The Expression of Connexins in Neurodegeneration

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ABSTRACT

Changes in connexin expression are thought to play a role in neurological disease and the possible effects of these changes in connexion expression in the disease process are therefore important. The need to screen for connexin expression quickly and cost-effectively has directed attention to microarray technology. The aim of the first section of this study was to design a 'boutique' microarray chip to screen for mRNA for all the reported connexins in the human. Where possible, the probes were designed to be compatible with the rat connexin mRNA. Twenty 50-base length oligonucleotide custom-designed probes, fourteen of them showing cross-species human/rat alignment, and twenty probes for the human connexin sequences designed by the German company MWG were included in the array. The array was tested on rat and on human tissue and the result validated by the Affymetrix GeneChips. Results confirmed that our array design was reliable, had a consistent target binding pattern and that it could detect differential connexin gene expression in both human and rat tissues.

As our goal was to screen human brain tissue for connexin expression, obtaining RNA of sufficiently high quality to be used in microarray experiments became a concern. We investigated whether it was possible to obtain high quality RNA from postmortem human tissue using samples obtained from the New Zealand Neurological Foundation Human Brain Bank. The principle finding was that RNA quality is most strongly affected by the pH of the tissue, with both the pH and the RNA quality being influenced by the mode of death. The best quality RNA samples were then used for the screening of human brain tissue for connexin expression.

One control and five disease samples from the secondarily affected regions of Huntington's (MC), Parkinson's (CN) and Alzheimer's disease, as well as hippocampus samples from two mesial temporal lobe epilepsy patients were screened on the custom-designed connexin array and on the Illumina Sentrix arrays to search for changes in the expression of connexins and inflammatory cytokines. It proved possible to obtain consistent data from the two platforms from samples with a wide range of RNA quality, and certain common patterns in the change of connexins and inflammatory markers were identified

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TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF TABLES ix

LIST OF FIGURES xiv

LIST OF ABBREVIATIONS xix

1 INTRODUCTION 1

1.1 Gap Junctions and Their Role in Neurological Disease 1

1.1.1 Gap junctions – structure and function 1

1.1.2 Connexins in the CNS – health and disease 4

1.1.2.1 Connexin expression in the CNS 4
1.1.2.2 Neurodegenerative diseases 7
1.1.2.3 Vascular effects in neurodegenerative diseases 9

1.2 RNA quality in the human brain 13

1.3 Microarrays 15

1.3.1 Screening techniques 15

1.3.2 Microarrays – outline 17

1.3.3 cDNA *vs* oligonucleotide microarrays 17

1.3.3.1 cDNA microarrays 17
1.3.3.2 Oligonucleotide microarrays 18
1.3.3.3 Two-colour and one-colour arrays 19
1.3.3.4 Oligonucleotide design 20
1.3.3.5 Sensitivity and specificity 20
1.3.3.6 Reproducibility 22
1.3.3.7 Accuracy 23
1.3.3.8 Comparison across platforms 23
1.3.3.9 The microarray chip: parameters 24
1.3.3.10 Oligonucleotide probe synthesis 26
1.3.3.11 Microarray slides 26
1.3.3.12 Microarray protocol 27
1.3.3.13 Microarray data: extraction, analysis and submission 28
1.3.3.14 Data analysis 29
1.3.3.15 Microarray data publication 34

1.4 Beyond microarrays 35

1.4.1 RNA-Seq 35

1.4.2 Non-coding RNAs 36

1.4.3 Biointegrative approaches to neurodegenerative diseases 37

Hypothesis 39

Aims 40

2 METHODOLOGY 41

2.1 Methods for Chapter 3 – Connexin Microarray Chip Design and Validation in Rat and Human 41

2.1.1 Design 41

2.1.1.1 Oligonucleotide probe design 41
2.1.1.2 Microarray chip Design 48

2.1.2 Validation 51

2.1.2.1 Handling RNA - general considerations 51
2.1.2.2 Preliminary validation in rat and human Tissue 51
2.1.2.3 Validation of the Custom Designed Microarray Chip in Rat Heart vs Human Heart 59
2.1.2.4 Validation of the custom-designed microarray chip using the commercial Affymetrix platform 67

