Version

This is the Accepted Manuscript version of the following article. This version is defined in the NISO recommended practice RP-8-2008 http://www.niso.org/publications/rp/

Suggested Reference


Copyright

Items in ResearchSpace are protected by copyright, with all rights reserved, unless otherwise indicated. Previously published items are made available in accordance with the copyright policy of the publisher.

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License.

For more information, see General copyright, Publisher copyright, SHERPA/RoMEO.
Symptom heterogeneity in Huntington’s disease correlates with neuronal degeneration in the cerebral cortex

Nasim F. Mehrabi, PhD,1,2 Henry J. Waldvogel, PhD,1,2 Lynette J. Tippett, PhD,1,3 Virginia M. Hogg, MD,1,3 Beth J. Synek, MD1,4, Richard L. M. Faull, MBCHB, PhD, DSc1,2

1 Centre for Brain Research, University of Auckland, Auckland, New Zealand
2 Department of Anatomy and Medical Imaging, University of Auckland, Auckland, New Zealand
3 Department of Psychology, University of Auckland, Auckland, New Zealand
4 Forensic Pathology, Auckland City Hospital, Auckland, New Zealand

Corresponding author: Professor Richard L. M. Faull
Corresponding author’s address: Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand.
Corresponding author’s phone and fax: Tel: +649 923 6708; Fax: +649 373 7484
Corresponding author’s E-mail: rlm.faull@auckland.ac.]

Running title: Cortical interneuron loss in HD

Financial Disclosure/Conflict of Interest concerning the research related to the manuscript:
The authors declare no competing financial interests, and no conflict of interest concerning the research presented in the manuscript.
Abstract

Background: Huntington’s disease (HD) is characterized by variable symptoms and neuropathology of the basal ganglia and cortex. Previously, we have shown that the pattern of pyramidal cell loss in 8 different cortical regions correlates with the phenotypic variability in HD. In the primary motor and anterior cingulate cortices, the pattern of interneuron degeneration correlates with pyramidal cell death and variable HD symptom profiles.

Objectives: This study aimed to examine the pattern of interneuron degeneration in 3 further regions of the HD cortex (primary sensory, superior frontal, superior parietal cortices) to determine whether HD neuropathogenesis was characterised by a general fundamental pattern of cortical interneuron loss, and explore the relationship between cortical interneuron loss with previously determined pyramidal cell loss and clinical heterogeneity.

Methods: Stereological counting was used to quantify 3 sub-populations of calcium-binding protein containing interneurons in 3 cortical human brain regions of 14 HD and 13 control cases as used in our previous studies (1, 2). The HD cases were grouped according to their predominant symptom profile (“motor”, “mood”, “mixed”).

Results: The present results demonstrated a heterogeneous loss of interneurons across the 3 cortical regions which, when compared with our previous studies, mirrored the pattern of pyramidal cell loss in the same cortical areas. Most interestingly, the pattern of neuronal loss in these regions correlated with the variable HD symptom profiles.

Conclusion: The overall findings in our present and previous cortical studies establish a clear correlative pattern of variable cortical neuronal degeneration in HD pathogenesis, which mirrors the heterogeneity of HD symptom phenotypes.

Key words: Huntington’s Disease, Cerebral Cortex, Interneurons, Pyramidal cells, Pathology, Variable Symptoms.
Introduction

Huntington’s disease (HD) is a progressive neurodegenerative disorder characterised by motor disturbances, cognitive loss, and psychiatric manifestations (3, 4). HD is caused by an expansion of CAG trinucleotide repeats in the IT15 (Interesting Transcript 15) gene on chromosome 4, which encodes a mutant protein called huntingtin (5). The exact mechanism through which the mutant huntingtin causes dysfunction and degeneration of neurons is not entirely understood. However, abnormal depositions of huntingtin fragments (huntingtin aggregates) in the nuclei and cytoplasm of neurons have been suggested to initiate a pathogenic cascade leading to neuronal death throughout widespread regions of the forebrain, especially the basal ganglia and the cerebral cortex (6, 7).

In the basal ganglia, the progressive loss of medium spiny GABAergic projection neurons of the striatum, with the slow atrophy of other associated nuclei were considered as the main neuropathological hallmark of Huntington’s disease (8, 9). However, it is now well established that the HD symptoms and brain dysfunction result from major neurodegeneration in both the cerebral cortex and basal ganglia (10-14). The cerebral cortex is closely interconnected with the striatum via the direct glutamatergic corticostriatal projections, and via the output striato-pallido-thalamo-cortical projection system. Thus, the degeneration in both the cortex and striatum may be intimately linked.

