UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH & LIFE SCIENCES

School of Medicine

Functional Study of Ubiquitin C-terminal Hydrolase-L1 Gene Promoter Haplotypes

by

Shane Sanassy

Thesis for the degree of Doctor of Philosophy

October 2007

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE, HEALTH & LIFE SCIENCES
SCHOOL OF MEDICINE

Doctor of Philosophy

FUNCTIONAL STUDY OF UBIQUITIN C-TERMINAL HYDROLASE-L1 GENE PROMOTER HAPLOTYPES

by Shane Sanassy

The Ubiquitin Conjugating System (UCS) describes a system in which the 96-amino acid residue Ubiquitin can be selectively covalently linked to intracellular proteins. This endows cells with an indispensable level of regulation to determine protein fate in a wide range of basic cellular events.

The abundant, neuron specific Ubiquitin Carboxyl-Terminal Hydrolase-L1 (UCH-L1) is intimately involved with the UCS – both in a hydrolase and ligase capacity. Mutations in *UCH-L1* have clearly been associated with various neurodegenerative disorders, including Alzheimer's, Huntington's and particularly Parkinson's disease.

The main and unique objective of this study was to identify any common Caucasian sequence variants in *UCH-L1's* promoter, and to investigate whether they are associated with neurodegenerative symptoms, and any change in *UCH-L1* transcriptional activity.

Seven novel *UCH-L1* Single Nucleotide Polymorphisms (SNPs), as well as the C54A documented coding region polymorphism (Ser18Tyr), were identified using both denaturing High Performance Liquid Chromatography (dHPLC) and DNA sequencing analysis. In relation to the translational start site, the novel SNPs elucidated were: A-307G, A-306G, G-234A, A-24G, C-16T, G12A and G21A. Restriction Fragment Length Polymorphism (RFLP) genotyping analysis was then employed within Caucasian DNA sample sets of 31 and 480 individuals, to firstly elucidate the common *UCH-L1* promoter haplotypes that exist within the population, and secondly, in an attempt to uncover any association between the polymorphic alleles and general neurodegenerative symptoms - no association was uncovered.

Using pGEM-T Easy as an initial 'holding vector', the three common *UCH-L1* promoter haplotypes elucidated – AAGAC, GAGGT and AGAAC - were incorporated into a modified pGL3 vector to ascertain transcriptional activity rates. This was done by Luciferase expression analysis, and the results identified the GAGGT promoter haplotype as having a significantly increased transcriptional activity in all human cell lines tested.

It is my contention, that the pronounced increase in transcriptional activity elucidated for the GAGGT *UCH-L1* promoter haplotypes, potentially indicates a primary genetic risk factor for sporadic Parkinson's disease in the Caucasian population – a novel pathogenic model of which is proposed in this thesis. The fact that RFLP genotyping analysis uncovered no association of the promoter polymorphic alleles with more general neurodegenerative symptoms, indicates the need for further studies to be focused more specifically towards Parkinson's disease.

