MICROARRAY STUDIES OF GENOME-WIDE CHANGES IN BRAIN AND HEART GENE EXPRESSION IN MOUSE MODELS OF IRON OVERLOAD

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B Biomed Sci (Hons)

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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FACULTY OF HEALTH
UNIVERSITY OF NEWCASTLE
STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Daniel Johnstone

DECLARATIONS

I hereby certify that some work embodied in this thesis has been done in collaboration with other researchers. I have included as part of the thesis a statement clearly outlining the extent of collaboration, with whom and under what auspices.
ACKNOWLEDGEMENTS

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PUBLICATIONS ARISING FROM THIS THESIS

PEER REVIEWED PUBLICATIONS


CONFERENCE ABSTRACTS


**AWARDS ARISING FROM THIS THESIS**

**RESEARCH AWARDS**

1. 2009 Australian Society for Medical Research (ASMR) National Research Award – Domestic

**TRAVEL AWARDS**

1. Alzheimer’s Association Travel Fellowship to attend International Conference on Alzheimer’s Disease, July 10-15, 2010, Honolulu, USA

2. Student Bursary to attend the Australian Society for Medical Research 48th National Scientific Conference, November 15-17, 2009, Hobart, Australia

3. Student Bursary to attend the International BioIron Society Meeting, June 7-11, 2009, Porto, Portugal

4. Student Bursary to attend the 19th International Conference on Genome Informatics December 1-3, 2008, Gold Coast, Australia

5. Alzheimer’s Association Travel Fellowship to attend International Conference on Alzheimer’s Disease, July 26-31, 2008, Chicago, USA
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<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APP</td>
<td>Amyloid precursor protein</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>Avg</td>
<td>Average normalisation</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood-brain barrier</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone morphogenetic protein</td>
</tr>
<tr>
<td>BS</td>
<td>BeadStudio</td>
</tr>
<tr>
<td>CADASIL</td>
<td>Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Calcium/calmodulin-dependent protein kinase II</td>
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<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
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<tr>
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<td>Cubic Spline normalisation</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>$C_t$</td>
<td>Threshold cycle</td>
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<tr>
<td>DAVID</td>
<td>Database for Annotation, Visualisation and Integrated Discovery</td>
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<td>Duodenal cytochrome b</td>
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<td>Divalent metal transporter 1</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>EGR</td>
<td>Early growth response</td>
</tr>
<tr>
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<td>Extracellular signal-regulated kinase</td>
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<tr>
<td>FDR</td>
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<td>Fe$^{2+}$</td>
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<td>Mitogen-activated protein kinase</td>
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<td>mRNA</td>
<td>Messenger RNA</td>
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<td>NBIA</td>
<td>Neurodegeneration with brain iron accumulation</td>
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<tr>
<td>STAT</td>
<td>Signal transducer and activator of transcription</td>
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<tr>
<td>TBI</td>
<td>Transferrin-bound iron</td>
</tr>
<tr>
<td>TE</td>
<td>Tris/EDTA</td>
</tr>
<tr>
<td>TfR1</td>
<td>Transferrin receptor 1</td>
</tr>
<tr>
<td>TfR2</td>
<td>Transferrin receptor 2</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>WT</td>
<td>Wildtype control mice</td>
</tr>
</tbody>
</table>
ABSTRACT

Iron is essential for life, having critical roles in oxygen transport, cellular energy production and as an enzyme cofactor, however too much iron can be detrimental to health. Approximately 10% of people in developed countries have body iron levels above normal reference ranges. This generally arises as a result of excessive dietary iron intake or genetic mutations that perturb systemic iron homeostasis, most commonly loss-of-function polymorphisms in the *HFE* gene. Abnormally high body iron levels can lead to the disorder haemochromatosis, which involves damage to the liver and possibly also other organs such as the heart and pancreas, however effects on the brain are not well understood. Although iron accumulation in particular brain regions due to certain rare genetic mutations is implicated in severe neurodegeneration and neurologic dysfunction, the blood-brain barrier is thought to protect the brain against the effects of high systemic iron levels in haemochromatosis.

To investigate the effects of iron overload disorders on the brain, microarray technology was used to assess genome-wide brain gene expression in mouse models of dietary or genetic iron overload. Three groups of mice were studied: wildtype control mice, wildtype mice fed a short-term iron-supplemented diet and mice with disruption of the *Hfe* gene (*Hfe* knockout). As there are many methods available for analysing microarray data, a range of different approaches were first evaluated for use with these datasets. To improve the robustness of the findings, several of the most suitable approaches were utilised for subsequent analyses. Various bioinformatics tools were then used to determine potential functional effects of the observed changes in gene expression. Select expression changes of interest were validated by real-time reverse transcription polymerase chain reaction (RT-PCR).

Neither model of iron overload showed increased brain non-haem iron levels nor expression changes for a large number of iron-related genes, however both models did show potentially important alterations in brain gene expression. Mice fed a short-term high-iron diet showed only restricted changes relative to control mice, however there was altered expression of genes relating to biological functions involving iron, such as
nitric oxide signalling and accumulation of the lysosomal waste product lipofuscin. Lipofuscin production is accelerated by high iron levels and excessive lipofuscin is associated with neurodegeneration in the disease class neuronal ceroid lipofuscinoses. Iron-supplemented mice also showed expression changes for a number of genes causatively linked to other rare neurologic disorders.

Similarly, the \textit{Hfe} knockout mouse model of genetic haemochromatosis showed brain gene expression changes relating to lysosomal lipofuscin accumulation and certain neurologic disorders. However, in contrast to the dietary iron-supplemented mice, \textit{Hfe} knockout mice showed extensive changes in brain gene expression relative to wildtype controls. These included expression changes for genes involved in key brain functions, such as neurotransmission, synaptic plasticity and transcriptional regulation, as well as processes that are influenced by iron, such as haem synthesis and degradation. In addition, analysis of molecular pathways revealed a disproportionately high number of expression changes for genes relating to Alzheimer’s disease, suggesting disruption of the \textit{Hfe} gene might influence disease processes. There was also evidence for effects on related pathways such as Notch signalling, which could potentially impair memory and other cognitive functions independent of Alzheimer’s-related effects.

Assessment of heart gene expression changes in these mouse models revealed few similarities with the brain, suggesting that many of the expression changes observed in the brain may be tissue-specific. However there were some notable changes in the heart that could have functional consequences, including expression changes for genes involved in cardiac pacemaking, intracellular calcium release and neurotransmitter degradation. Physiological investigations of sinoatrial node preparations from \textit{Hfe} knockout mice were indicative of a lower heart rate at baseline compared to wildtype control mice but no difference in contractile responses to either stimulatory (noradrenaline) or inhibitory (carbachol) agents.

Together these findings provide evidence for important brain and heart gene expression changes in disorders of iron overload. The nature and extent of these changes appears dependent on the cause (dietary or genetic) and duration (acute or chronic) of iron loading. These changes could have consequences for both normal functioning and disease pathogenesis and might help explain some of the problems experienced by
patients with iron overload disorders. The findings suggest a range of new research
directions and are likely to alter the way that haemochromatosis and other iron overload
disorders are perceived by clinicians, possibly leading to improved monitoring and
treatment of the large number of patients with these conditions.