Despite the single-gene aetiology, individuals with HD can show clear and considerable phenotypic variability. Previous studies from our laboratory have established that variation in symptom profile correlates very closely with the pattern of cortical and striatal degeneration (1, 2, 13, 14). The cerebral cortex comprises of two types of neurons: the efferent glutamatergic pyramidal projection neurons which project in part to the striatum, and the GABAergic interneurons which modulate the output of the pyramidal neurons. Our studies, to date, have shown that the heterogeneous loss of pyramidal cells across 8 regions of the cortex correlates with variable symptom profiles in HD (1, 14). In 2 of these cortical regions so far investigated (the primary motor cortex and the anterior cingulate gyrus), interneuronal degeneration occurs in concert with pyramidal cell death, and that this pattern of cell death correlates with HD symptom profile (2). That is, interneuron and pyramidal cell loss occur in: i) the primary motor cortex in HD cases dominated with motor
symptoms; and ii) the cingulate cortex in HD cases dominated by mood symptoms. The present study advances this overview of cortical cell degeneration in HD by specifically examining whether the pattern of interneuronal degeneration in 3 further major functionally diverse regions of the cortex (primary sensory cortex, superior frontal cortex, superior parietal cortex) correlates with our previous findings on pyramidal cell studies in the same cortical regions of the same HD cases (1, 2, 14). The overall aim of this study is to determine what role interneurons may play in HD, and whether there is a fundamental principle link between the variable pattern of interneuron and pyramidal cell death across the cortex which correlates with symptom profile in HD. That is, whether cortical interneuron loss always accompanies pyramidal cell loss and striatal pathological degeneration, and whether the variable widespread cortical pathogenesis correlates with HD symptom heterogeneity in the human brain.
Materials and Methods

The brain tissue was obtained with the full consent of all families, and the research protocols were approved by the University of Auckland Human Participants Ethics Committee (2008/279).

Processing of human brain tissue (Figure 1)

In this study, cortical tissue blocks from the right hemisphere of a total of 14 Huntington’s disease (HD) cases, and 13 neurologically normal control brains were used. The HD and control cases were matched as closely as possible for age and post-mortem interval (PMI), age and gender (see Table 1). The HD and control cases used in this study were the same cases previously used in the determination of cortical pyramidal cell loss (1, 2, 14).

The brains were perfused according to previously published protocols (15), and blocks from the superior parietal cortex (Brodmann area 7, Figure 1A), primary sensory cortex (Brodmann area 3, Figure 1B), and superior frontal cortex (Brodmann area 6, Figure 1C) were carefully dissected as described previously (1, 2).

Clinical Assessments

Clinical data to assess symptom profiles for each case was collected retrospectively from clinical records and from family members of the 14 cases who had died with HD, as detailed previously (11, 12). The clinical data for each HD case was reviewed independently by two neuropsychologists (L.J.T and V.M.H) experienced with HD symptom assessments, and cases were classified using the following definitions:¹

**HD Motor**: Individuals that displayed a clear movement disorder with no significant presence of mood symptoms during the symptomatic phase of the disease.

**HD Mood**: Individuals that demonstrated predominant mood disturbances during the symptomatic phase of the disease. However, some degree of motor symptoms was also present, but these were either very mild or only emerged during the very late stages of the disease.

¹ Due to the retrospective nature of data collection, valid assessment of cognitive symptoms was not possible.
**HD Mixed (motor and mood):** Individuals that clearly demonstrated significant levels of both motor and mood symptoms during a large part of the symptomatic duration of the disease.

**Immunohistochemistry and stereology**

The entire fixed tissue block from each of the 3 cortical regions were cut in the coronal plane into 50 µm serial sections. Standard immunohistochemical procedures were performed to identify the 3 main subpopulations of cortical interneurons as detailed previously (14, 15), using rabbit anti-calbindin-D28k (CB) 1:5,000 (gift from Dr Emson, Babraham institute, Cambridge, UK), rabbit anti-calretinin (CR) 1:5,000 (Swant, Marly, Switzerland), and mouse anti-parvalbumin (PV) 1:15,000 (Swant). To obtain an unbiased estimate of the total number of interneurons in the 3 cortical regions of interest, double-blind unbiased stereological methods were performed as detailed previously (1, 2). To quantify any changes in the average cell soma size of interneurons, the isotropic nucleator was used to estimate the cross-sectional area and volume of every 8th cell counted for all 3 sub-populations of interneurons in the 3 cortical regions of interest (16).