2.2 Methods for Chapter 4 - RNA Quality of Human Brain Tissue. 72

2.2.1 Tissue collection and preparation 72

2.2.2 RNA extraction and purification 72

2.2.3 RNA quality assessment 72

2.2.4 Tissue pH measurement 72

2.2.5 Statistical analysis 73

2.3 Methods for Chapter 5 – Screening of Human Brain Tissue for Connexin Expression 76

2.3.1 Using the custom-designed microarray chip to screen for connexin expression 76

2.3.1.1 RNA sources 76
2.3.1.2 cDNA target synthesis and fluorescent dye labelling 76
2.3.1.3 Microarray chip 76
2.3.1.4 Data Analysis 77

2.3.2 Using the Illumina Sentrix® commercial platform to validate the custom-designed microarray chip data for the human brain. 81

2.3.2.1 Array manufacturing technology 81
2.3.2.2 Controls 83
2.3.2.3 Overview of the Illumina BeadChip Assay hybridisation and labelling procedure .84
2.3.2.4 Data Analysis 84

3 CUSTOM CONNEXIN MICROARRAY CHIP DESIGN AND VALIDATION 85

3.1 Microarray Chip Design 85

3.1.1 MWG-designed connexin oligonucleotides 85

3.1.2 Custom-designed oligonucleotides 85

3.2 Preliminary Validation in Rat and Human Tissue 88

3.2.1 Microarray slides 88

3.2.1.1 Rat heart 2 (Cy3) vs rat heart 2 (Cy5) array 89
3.2.1.2 Rat heart 2 (Cy3) vs rat heart 3 (Cy5) 89
3.2.1.3 Rat heart (Cy3) vs rat lens (Cy5) array 92
3.2.1.4 Rat lens (Cy3) vs rat heart (Cy5) array 92
3.2.1.5 Rat brain (Cy3) vs rat heart (Cy5) array 95
3.2.1.6 Rat liver (Cy3) vs rat brain (Cy5) array 97
3.2.1.7 Rat brain (Cy3) vs rat liver (Cy5) array 97
3.2.1.8 Human brain (Cy3) vs rat brain (Cy5) array 99
3.2.1.9 Rat brain (Cy3) vs human brain (Cy5) array 99

3.2.2 Summary 101

3.3 Validation in Rat Heart and Rat Liver 112

3.3.1 Custom-designed array 112

3.3.2 Connexin expression 112

3.3.2.1 Cx43 112
3.3.2.2 Cx40 113
3.3.2.3 Cx32 113
3.3.2.4 Cx26 114

3.3.3 Connexins summary 114

3.3.4 Controls 115

3.3.5 Correlation coefficients **..... 115**

3.3.6 Affymetrix arrays 121

3.3.6.1 Rat heart array 121
3.3.6.2 Rat liver array 121
3.3.6.3 Connexins 122

4 RNA QUALITY OF THE HUMAN BRAIN TISSUE 127

4.1 Normal Controls 127

4.2 Alzheimer's Disease 128

4.3 Parkinson's Disease 128

4.4 Huntington's Disease 128

4.5 Epilepsy 129

4.6 The Postmortem Delay Range Study 129

4.7 The Effect of the Agonal State 129

4.8 The Variables Associated with the Cerebellar RNA Integrity 129

5 SCREENING THE HUMAN BRAIN 139

5.1 Screening Human Brain for Connexin Expression Using the Custom-Designed Array 140
5.1.1 Huntington's disease 141
5.1.1.1 Controls 141
5.1.1.2 Connexins 141
5.1.2 Parkinson's disease 142
5.1.2.1 Controls 142
5.1.2.2 Connexins 142
5.1.3 Alzheimer's disease 143
5.1.3.1 Controls 143
5.1.3.2 Connexins 144
5.1.4 Epilepsy 145
5.1.4.1 Controls 145
5.1.4.2 Connexins 145
5.1.5 Summary 146
5.1.5.1 Controls 146
5.1.5.2 Connexins 146
5.2 Screening Human Brain Tissue Using the Commercially Designed Illumina Array. 148
5.2.1.1 Huntington's disease 148
5.2.1.2 Parkinson's disease 148
5.2.1.3 Alzheimer's disease 149
5.2.2 Connexins 149
5.2.2.1 Huntington's disease 149
5.2.2.2 Parkinson's disease 150
5.2.2.3 Alzheimer's disease 150
5.2.3 Inflammatory markers 151
5.2.3.1 Huntington's disease 151
5.2.3.2 Parkinson's disease 151
5.2.3.3 Alzheimer's disease 152
5.2.4 Summary 152
5.2.5 Connexin expression relative to cell types 153
5.2.6 Platform comparison 154
Tables 156
Figures 171
6 DISCUSSION 204
6.1 Microarrays 204
6.1.1 Oligonucleotide design 204
6.1.2 Microarray chips 205