Table of Contents

Abstract i

Declaration of Authorship ii

Acknowledgements iii

Abbreviations iv

1 Introduction 1

1.1 The Ubiquitin-Conjugating System 1

1.1.1 Polyubiquitination 4

1.1.2 Monoubiquitination and Endocytic Trafficking 6

1.1.3 A Fourth UCS Enzyme? 7

1.1.4 The Proteasome (inv. UPS) 8

1.2 Deubiquitinating Enzymes (DUBs) 11

1.2.1 Ubiquitin-Specific Proteases (UBPs/ USPs) 12

1.2.2 Ubiquitin C-Terminal Hydrolases (UCHs) 14

1.2.3 The Ovarian Tumour (OTU)- Related Proteases 16

1.2.4 Machando-Joseph Disease Proteins (or Josephin) Domain Protease (MJDs) 17

1.2.5 JAMM Motif Proteases (Metalloprotease Subclass) 17

1.3 Ubiquitin Carboxyl-Terminal Hydrolase L1 (UCH-L1) 19

1.3.1 UCH-L1's Isoleucine93Methionine Mutation (Ile93Met) and Parkinson's Disease 20

1.3.2 UCH-L1's Serine18Tyrosine Polymorphism (Ser18Tyr) and Parkinson's Disease 25

1.3.2.1 UCH-L1's Ser18Tyr Polymorphism and Alzheimer's Disease 29

1.3.2.2 UCH-L1's Ser18Tyr Polymorphism and Huntington's Disease 30

1.3.3 UCH-L1's Methionine124Leucine Mutation (Met124Leu) and Parkinson's Disease 30

1.3.4 UCH-L1 and Dementia With Lewy Bodies 31

1.3.5 Gracile Axonal Dystrophic Mouse - UCH-L1 Intragenic Deletion 31

1.3.6 Ubiquitin Carboxyl-Terminal Hydrolase-L1 – Discussion 33

1.3.7 Summary of *UCH-L1* Neurodegenerative Disease Association 38

1.4 Other UCS Associated Genes Implicated in Parkinson's Disease 39

1.4.1 *α-Synuclein* 39

1.4.2 *Parkin* 41

1.4.3 *Synphilin-1* 42

1.5 Parkinson's Disease 44

1.5.1 Physiological Characteristics of Parkinson's Disease 44

1.5.2 Parkinson's Disease Epidemiology 45

1.5.3 Pathology of Parkinson's Disease 46

1.5.4 Environmental Factors in Parkinson's Disease 50

1.5.5 Parkinson's Disease Therapy 53

1.6 Basic Overview of other Neurodegenerative Diseases (Dementias) Associated with UCH-L1 55

1.6.1 Alzheimer's Disease 55

1.6.2 Dementia with Lewy Bodies 58

1.6.3 Huntington's Disease 62

1.7 Basic Relevant Molecular Overview 66

1.7.1 Eukaryotic Transcription Regulation 66

1.7.1.1 The Core Promoter 67

1.7.1.2 Upstream Regulatory Elements 68

1.7.1.3 Distant Regulatory Elements 68

1.7.1.4 DNA Methylation 69

1.7.2 Genome Variation 70

1.7.2.1 Hardy-Weinberg Equilibrium 72

1.8 *UCH-L1*'s Expression 73

1.9 This Study's Importance 77

1.10 Objectives 78

2. Materials & Methods 79

2.1 Polymorpism Identification 79

2.1.1 DNA Arrays 79

2.1.1.1 x26 Control DNA Array 79

2.1.1.2 x64 Control DNA Array 79

2.1.1.3 x480 DNA Array with Cognitive Function Data 80

2.1.2 Polyacrylamide MADGE Gels 80

2.1.2.1 MADGE Background 80

2.1.2.2 MADGE Gel Preparation 81

2.1.2.3 MADGE Gel Loading and Running 82

2.1.2.4 DNA Ladders 83

2.1.3 Polymerase Chain Reaction 83

2.1.3.1 Theory 83

2.1.3.2 Protocol 85

2.1.3.3 Polymerase Chain Reaction + Betaine 86

2.1.3.3.1 Theory 86

2.1.3.3.2 Protocol 86

2.1.3.4 PCR Primer Pairs Utilised to Amplify *UCH-L1* Regions 87

2.1.4 denaturing High Performance Liquid Chromatography 88

2.1.4.1 Basic HPLC Theory 88

2.1.4.2 dHPLC Theory 89

2.1.4.3 Setting up a dHPLC Method in 'Wavemaker™' 90

2.1.4.4 dHPLC Protocol 92

2.1.5 DNA Sequencing 93

2.1.5.1 Underlying Theory 93

2.1.5.2 Theory 94