**Statistical analysis**

Average changes in total interneuron number and interneuron soma volume in HD cases compared to control cases were assessed using the Student’s t-test. Multiple comparisons between subgroups (classified according to grade or symptoms) were carried out using one-way analysis of variance (ANOVA), followed by Tukey’s HSD post-hoc test. Also, HD subgroups were compared against the control group using one-way ANOVA, followed by Dunnett’s post-hoc test. P-values < 0.05 were considered statistically significant (statistical significance expressed as *P < 0.05, **P < 0.01, ***P < 0.001).
Results

Overall loss of interneurons in three diverse cortical regions in Huntington’s disease (Figure 2)

When comparing the pooled results for 14 HD cases with 13 control cases (Figure 2), in the primary sensory cortex, there was no overall significant loss in any of the three main interneuron populations (CB, PV, CR) (Figure 2A). However, loss of specific interneuron types was found when the HD cases were further sub-grouped according to the predominant HD symptomatology, as detailed below (Figure 3). By contrast, in the superior frontal cortex, the population of all calcium-binding protein containing interneurons (CB, PV, CR) was significantly reduced in 14 HD cases compared to 13 controls (Figure 2B). In the superior parietal cortex, there were 21% fewer PV+ interneurons ($P <0.05$) in the 14 HD cases, with no significant changes in the population of CB+ and CR+ interneurons (Figure 2C). The pattern of interneuron loss in the superior frontal and superior parietal cortices in different HD symptom sub-groups is also detailed below (Figure 3).

For all stereological analyses of the HD and control cases, the average coefficient of error (CE) for the total number of interneurons (N) for each marker was less than 0.10. This indicates that the observed variability is due to a true difference in the number of interneurons between cases rather than a lack of precision in the stereological counting methods employed (Gundersen et al., 1999, West, 2002).

The relationship between the total number of interneurons in different cortical regions with respect to CAG repeat length, post-mortem interval (PMI), and age at death was also investigated by calculating Pearson’s correlations. There were no significant correlations between total number of interneurons in the 3 cortical regions with any of these variables.
**Relationship between differential cortical interneuron loss and cell soma volume changes with clinical symptoms (Figure 3, Supplementary Figure 1)**

Next, we investigated whether the differential pattern of interneuron loss in the 3 cortical regions was associated with the variable symptom profiles of the HD cases. To achieve this, the average total interneuron loss in each of the three symptom groups (motor, mood, mixed) was compared to the control group. Prior to this analysis, we confirmed that there were no statistical differences between the HD symptom groups and their average pathological grade (Kruskal-Wallis Anova analysis, \( P < 0.89 \)). Hence the average pathological grade did not confound the results for HD symptoms. Also, post-hoc analysis with Tukey’s test revealed no significant changes in the total number (Figure 3) and average soma volume (Supplementary Figure 1) of interneurons between the three symptom subgroups.

**Primary sensory cortex (Figure 3A):**

The HD motor cases showed a significant 67% loss of CB interneurons in the primary sensory cortex compared to the control group (\( P=0.04 \)), with no significant loss of CR and PV interneurons (Figure 3A-j). In contrast, in the HD mood and HD mixed symptom sub-groups, no significant changes were found in the population of interneurons (CB, PV, CR) in the primary sensory cortex (Figure 3A-j). The stereological cell soma volume quantification results showed no significant changes in the average soma volume of interneurons (CB, PV, or CR) in the primary sensory cortex, when comparing HD symptom sub-groups with controls (supplementary Figure 1A).