6.2 RNA quality 209
6.3 Connexins Expression and Inflammation in the Human Brain 211
6.3.1 Expression of connexins 211
6.3.2 Inflammatory cytokines and their relationship to connexin expression 213
6.3.3 Microarray experiments in human neurodegenerative diseases 216
6.4 Limitations of this study 219
CONCLUSION 223
FUTURE DIRECTIONS 224
REFERENCES 225

LIST OF TABLES

Table 1. The summary table of the connexins found in the brain by the cell type in which they are expressed. 12

Table 2. MWG connexin oligonucleotide probes. 43

Table 3. Array controls. 44

Table 4. Source of human tissue for RNA extraction. 51

Table 5. Normal control cases. 74

Table 6. Alzheimer's disease cases 74

Table 7. Parkinson's disease cases 74

Table 8. Huntington's disease cases 74

Table 9. Epilepsy cases 75

Table 10. Cases for the postmortem delay range study. 75

Table 11. MWG-designed oligos. 79

Table 12. Positive and negative controls. 79

Table 13. Custom-designed oligos. 80

Table 14. Custom-designed connexin oligonucleotides (human –rat base mismatches are highlighted in red. 87

Table 15. Connexins expressed in the rat heart slides. 91

Table 16.Connexins expressed in the rat heart and rat lens slide pair. 94

Table 17. Connexins expressed in the rat heart and rat brain slide. 96

Table 18. Connexins expressed in the rat liver and rat brain slide pair. 98

Table 19. Connexins expressed in the human brain and rat brain slide pair. 100

Table 20. Pearson correlation coefficients for normalised log mean intensities of RNA samples on different arrays. 102

Table 21.Experimental design - three biological repeats, three sample repeats for each of the animals and tree dye reversal repeats for one of the animals. 115

Table 22. Average connexin expression values, nine heart Cy5 vs liver Cy3 slides and three heart Cy3 vs liver cy5 slides (dye reversals). Orange – significantly higher expression in the heart, green – significantly higher expression in the liver, grey – no change. LogDif – log difference. 116

Table 23. Correlation coefficients for dye reversals, sample repeats and biological repeats. ..117

Table 24. The expression levels of housekeeping controls for the heart and liver Affymetrix arrays. Detection: P - present, A - absent, M - marginal. 123

Table 25. The expression levels of hybridisation controls for the heart and liver Affymetrix arrays. Detection: P - present, A - absent, M - marginal. 123

Table 26. The expression levels of unlabelled polyA controls for the heart and liver Affymetrix arrays. Detection: P - present, A - absent, M - marginal. 124

Table 27. The expression levels for connexins in the heart and liver Affymetrix arrays. Detection: P - present, A - absent, M - marginal. The genes for connexins analysed for both the Affymetrix and the custom-designed arrays are highlighted in blue. The signal levels for probes detected as present on the array are highlighted in green, for probes detected as marginally present – in blue, and for probes which were not detected – in grey. 125

Table 28. The patient data and the RNA quality (RIN) values for the normal control cases (h - hours).....130

Table 29. The patient data and the RNA quality (RIN) values for the Alzheimer's disease cases (h - hours).....130

Table 30. The patient data and the RNA quality (RIN) values for the Parkinson's disease cases (h - hours).....131

Table 31. The patient data and the RNA quality (RIN) values for the Huntington's disease cases (h - hours).....131

Table 32. Case ID and the RNA quality (RIN) values for epilepsy.....132

Table 33. The patient data and the RNA quality (RIN) values for the long postmortem delay range cases (h - hours).....132

Table 34. The samples used in the human brain screening experiments.....140

Table 35. Differential expression of housekeeping controls in Huntington's disease, custom-designed array. Each of the five Huntington's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....156

Table 36. Differential expression of connexins in Huntington's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....156

Table 37. Differential expression of connexins in Huntington's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....157

Table 38. Differential expression of connexins in Huntington's disease, custom-designed set, includes the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....157

Table 39. Differential expression of connexins in Huntington's disease, MWG-designed set, includes the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....158

Table 40.Differential connexin expression in Huntington's disease, Illumina array. Five Huntington's disease samples were screened against one control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values.....158

