their predicted target gene(s) in vitro. Several miR-15 family members, which share a seed region and therefore many mRNA targets, regulated schizophrenia candidate genes such as visinin-like 1 (VSNL1) and reelin (RELN).

Gene and miRNA expression was analysed in a larger cohort of participants with schizophrenia and non-psychiatric controls in peripheral blood mononuclear cells (PBMCs) from the Australian Schizophrenia Research Bank (ASRB). The expression of 83 miRNA was significantly down-regulated in schizophrenia relative to controls including several brain expressed miRNA that have previously been reported to be altered in the brain in schizophrenia (miR-128, miR-134 and miR-181b). Importantly, almost a quarter of the down-regulated miRNA reside within an imprinted cluster, at chromosome at 14q32, which has previously been associated with schizophrenia. Down-regulation of the 14q32 cluster was not the result of a copy number variation suggesting other mechanisms, possibly epigenetic, may be involved. Further analysis suggested that these miRNA regulated several nervous-system related pathways and also implicated dysregulation of immune-associated pathways in schizophrenia. Functional analysis of differential gene expression in the same cohort also revealed enrichment of processes and pathways associated with immune and inflammation responses: 105 genes were down-regulated (e.g. Chemokine (C–C motif) receptor 7; CCR7) and 59 up-regulated (e.g. Defensin alpha-4; DEFA4) in participants with schizophrenia compared to controls.
Since medication could potentially contribute to altered expression in these cohorts, differential gene expression was assessed in PBMCs from antipsychotic naïve schizophrenia patients and post-treatment. Several genes that were differentially expressed in the schizophrenia patients appeared to be responsive to medication including the schizophrenia candidate gene v-akt murine thymoma viral oncogene homolog 1 (AKT1), incidentally located in the 14q32 chromosomal region, which was common to several dysregulated canonical pathways implicated in this study. Again, immune and inflammation-associated pathways were also altered in schizophrenia patients both prior to and following antipsychotic treatment, consistent with the analysis of the ASRB cohort, providing further evidence of immune dysfunction in schizophrenia. Finally, assessment of the impact of acute and subacute exposure to 3 antipsychotics (chlorpromazine, clozapine and haloperidol) in JM-Jurkat T-lymphocytes on gene and miRNA expression showed minimal overlap with the differential expression observed in PBMCs, suggesting that expression patterns observed in the PBMCs were associated with schizophrenia as opposed to a consequence of medication. Moreover functional analysis indicated that antipsychotics dysregulate genes involved in oxidative stress which may reflect the molecular mechanism(s) underlying some of their side effects such as extra-pyramidal symptoms, weight gain and in the case of clozapine, agranulocytosis.

The molecular profiles obtained from the gene and miRNA expression analyses in post-mortem brain, PBMCs from living schizophrenia patients and healthy controls, as well as in human tissue after antipsychotic exposure, provide insight into the underlying molecular biology of schizophrenia and antipsychotics, which could lead to targets for therapeutic intervention. Furthermore, functional analysis of the differential expression in these aforementioned studies implicated dysregulation of several biological processes and pathways such as immune and inflammation pathways.