**Superior frontal cortex (Figure 3B):**

In the superior frontal cortex, the HD motor symptom group showed a significant loss of CB (44%, \( P=0.03 \)), PV (64%, \( P=0.00 \)), and CR (48%, \( P=0.001 \)) interneurons (Figure 3B) when compared to the controls. In the HD mood cases, a significant 43% loss of CR interneurons (\( P=0.01 \)) was observed, with no significant changes in the population of the other interneuron sub-types (CB, PV) (Figure 3B-j). The results from HD mixed cases revealed a significant loss of PV (66%, \( P=0.001 \)) and CR (44%, \( P=0.01 \)) interneurons, with no change in the total number of CB interneurons (Figure 3B-j). Furthermore, the stereological cell soma volume quantification results reflected major changes by all interneurons. These results showed a significant reduction in the soma volume of the surviving interneurons when different symptom sub-groups were compared with the control cohort. In particular, in HD motor cases, the average soma volume of CB and PV interneurons was reduced significantly by 21% (\( P=0.04 \)) and 34% (\( P=0.03 \)), respectively. In the HD mixed cases, the average soma volume of PV interneurons was reduced by 34% (\( P=0.03 \)) (supplementary Figure 1B).
**Superior parietal cortex (Figure 3C):**

In contrast, in the superior parietal cortex there were no significant changes in the 3 main interneuron populations, when any of the 3 HD symptom groups (motor, mood, mixed) were compared with the controls (Figure 3 C-j). Also, no significant changes were observed in the cell soma volume of CB, PV, or CR interneurons in the superior parietal cortex in any of the HD symptom sub-groups compared to the control cases (supplementary Figure 1C).

**Relationship between the pattern of cell loss in the cerebral cortex and striatal neuropathological grades**

In order to investigate the relationship between striatal neuropathology and cortical interneuron loss, the HD stereological counting results were compared with the Vonsattel striatal neuropathological grades (grade 1, and grade 2-3). No clear statistical association was evident between the total number of interneurons in the 3 cortical regions studied and the Vonsattel striatal neuropathological grades.
Discussion

The results of this study show that specific subclasses of GABAergic interneurons degenerate according to the cortical area and predominant HD symptomatology. The relationship demonstrated between the degeneration of interneurons and symptom profiles provides a new perspective on the cellular basis of clinical heterogeneity in HD.

Relationship between differential cell loss in the cortex and clinical symptoms

A great deal of variability can be observed in the type of symptoms that individual HD patients exhibit during the symptomatic phase of the disease. Some patients show major motor dysfunction at clinical onset with minimum changes in mood and cognition, others show major mood and cognitive-related changes with minimum motor dysfunction, while many show a variable mix of both motor and mood/cognition symptoms (17, 18).

Major alterations in GABA neurotransmission and the inhibitory function of GABAergic interneurons have been implicated in the striatum and cortex of HD (2, 19-21). Therefore, the present study was designed to investigate the cellular changes of the GABAergic interneurons (CB, PV, CR) in three different areas of the cortex, and to explore the relationship between the pattern of cell loss in these areas and symptom variability in HD. Our results show a heterogeneous pattern of GABAergic interneuron loss in three functionally different cortical regions which is related to variable symptom profiles in HD.

This is the first study to show that in the primary sensory cortex there was a selective major loss of calbindin-D28k (CB) interneurons (67% loss) in HD cases with a predominant motor disorder (Figure 3A), with no significant loss of interneurons in HD cases with predominantly mood symptoms. Interestingly, a previous study on the same cases from our laboratory has demonstrated a similar association between motor symptoms in HD and loss of CB interneurons (57%) in the primary motor cortex, which is the primary cortical area involved in the control of movement (2). The similar changes observed in the primary motor and primary sensory cortex underpin the close association between the function of these two cortical regions, which is especially emphasised by the fact that the sensory and motor corticostriate projections completely overlap in the striatum in a corresponding somatotopic
fashion (22). By contrast, our previous study also showed a significant loss of all three subclasses of calcium-binding protein containing interneurons in the anterior cingulate cortex (a limbic area that is involved in mood and behaviour) in HD mood cases, but no loss of interneurons in HD motor cases (2).

Furthermore, the present study showed that in the superior frontal cortex, also known as the supplementary motor cortex, there was a significant loss of all three subclasses of calcium-binding protein containing interneurons (CB: 44%, PV: 64%, CR: 48%) in HD cases with predominantly motor symptoms, and loss of CR (43%) interneurons in HD mood cases (Figure 3B). The mixed cases were intermediary between the motor and mood cases, with significant loss of PV (66%) and CR (44%) interneurons. The superior frontal cortex is an association area that consists of cytoarchitectonically transitional areas (Brodmann areas 6, 8, and 9) which are involved in integrating motor, mood and cognitive information based on their connectivity with the relevant sensory, motor and limbic areas of the brain (23, 24). Brodmann area 8 (BA8), which has been used in this study, has been shown to be connected with the primary motor cortex and premotor area (BA6), and therefore is thought to be involved in the initiation and control of movement, especially eye movements (25-29). However, human neuroimaging studies have shown that the superior frontal cortex is active not only during demanding motor tasks, but also during motor-related cognitive tasks i.e. learning associated with movement (30, 31). Also, neuroanatomical evidence has revealed that the caudal parts of the superior frontal cortex have a close relationship with motor cortices; whereas the rostral parts of this area have a close connectional relationship with the prefrontal cortex (32, 33). Based on these studies and our results, we suggest that this area of the cortex is also involved in the cognitive aspects of movement control.