Table 41. Differential expression of inflammatory markers in Huntington's disease, Illumina array. Five Huntington's disease samples were screened against one control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. 159

Table 42. Differential expression of housekeeping controls in Parkinson's disease, custom-designed array. Each of the five Parkinson's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....160

Table 43. Differential expression of connexins in Parkinson's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....160

Table 44. Differential expression of connexins in Parkinson's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....161

Table 45. Differential expression of connexins in Parkinson's disease, cusom-designed set, includes the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....161

Table 46. Differential expression of connexins in Parkinson's disease, MWG-designed set, includes the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....162

Table 47. Differential expression connexins in Parkinson's disease, Illumina array. Five Parkinson's disease samples were screened against one control sample. . LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values.....162

Table 48. Differential expression of inflammatory markers in Parkinson's disease, Illumina array. Five Parkinson's disease samples were screened against one control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. 163

Table 49. Differential expression of housekeeping controls in Alzheimer's disease, custom-designed array. Each of the five Alzheimer's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....164

Table 50. Differential expression of connexins in Alzheimer's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change.....164

Table 51. Differential expression of connexins in Alzheimer's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are

also expressed on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change. 165

Table 52. Differential expression of connexins in Alzheimer's disease, cusom-designed set, includes only the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change..... 165

Table 53 Differential expression of connexins in Alzheimer's disease, MWG-designed set, includes the connexins that are expressed on the custom-designed array, but not on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change..... 166

Table 54. Differential expression connexins in Alzheimer's disease, Illumina array. Five Alzheimer's disease samples were screened against one control sample. . LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. 166

Table 55. Differential expression of inflammatory markers in Alzheimer's disease, Illumina array. Five Alzheimer's disease samples were screened against one control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. 167

Table 56. Differential expression of housekeeping controls in epilepsy, custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change..... 168

Table 57. Differential expression of connexins in epilepsy, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array for the other diseases. Each of the two epilepsy samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change. 168

Table 58. Differential expression of connexins in epilepsy, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array for the other diseases. Each of the two epilepsy samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change. 169

Table 59. Differential expression of connexins in epilepsy, cusom-designed set, includes the connexins that are expressed only the custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. LogDif - log difference between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change. 169

Table 60. Differential expression of connexins in epilepsy, MWG-designed set, includes the connexins that are expressed only the custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. . LogDif - log difference

between the disease sample and the control sample values. FC - fold change between the disease sample and the control sample values. Colours: orange – upregulated; green – downregulated, - grey – no change. 170

LIST OF FIGURES

Figure 1. The structure of a gap junction, on the left; the molecular structure of a connexin, on the right (picture kindly provided by Professor Colin R. Green). 2

Figure 2. The principle of microarrays: the probe immobilised on the slide and a cDNA or cRNA target incorporating a tag for visualisation (Adapted from the High-Light Microarray System (Qiagen, 2003)).....16

Figure 3. Overview of the oligonucleotide design protocol. 47

Figure 4. Microarray printing pattern: one section. 50

Figure 5. A denaturing RNA gel of the rat liver, rat heart, rat brain, rat lens and human brain samples, showing the 28s, 18s and 5s bands with the 28s/18s band ratio of ~2. 55

Figure 6. An example of an electropherogram generated by the Agilent Bioanalyser 2100.....61

Figure 7. Photolithographic synthesis of Affymetrix arrays. (Picture source (Tomlinson et al., 2008)). 69

Figure 8. New custom array layout. 78

Figure 9. Picture of the Illumina Sentrix® array surface showing nucleotide-coated beads embedded in wells on the silicon wafer surface. (Picture source http://www.centerforgenomicsciences.org). 82

Figure 10. The rat heart2 *vs* rat heart3 normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different heart samples. 103

Figure 11. The rat heart 2 *vs* rat heart 2 normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing the same sample labelled with Cy3 and Cy5. 104

Figure 12. The rat lens *vs* rat heart normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 105

Figure 13. Rat lens *vs* rat heart (dye reversal) normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 106

Figure 14. The rat heart *vs* rat brain normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 107

Figure 15. The rat brain *vs* rat liver normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 108

Figure 16. The rat brain *vs* rat liver (dye reversal) normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 109

Figure 17. The rat brain *vs* human brain normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 110

Figure 18. The rat brain vs human brain (dye reversal) normalised log intensity data shows the relative distribution of signal intensities in the red (x axis) and green (y-axis) channel representing two different tissue samples. 111

Figure 19. Average difference in connexin expression in the heart and the liver. Three rats (biological replicates) and three sample replicates for each.of the animals were used.