2.1.5.3 Shrimp Alkaline Phosphatase/ Exonuclease I Sample Preparation 95

2.1.5.4 Sequencing Reaction Protocol 96

2.1.5.5 Ethanol Precipitation Protocol 96

2.1.5.6 Sequencing Gel Preparation 97

2.1.5.7 Sample Preparation/ Loading 99

2.1.5.8 Sequence Run 99

2.2 Single Nucleotide Polymorphism 100

2.2.1 Restriction Endonucleases 100

2.2.2 PCR Product Endonuclease Digestion 101

2.2.3 Primer-Intro. Restriction Analysis-Polymerase Chain Reaction 101

2.2.4 Restriction Fragment Length Polymorphism Genotyping Methods 102

2.2.4.1 C-16T SNP Genotyping 102

2.2.4.2 G-234A SNP Genotyping 103

2.2.4.3 A-307G SNP Genotyping 104

2.2.4.4 C54A (S18Y) SNP Genotyping 105

2.2.4.5 A-24G SNP Genotyping 105

2.2.4.6 A-306G SNP Genotyping 107

2.3 Cloning Methodologies 109

2.3.1 Plasmid DNA Vectors 109

2.3.1.1 pGEM-T Easy Theory 109

2.3.1.2 pGL3-Basic Vector 111

2.3.1.3 pGL3-*Eco* RI Construction 112

2.3.1.4 pGL3-*Eco* RI Modified Vector 113

2.3.2 Agarose Gels 114

2.3.2.1 Gel Loading and Running 114

2.3.3 Plasmid DNA Endonuclease Digestion 115

2.3.4 General DNA Isolation/ Purification Protocols 115

2.3.4.1 DNA Isolation from Agarose Gels 115

2.3.4.2 Miniprep Protocol 116

2.3.4.3 Midiprep Protocol 117

2.3.5 Polymerase Chain Reaction from Bacterial Colony 119

2.3.6 Plasmid DNA Endonuclease Digestion 119

2.3.7 Preparation of Competent Cells – Calcium Chloride Method 120

2.3.8 Ligations Using the pGEM-T Easy Vector 121

2.3.9 Ligations Using the Modified pGL3-*Eco* RI Vector 122

2.3.10 Transformations Using the pGEM-T Easy Vector System 123

2.3.11 Transformations Using the pGL3 Vector Ligation Products 124

2.4 Cell Culture Protocols 125

2.4.1 Mammalian Cell Lines and Required Growth Media 125

2.4.1.1 - A2058 (Human Skin Melanoma) 125

2.4.1.2 – MCF-7 (Human Breast Adenocarcinoma) 125

2.4.1.3 – ND-7 (Mouse Neuroblastoma/ Rat Basal Ganglia Neuron Hybrid) 125

2.4.1.4 – HCN-1A (Human Brain Cortical Neuron) 126

2.4.2 Cell line Maintenance 126

2.4.2.1 Propagation 126

2.4.2.2 Subculturing Protocol 127

2.4.3 Vector Transfection into Adherent Mammalian Cells 127

2.4.3.1 Transfection Related Chemicals 127

2.4.3.1.1 GeneJuice Theory 127

2.4.3.2 Transfection Preparation 128

2.4.3.3 Transfection Procedure 128

2.4.4 Dual-Luciferase Reporter Assay 129

2.4.4.1 Dual-Luciferase Reporter Assay Theory 129

2.4.4.2 Dual-Luciferase Reporter Assay Buffer Preparation 130

2.4.4.3 Assay Preparation – Lysis of Cultured Cells 130

2.4.4.4 Luciferase Assay Protocol 131

2.5 Statistical Analysis **132**

2.5.1 Statistical Analysis of Luciferase Data 132

2.5.1.1 Standard Error of Mean Calculations **132**

2.5.1.2 *p*-value Calculations **132**

2.5.2 Cognitive Function Tests **132**

2.5.2.1 AH4 Cognitive Function Test 132

2.5.2.2 Mill Hill Cognitive Function Test 132

2.5.2.3 Immediate Recall Section of Wechsler Logical Memory Cognitive Test 133

2.5.2.4 Delayed Recall Section of Wechsler Logical Memory Cognitive Test 133

2.5.2.5 Regression of AH4 Test Scores on Mill Hill Test Scores (theory of) 133

2.5.2.6 *p*-value Calculations 133

3 Results **134**

3.1 Results I – Mutation Screening **134**

3.1.1 dHPLC Analysis **134**

3.1.1.1 dHPLC Analysis of Promoter Region A **136**