The superior parietal cortex is another association area that is known to be affected in HD, and therefore was investigated in the present study (10, 34). Our results showed a significant 21% loss in the population of PV+ interneurons in the superior parietal cortex, when 14 HD cases were compared with 13 controls. However, when the HD cases were grouped according to their predominant symptom profile, no significant interneuron loss was observed in the superior parietal cortex in either motor or mood cases (Figure 3C). Collectively, these data show that loss of interneurons in different cortical areas is variable,
but is associated with motor or mood symptoms in HD. These findings are generally consistent with our knowledge of the functionality of the cortical areas investigated.

Cortical interneurons are critical for modulating the excitability of cortical pyramidal neurons. Enhanced cortical excitability, which may be related to loss of inhibition due to reduced GABAergic interneuronal inputs, has been observed in early stages of the disease, i.e. seizures in juvenile HD patients (3), and in HD mice (35). In turn, enhanced cortical excitability, via the glutamatergic corticostriatal projection system, in HD has been postulated to promote excitotoxicity in striatal medium spiny neurons and contribute to their degeneration (36). The dysfunction of cortical interneurons in HD has been documented previously in mouse models of HD (21, 37, 38). Furthermore, a recent transcranial magnetic stimulation (TMS) study has shown a significant reduction in cortical inhibition in the primary motor cortex in pre-symptomatic and symptomatic HD patients (39). This could be indicative of early involvement of cortical GABAergic interneurons in HD pathology. Taken together, these observations suggest that loss of inhibition by GABAergic interneurons plays a crucial role in pyramidal cell dysfunction, symptom variability, and pathogenesis of HD.

**Relationship between the pattern of interneuron loss and pyramidal neurons**

The present study extends our previous findings (1, 14), which demonstrate a surprisingly close association between the loss of pyramidal neurons in different cortical regions and the dominant motor or mood symptom profiles. In particular, our results from previous and present stereological studies on the same HD cases show a close relationship between the pattern of both interneuron loss and pyramidal cell loss with variable HD symptoms, as summarised in Figure 4. For example, in the cortical areas which are known to be involved in control of movement (the primary motor cortex, the primary sensory cortex, and the superior frontal cortex) there was a significant loss of both interneurons and pyramidal cells in the HD motor cases, but not in HD mood cases (Figure 4). In contrast, in the limbic anterior cingulate cortex, known to be involved in mood and behaviour, there was a significant loss of all calcium-binding protein containing interneurons and pyramidal cells in the HD mood cases, but not in the HD motor cases (Figure 4).
It is highly likely that the HTT gene mutation in HD causes a linked disruption of cellular function in both cortical interneurons and pyramidal cells simultaneously in the same cortical regions, which might lead to a coordinated pattern of cortical degeneration. Furthermore, the association between cortical interneuron loss and pyramidal cell loss is indicative of the important role of interneuron-pyramidal cell interaction in the neuropathology of HD. There is strong evidence to support this hypothesis from Cre/LoxP conditional HD mice, where the motor deficits and cortical neuropathology was only observed when the mutant huntingtin was expressed in multiple neuronal types (pyramidal cells and interneurons), but not when it was only restricted to the cortical pyramidal cells (21). In addition, the conditional HD mice showed a reduction in the cortical GABAergic inhibitory input onto cortical pyramidal neurons. This is thought to play a major role in the dysfunction of cortical pyramidal neurons. Loss of interneuron inhibitory input and interneuron/pyramidal synaptopathy has also been detected in BACHD transgenic mice (37). Similar studies using other HD mouse models (R6/2, YAC128, CAG140 knock-in) have shown alterations in the patterns of inhibitory postsynaptic potentials and the discharge frequency of GABAergic interneurons in the somatosensory cortex (38). These results suggest that increased cortical pyramidal cell excitability, as a result of reduced inhibition by GABAergic interneurons, could be a major contributing factor in the neuropathogenesis of HD.