Positive difference indicates higher expression in the heart and negative difference indicates higher expression in the liver. 118

Figure 20. Connexin expression for the nine heart (Cy5) *vs* liver (Cy3) slides. Three rats (biological replicates) and three sample replicates for each.of the animals were used. ...119

Figure 21. Connexin expression for the three heart (Cy3) *vs* liver (Cy5) slide. Three sample replicates were used. 120

Figure 22. Average difference in connexin expression in the heart and the liver. Positive difference indicates higher expression in the heart and negative difference indicates higher expression in the liver. One heart and one liver sample from the same rat was used. Ht Liv – expressed in both the heart and the liver. Liv – expressed in liver only. 126

Figure 23. The RNA quality values and trends in relation to the length of postmortem delay for the normal control cases. Six cerebellum samples, four CN sample, one MC sample, one MTG samples were screened. 133

Figure 24. The RNA quality values and trends in relation to the length of postmortem delay for the Alzheimer's disease cases. Cerebellum samples from seven patients and MTG samples from five patients were screened. 134

Figure 25. The RNA quality values and trends in relation to the length of postmortem delay for the Parkinson's disease cases. Cerebellum samples from six patients and CN samples from four patients were screened. 135

Figure 26. The RNA quality values and trends in relation to the length of postmortem delay for the Huntington's disease cases. Cerebellum samples from five patients and MC samples from five patients were screened. 136

Figure 27. Trends in cerebellar RNA quality by disease and control group in relation to the length of postmortem delay. Normal control samples from five patients, Alzheimer's disease samples from seven patients, Parkinson's disease samples form six patients and Huntington's disease samples from five patients were used. Individual RNA quality was variable both within and between all groups with Huntington's disease cases having the highest mean for RNA quality (mean RIN=8.6), second only to normal controls (mean RIN=8.2). Although the sample sizes were small and variable between disease groups, there is a trend towards a decrease in RNA quality relative to postmortem delay in disease groups with the correlation between postmortem delay and cerebellar quality inverted in the control group. 137

Figure 28. A Bland and Altman plot of the differences between cerebellar and secondary region RNA quality plotted against the mean RIN value for the cerebellum and the secondary region RNA pairs in each case. Five patient sample pairs for each disease were used. The higher the RNA quality for the two samples, the smaller the difference is likely to be. For example, in Huntington's disease and normal controls, both cerebellar and motor cortex samples had high mean values and RIN differences between the sample pairs were close to zero. By contrast, for Alzheimer's disease where the cerebellar RNA quality and that of the medial temporal gyrus were low, the difference between the pairs was large. Thus higher quality cerebellar RNA appears to be a better predictor of RNA quality in the secondarily affected brain region. 138

Figure 29. Differential expression of positive controls in Huntington's disease, custom-designed array. Each of the five Huntington's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 172

Figure 30. Differential expression of connexins in Huntington's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 173

Figure 31. Differential expression of connexins in Huntington's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 174

Figure 32. Differential expression of connexins in Huntington's disease, custom-designed set, includes the connexins that are expressed on the custom-designed array but not on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 175

Figure 33. Differential expression of connexins in Huntington's disease, MWG-designed set, includes the connexins that are expressed on the custom-designed array but not on the Illumina array. Each of the five Huntington's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 176

Figure 34. Differential connexin expression in Huntington's disease, Illumina array. Five Huntington's disease samples were screened against one control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 177

Figure 35. Differential expression of inflammatory markers in Huntington's disease, Illumina array. Five Huntington's disease samples were screened against one control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 178

Figure 36. Differential expression of positive controls in Parkinson's disease, custom-designed array. Each of the five Parkinson's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 179

Figure 37. Differential expression of connexins in Parkinson's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample 180

Figure 38. Differential expression of connexins in Parkinson's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 181

Figure 39. Differential expression of connexins in Parkinson's disease, custom-designed set, includes the connexins that are expressed on the custom-designed array but not on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 182

Figure 40. Differential expression of connexins in Parkinson's disease, MWG-designed set, includes the connexins that are expressed on the custom-designed array but not on the Illumina array. Each of the five Parkinson's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 183