3.1.1.2 dHPLC Analysis of Promoter Region B **137**

3.1.1.3 dHPLC Analysis of Promoter Region C **138**

3.1.1.4 dHPLC Analysis of Promoter Region D **139**

3.1.1.5 Interpretation of dHPLC Analysis **140**

3.1.1.6 Confirming the Presence of Exon 3's Polymorphic +54A allele **141**

3.1.1.6.1 RFLP of Exon 3's +54A Allele - Interpretation 142

3.1.2 Sequencing Results 143

3.1.2.1 DNA Sequencing of Promoter Region A 144

3.1.2.2 DNA Sequencing of Promoter Region B 147

3.1.2.3 DNA Sequencing of Promoter Region C 149

3.1.2.4 Exon 3 (C54A) Sequencing Results 152

3.1.2.5 Interpretation of DNA Sequencing 154

3.1.3 Determination of Allele Frequency and *UCH-L1* Promoter Haplotypes 155

3.1.3.1 RFLP Genotyping Strategies 155

3.1.3.2 x64 DNA Sample Set (62 Chromosomes) Genotyping Results and Analysis 166

3.1.3.2.1 Variant Allele Frequency Table 166

3.1.3.2.2 Genotype Frequency Table 167

3.1.3.2.3 Hardy-Weinberg Equilibrium Consistency of Genotpye Frequencies 168

3.1.3.2.4 Haplotype Frequency Diagrams 169

3.1.3.2.5 Interpretation of x64 DNA Sample Set Genotyping Analysis 170

3.1.3.3 x480 DNA Sample Set (960 Chromosomes) Genotyping Results and Analysis 171

3.1.3.3.1 Variant Allele Frequency Table 171

3.1.3.3.2 Genotype Frequency Table 172

3.1.3.3.3 Hardy-Weinberg Equilibrium Consistency of Genotpye Frequencies 173

3.1.3.3.4 Haplotype Frequency Diagrams 174

3.1.3.3.5 Interpretation of x480 DNA Sample Set Genotyping Analysis 176

3.1.4 Discussion of Mutation Screening 177

3.2 Results II – Functional Studies 180

3.2.1 Construction of pGL3/ *UCH-L1* Promoter Vectors 181

3.2.1.1 Initial Cloning Strategy 181

3.2.1.2 Revised Cloning Strategy 183

3.2.1.2.1 – Incorporation of Haplotypes into pGEM-T Easy 183

3.2.1.2.2 Transfer from pGEM-T Easy into pGL3-*Eco* RI 185

3.2.2 Transcriptional Activity of the *UCH-L1* Promoter Haplotypes 191

3.2.2.1 A2058 Dual-Luciferase Reporter Assays 192

3.2.2.2 MCF-7 Dual-Luciferase Reporter Assays 194

3.2.2.3 ND-7 Dual-Luciferase Reporter Assays 196

3.2.2.4 HCN-1A Dual-Luciferase Reporter Assays 198

3.2.2.5 Luciferase Reporter Assays – Discussion 199

3.2.3 Discussion of Functional Studies 200

3.3 Results III – Population Studies 202

3.3.1 Population Genetics Discussion 209

4 Discussion 211

5 Future Work 221

6 Appendix 225

7 References 242

Table of Figures

Figure 1 – Schematic diagram of the ubiquitin-proteasome system **2**

Figure 2 – Ribbon structure of Ubiquitin **4**

Figure 3 – Schematic representation of the 26S proteasome's subunits **8**

Figure 4 – The family in which the Ile93Met mutation was discovered demonstrating the Ile93Met's penetrance. **22**

Figure 5 – A structural model for the UCH-L1-ubiquitin complex. **24**

Figure 6 – The dopaminergic diffuse modulatory systems arising from the substantia nigra and the ventral tegmental area in the midbrain **46**

Figure 7 – A substantia nigra neuron realising a Lewy body inclusion **48**

Figure 8 – The Hardy-Weinberg principle for two alleles. **72**

Figure 9 – Basic depiction of *UCH-L1*'s main promoter elements **73**

Figure 10 – Comparison of the nucleotide sequences at the 5' ends of the human and Monodelphis UCH-L1 genes by optimal sequence alignment. **76**