Based on these animal studies and our findings using HD human post-mortem tissue, we can suggest that the loss of GABAergic interneurons in different cortical regions leads to disinhibition of cortical pyramidal cells, and thereby results in pyramidal cell dysfunction. The dysfunction of pyramidal cells leads to disinhibition of the cortico-striatal projections, which in turn could contribute to excitotoxic medium striatal neuron dysfunction and death. Therefore, by considering our collective results in the human cerebral cortex (1, 2, 14) and striatum (40), we can suggest that alterations in interneuron/pyramidal cell interactions play a major role in the pattern of degeneration in these areas of the brain in HD. Variable specific loss of cortical interneurons and pyramidal neurons in the corresponding functional regions of the cortex, combined with the variable striatal compartmental degeneration seen in HD brain results in a coordinated cortico-striatal pathogenesis which reflects the heterogeneity of HD symptoms (Figure 4).
**Conclusion**

Our results from the present and previous studies have demonstrated a significant variable, but linked, loss of both GABAergic interneurons and pyramidal cells across different cortical regions in symptomatically different HD cases. These studies, together with our previous studies on the HD striatum showing variable striosome/matrix compartmental degeneration which links to symptom profile (13), provide further support for the notion that the variable cortical degeneration in concert with the variable striatal pathology contribute significantly to the neuropathology and symptomatology of HD.
Figure 1: Diagram showing the lateral view of the location of blocks that were used for stereological analysis: (A) superior frontal cortex (SF); (B) primary sensory cortex (S); and (C) superior parietal cortex (SP).
Figure 2: Graphs showing the average total number of each interneuron sub-type (CB, PV, CR), as a percentage of control total interneuron number, when comparing 14 HD cases with 13 controls in the: (A) primary sensory cortex; (B) superior frontal cortex; and (C) superior parietal cortex.
Figure 3: A, B, C (a-i): representative photomicrographs illustrating morphological changes of interneurons in the three cortical regions studied. Scale bar: 50 µm.

A, B, C (j): Graphs showing the total number of interneurons, as percentage of control, in different HD symptom sub-groups in the three cortical regions studied.
Figure 4: This diagram summarises the average loss of interneurons and pyramidal cells in HD cases, classified as motor or mood cases, in five different cortical regions. Note that, the loss of interneurons was always accompanied by the loss of pyramidal cells, except in the SF mood cases.

GPe, globus pallidus external segment; GPi, globus pallidus internal segment; STN, subthalamic nucleus; VS, ventral striatum; VP, ventral pallidum; VA/VL, ventral anterior/ventral lateral nuclei; MD, mediodorsal nucleus; AM, anteriomedial nucleus.
Supplementary Figure 1 (A-C): Graphs showing the mean soma volume of interneurons, as percentage of control, in different HD symptom sub-groups in the three cortical regions studied.
Acknowledgements:

We express our appreciation to all the donor Huntington’s families in New Zealand, who through their generosity have enabled this study to be undertaken. We also thank the Huntington’s Disease Association for their generous and invaluable assistance, and the Neurological Foundation of New Zealand Human Brain Bank. This work was supported by the Health Research Council of New Zealand (11-802), Neurological Foundation of New Zealand (1102-PG), as well as funding from the Matthew Oswin Memorial Trust and the TM Pacey Trust. Further support was from The University of Auckland Doctoral Scholarship to Nasim F Mehrabi.

Authors’ roles:

**Nasim F Mehrabi:** Experimental work, data acquisition (stereology), data analysis; drafting the manuscript and subsequent revisions.

**Lynette J. Tippett and Virginia M. Hogg:** Neuropsychological clinical assessments, statistical analysis, manuscript revisions.

**Beth J. Synek:** Neuropathological grading of HD cases and neuropathological examination of control cases.

**Richard L. M. Faull and Henry J. Waldvogel:** conception, design and overall supervision of the project, revision of manuscript drafts.
Funding sources for study: This research was conducted with the support of the Health Research Council of New Zealand (HRC), Neurological Foundation of New Zealand, as well as funding from the Matthew Oswin Memorial Trust, the TM Pacey Trust, and the Freemasons of New Zealand. Further support was from The University of Auckland Doctoral Scholarship to Nasim Mehrabi.

Financial disclosure of all authors: Nothing to report.
References