Figure 41. Differential connexin expression in Parkinson's disease, Illumina array. Five Parkinson's disease samples were screened against one control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 184

Figure 42. Differential expression of inflammatory markers in Parkinson's disease, Illumina array. Five Parkinson's disease samples were screened against one control sample. Y axis

- the expression level in the diseased sample minus the expression level in the normal control sample.....185

Figure 43. Differential expression of positive controls in Alzheimer's disease. Each of the five Alzheimer's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 186

Figure 44. Differential expression of connexins in Alzheimer's disease, custom-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 187

Figure 45. Differential expression of connexins in Alzheimer's disease, MWG-designed set, includes only the connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 188

Figure 46. Differential expression of connexins in Alzheimer's disease, custom-designed set, includes the connexins that are expressed only on the custom-designed array but not on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 189

Figure 47. Differential expression of connexins in Alzheimer's disease, MWG-designed set, includes the connexins that are expressed only on the custom-designed array but not on the Illumina array. Each of the five Alzheimer's disease samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 190

Figure 48. Differential connexin expression in Alzheimer's disease, Illumina array. Five Alzheimer's disease samples were screened against one control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 191

Figure 49. Differential expression of inflammatory markers in Alzheimer's disease. Five Alzheimer's disease samples were screened against one control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 192

Figure 50. Differential expression of positive controls in epilepsy, custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 193

Figure 51. Differential expression of connexins in epilepsy, custom-designed set, includes only the connexins that are expressed on the custom-designed array and the Illumina arrays for the other diseases. Each of the two epilepsy samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample.....194

Figure 52. Differential expression of connexins in epilepsy, MWG-designed set, includes only the connexins that are expressed on the custom-designed array and the Illumina arrays for the other diseases. Each of the two epilepsy samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample.....195

Figure 53. Differential expression of connexins in epilepsy, custom-designed set, includes the connexins that are expressed only in the custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 196

Figure 54. Differential expression of connexins in epilepsy, MWG-designed set, includes the connexins that are expressed only in the custom-designed array. Each of the two epilepsy samples was screened against the normal control sample. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 197

Figure 55. Average differential expression of positive controls in Huntington's, Parkinson's, Alzheimer's disease and epilepsy, custom-designed array. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 198

Figure 56. Average differential expression of positive controls in Huntington's, Parkinson's and Alzheimer's disease, Illumina array. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 199

Figure 57. Average positive control expression for Huntington's, Parkinson's, Alzheimer's disease and epilepsy, custom-designed array. Y axis – absolute probe signal in the samples minus the average signal for the negative controls for the disease. 200

Figure 58. Average expression of positive controls in Huntington's, Parkinson's and Alzheimer's diseases, Illumina array. Y axis – absolute probe signal in the samples minus the average signal for the negative controls for the disease. 201

Figure 59. Average differential connexin expression for Huntington's, Parkinson's, Alzheimer's disease and epilepsy, custom-designed array, connexins that are expressed on the custom-designed array that are also expressed on the Illumina array. Y axis – the expression level in the diseased sample minus the expression level in the normal control sample. 202

Figure 60. Average differential connexin expression for Huntington's, Parkinson's, Alzheimer's disease and epilepsy, custom-designed array, connexins that are expressed only on the custom-designed array but not on the Illumina array. Y axis - the expression level in the diseased sample minus the expression level in the normal control sample. 203

LIST OF ABBREVIATIONS

CCD – charge-coupled device

CD11A – alpha L integrin

CD11B – alpha M integrin

CN – caudate nucleus

CNS – central nervous system

DNA – deoxyribonucleic acid

DNase - deoxyribonuclease

GAPDH – glyceraldehyde-3-phosphate dehydrogenase

GCOS – Affymetrix GeneChip® Operating Software

GFAP – glial fibrillary acidic protein

IL-1β – interleukin 1 beta

IL-6 – interleukin 6

IL-10 – interleukin 10

MC – motor cortex

MTG – medial temporal gyrus

HNRPC – heterogeneous nuclear ribonucleoprotein C1/C2

HPRT – hypoxanthine-guanine phosphoribosyltransferase

Oligo – oligonucleotide probe

PMT – photomultiplier tube

qRT-PCR – quantitative real-time polymerase chain reaction

RNA – ribonucleic acid

RNase – ribunuclease

RPS9 – ribosomal protein S9

TCEA1 – transcription elongation factor A (SII) 1

TNF-α – tumour necrosis factor alpha