Figure 11 – 96-well MADGE gel showing direction of electrophoresis. **81**

Figure 12 – The 100bp ladder **83**

Figure 13 – The 1kb DNA extension ladder **83**

Figure 14 – Basic outline of the PCR reaction **84**

Figure 15 – Creation of a mixture of hetero- and homoduplexes through dHPLC hybridisation **89**

Figure 16 – Temperature-dependent resolution of dHPLC heteroduplexes from homoduplexes **90**

Figure 17 – Chemical structure of deoxythymidine triphosphate **93**

Figure 18 – Chemical structure of dideoxythymidine triphosphate **93**

Figure 19 – Pictorial summary of the dideoxy DNA sequencing method **94**

Figure 20 – Diagram outlining the assembly of the DNA sequencing plates **97**

Figure 21 - Linear representation of promoter region A and the *Nci* I restriction sites **103**

Figure 22 - Linear representation of promoter region B and the *Sgr* AI restriction site **103**

Figure 23 - Linear representation of promoter region C and both *Taq* I's restriction sites. **104**

Figure 24 - Linear representation of the amplified exon 3 sequence and the *Rsa* 1 restriction site **105**

Figure 25 - Linear representation of the specified A-24G mismatch PCR region and *Fok* I's restriction site **106**

Figure 26 - Linear representation of the specified A-306G mismatch PCR region and *Mbo* II's restriction site **108**

Figure 27 – Schematic representation of the pGEM-T Easy vector **110**

Figure 28 – Schematic representation of the pGL3-Basic vector **111**

Figure 29 – pGL3-Eco RI construction strategy. **112**

Figure 30 – Schematic representation of the pGL3-Eco RI vector **113**

Figure 31 – Comparative dHPLC scans for Promoter Region A **136**

Figure 32 – Comparative dHPLC scans for Promoter Region B **137**

Figure 33 – Comparative dHPLC scans for Promoter Region C **138**

Figure 34 – Comparative dHPLC scans for Promoter Region D **139**

Figure 35 – A 5% polyacrylamide gel displaying *UCH-L1* exon 3 *Rsa* I RFLP analysis of x16 control DNA samples **141**

Figure 36 – Comparative DNA sequencing scans for the *UCH-L1* anti-sense nucleotide sequence -11 to -30 of promoter region A **145**

Figure 37 – Comparative DNA sequencing scans for the *UCH-L1* anti-sense nucleotide sequence +28 to +5 of promoter region A **146**

Figure 38 – Comparative DNA sequencing scans for the *UCH-L1* anti-sense nucleotide sequence -223 to -245 of promoter region B **148**

Figure 39 – Comparative DNA sequencing scans for the *UCH-L1* sense nucleotide sequence -318 to -296 of promoter region C **150**

Figure 40 – Comparative DNA sequencing scans for the *UCH-L1* sense nucleotide sequence -318 to -296 of promoter region C **151**

Figure 41 – Comparative DNA sequencing scans for the *UCH-L1* anti-sense nucleotide sequence +65 to +45 of exon 3 **153**

Figure 42 – A 5% polyacrylamide gel displaying *UCH-L1* promoter region A *Nci* I RFLP analysis of x96 samples **156**

Figure 43 – A 5% polyacrylamide gel displaying *UCH-L1* promoter region B *Sgr* AI RFLP analysis of x96 samples **157**

Figure 44 – A 5% polyacrylamide gel displaying *UCH-L1* promoter region C *Taq* I RFLP analysis of x96 samples **158**

Figure 45 – A 5% polyacrylamide gel displaying *UCH-L1* exon 3 *Rsa* I RFLP analysis of x64 samples **159**

Figure 46 – Comparative DNA sequencing scans for the *UCH-L1* anti-sense nucleotide sequence -11 to -30 of promoter region A **161**

Figure 47 – Comparative DNA sequencing scans for the *UCH-L1* sense nucleotide sequence -318 to -296 of promoter region C **163**

Figure 48 – Comparative DNA sequencing scans for the *UCH-L1* sense nucleotide sequence -318 to -296 of promoter region C **164**

Figure 49 - The four *UCH-L1* haplotypes are diagrammatically represented with reference to the distribution of the six polymorphic alleles **169**

Figure 50 - The ten *UCH-L1* promoter haplotypes are indicated with reference to the distribution of the five promoter/ 5' UTR polymorphic alleles **175**

Figure 51 – Diagrammatic representation of required *UCH-L1* promoter sequences **182**

Figure 52 – Agarose gel identifying three pGEM-T Easy constructs harbouring the *UCH-L1* promoter inserts **184**

Figure 53 – Basic outline of the cloning strategy devised. **186**

Figure 54 – 'Modified TAE agarose gel' showing the pGL3-Eco RI vector and the three *UCH-L1* promoter haplotype inserts after *Spe* I → *Eco* RI digestion **188**

Figure 55 - Agarose gel identifying three pGL3-Eco RI constructs harbouring the UCH-L1 promoter inserts for all three promoter haplotypes **189**

Figure 56 – A bar graph displaying the mean firefly/ renilla luciferase activity ratios realised for the three pGL3/ *UCH-L1* promoter constructs and a control pGL3 vector with no insert, in the A2058 human skin melanoma cell line **192**

Figure 57 – A bar graph displaying the mean firefly/ renilla luciferase activity ratios realised for the three pGL3/ *UCH-L1* promoter constructs and a control pGL3 vector with no insert, in the MCF-7 human breast adenocarcinoma cell line **194**

Figure 58 – A bar graph displaying the mean firefly/ renilla luciferase activity ratios realised for the three pGL3/ *UCH-L1* promoter constructs and a control pGL3 vector with no insert, in the ND-7 mouse neuroblastoma/ rat basal ganglia neuron hybrid cell line. **196**

Figure 59 – A bar graph displaying the mean firefly/ renilla luciferase activity ratios realised for the three pGL3/ *UCH-L1* promoter constructs and a control pGL3 vector with no insert, in the HCN-1A human brain cortical neuron cell line. **198**

Figure 60 – A bar graph displaying the mean increase in firefly/ renilla luciferase mean activity ratios **200**

Figure 61 – Comparison of the nucleotide sequences at the 5' end of the human (H) and *Monodelphis domestica* (M) UCH-L1 genes **212**

Figure 62 - The three *UCH-L1* promoter haplotypes are diagrammatically represented with reference to the distribution of the five novel promoter/ 5' UTR polymorphic alleles **214**

Table of Tables

Table 1 – Indicates the main reported mutations or genetic loci associated with familial Parkinson's disease **21**

Table 2 – Summarises the association that UCH-L1 has with various neurodegenerative diseases **38**

Table 3 – Indicates genes (UCS unrelated), and their respective genetic loci, that are associated with sporadic Parkinson's disease **51**

Table 4 **– Indicates the sample duplicity and quadruplicity in the x64 control DNA array** **79**

Table 5 – Lists and details the PCR primer pairs used to amplify the respective *UCH-L1* promoter and coding regions **87**

Table 6 – Displays all the relevant information for each restriction endonuclease used in this study **100**

Table 7 – Displays the four *UCH-L1* regions in which dHPLC heteroduplexes were elucidated **135**

Table 8 – Summarises the novel *UCH-L1* SNPs elucidated in this result's section. **143**

Table 9 –Displays the frequency of variant alleles obtained from the x 31 DNA sample set through both RFLP and sequencing analysis **166**

Table 10 – Collation of the RFLP and sequencing results with respect to the genotypes exhibited by each of the x31 individuals within the x64 DNA sample set **167**

Table 11 – Exhibits the genotype frequencies for the six *UCH-L1* SNPs investigated for this sample set with the Hardy-Weinberg formula applied **168**

Table 12 –Displays the frequency of variant alleles obtained from the x 480 DNA sample set **171**

Table 13 – Collation of the results with respect to the (determinable) genotypes exhibited within the x480 DNA sample set (960 chromosomes) **172**

Table 14 – Exhibits the genotype frequencies for the five *UCH-L1* SNPs investigated for this sample set (x480) with the Hardy-Weinberg formula applied **173**

Table 15 – Outlines the predicted changes in transcription factor binding that the five promoter *UCH-L1* SNP variant alleles realised from that of the reference alleles utilising the 'TFSEARCH' software **179**

Table 16 – Table displaying a summary of the statistical analysis (from x480 DNA array) for the AH4 cognitive function test scores in relation to each *UCH-L1* allele investigated in this study, and their respective genotypes **204**

Table 17 – Table displaying a summary of the statistical analysis (from x480 DNA array) for the Mill Hill cognitive function test scores in relation to each *UCH-L1* allele investigated in this study, and their respective genotypes **205**

Table 18 – Table displaying a summary of the statistical analysis (from x480 DNA array) for the immediate recall section of Wechsler logical memory cognitive function test scores in relation to each *UCH-L1* allele investigated in this study, and their respective genotypes **206**

Table 19 – Table displaying a summary of the statistical analysis (from x480 DNA array) for the delayed recall section of Wechsler logical memory cognitive function test scores in relation to each *UCH-L1* allele investigated in this study, and their respective genotypes **207**

Table 20 – Table displaying a summary of the statistical analysis (from x480 DNA array) for the residuals of a regression of AH4 Test Scores on Mill Hill cognitive function test scores, in relation to each *UCH-L1* allele investigated in this study, and their respective genotypes**.** **208**

DECLARATION OF AUTHORSHIP

I, ……., [please print name]

declare that the thesis entitled [enter title]

…….....

…….....

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done wholly or mainly while in candidature for a research degree at this University;
- where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- none of this work has been published before submission.

Signed: ……

Date:……

Acknowledgements

Primarily, I would like to thank the Parkinson's Disease Society for funding this research; without which this work would not have been possible.

I would also like to thank my two main supervisors during the course of this study - Dr Shu Ye & Dr Beatrice Zhang - whose constant friendly support and guidance have allowed me to reach the high standards that I have attained.

Professor Ian Day, Dr John Holloway, Dr Eve Pearce, Tricia Briggs, Kelly Wilkinson and Ross Laxton (Department of Human Genetics (Southampton University) – past and present) all deserve a special mention for their continual interest and willingness to help me throughout my research project.

Finally, appreciation indeed, to my friends and family for their unyielding support, particularly my parents for providing me with this opportunity.

Abbreviations

- A - adenine
- ABI - Applied Biosystems
- AchEI – cholinesterase inhibitors
- AMC - amido-4-methylcoumarin
- AMP - adenosine monophosphate
- ANOVA - Analysis of Variance
- ANT HBS - antennapaedia homeobox binding site
- APP - amyloid precursor protein
- APS - ammonium persulphate solution
- AS – anti-sense
- ATP – adenosine triphosphate
- βAPP - amyloid precursor protein
- BAG-1 - bcl-2-asscociated athanogene-1
- Bard1 - Brca-associated RING domain 1
- Bp – base pairs
- Brca1 - breast-cancer susceptibility gene 1
- BSA - bovine serum albumin
- C - cytosine
- CIAP - Calf Intestinal Alkaline Phosphatase
- CNS – central nervous system
- CO_2 – carbon dioxide
- CP – core particle
- CSPα - cysteine-string protein α
- DAT - dopamine transporter
- dATP - 2'-deoxyadenosine 5'-triphosphate
- dCTP – deoxycytosine triphosphate
- ddTTP - dideoxynucleotide dideoxythymidine triphosphate
- dGTP – deoxyguanine triphosphate

- dHPLC – denaturing high performance liquid chromatography
- DLR™ - Dual-Luciferase Reporter
- DMSO - 1Dimethyl sulfoxide
- DNA - deoxyribonucleic acid
- DNTPs - deoxynucleosides
- DOPAC - 3, 4-dihydroxyphenylacetate
- DTTP - deoxythymidine triphosphate
- DUBs - deubiquitinating enzymes
- EDTA - ethylenediaminetetraacetic acid disodium salt
- EGFR - epidermal growth factor receptor
- ER - endoplasmic reticulum
- ESCRT - endosomal sorting complex required for transport
- EXO1 - exonuclease 1
- FGF - fibroblast growth factor
- G - guanine
- GABA - γ-amino butyric acid
- Gad - gracile axonal dystrophic
- GRE - glucocorticoid response element
- H-Page - horizontal polyacrylamide
- HAUSP - herpesvirus-associated ubiquitin-specific protease
- Hbp - Hrs-binding protein
- HECT - homologous to E6-associated protein c-terminus
- HGF - hepatocyte growth factor
- HPLC – high performance liquid chromatography
- HSTF - heat shock transcription factor
- HVA - homovanillic acid
- Hz - hertz
- Inr - initiator
- JAMM - JAB1/ MPN/ Mov34 metalloenzyme
- Kb - kilobase

- LB - Luria-Bertoni
- LD - Linkage Disequilibrium
- MADGE - Microplate Array Diagonal Gel Electrophoresis
- MBF - metal binding factor
- Mda - Megadalton
- MDM2 - murine double minute clone 2 oncoprotein
- MEF - mouse embryonic fibroblast
- $MgCl_2$ – magnesium chloride
- MJD - Machado-Joseph disease
- MPTP - 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
- MRI - magnetic resonance imaging
- MRNA – messenger ribonucleic acid
- mV - millivolts
- MVB - multi-vesicular bodies
- *n* – number
- ns – not significant
- NEB – New England Biolabs
- NF-kB - nuclear factor-kappa B
- OR – odds ratio
- OUT - ovarian tumour
- p - probability
- Pael-R - parkin-associated endothelin receptor-like receptor
- PCNA - proliferating cell nuclear antigen
- PCR – polymerase chain reaction
- PD – Parkinson's disease
- PDGFR - platelet derived growth factor receptor
- PGP - protein gene product
- PIRA-PCR - primer-introduced restriction analysis
- PSN - perfectly conserved 12bp sequence
- RC - regulatory complex

- REMSBD - rapid eye movement sleep behaviour disorder
- RFLP – Restriction Fragment Length Polymorphism
- RING - really interesting new gene
- RNA - ribonucleic acid
- RTK - receptor tyrosine kinases
- S - sense
- S.D. – standard deviation
- SANS - small-angle neutron scattering
- SAP - shrimp alkaline phosphatase
- SDEV - standard deviation
- SEM - standard error of mean
- SNP - single nucleotide polymorphism
- T - thymine
- TBP - TATA box binding protein
- TE - transformation efficiency
- TEMED - N, N, N', N'-Tetramethyl-1-, 2-diaminomethane
- TF - transcription factors
- TFIID - transcription factor D complex
- TH - tyrosine hydroxylase
- TIC - transcription initiation complex
- UBA - ubiquitin-associated
- UBL - ubiquitin-like
- UBMC - ubiquitin-7-amido-4-methycoumarin
- UBPs - Ubiquitin-Specific Proteases
- UCH-L1 - ubiquitin carboxyl-terminal hydrolase-L1
- UCH-L2 - ubiquitin carboxyl-terminal hydrolase-L2
- UCH-L3 - ubiquitin carboxyl-terminal hydrolase-L3
- UCH-L4 - ubiquitin carboxyl-terminal hydrolase-L4
- UCH-L5 - ubiquitin carboxyl-terminal hydrolase-L5
- UCHs - ubiquitin carboxyl-terminal hydrolases

- UCS – ubiquitin-conjugating system
- UEV - ubiquitin E2 variant
- UFD2 - ubiquitin fusion degradation model 2
- UIM - ubiquitin-interacting motif
- UPS – ubiquitin-proteasome system
- URE - upstream regulatory elements
- USPs - Ubiquitin-Specific Proteases
- UTR – untranslated region
- UV – ultra violet
- VNTRs - variable number of tandem repeats
- Wt – wild type