Identification of markers of disease onset and progression in Huntington’s Disease

Nayana Lahiri
“There is no gene for the Human Spirit.”

GATTACA. Dir. Andrew Niccol. Columbia Pictures. 1997. Film
DECLARATION OF AUTHORSHIP AND ORIGINALITY

I, Nayana Lahiri, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.
ACKNOWLEDGEMENTS

Firstly, I would like to thank the patients, carers and relatives who gave up their time to take part in the research studies presented in this thesis. Their stories as well as their hope for and dedication to the pursuit of a cure for Huntington’s disease have moved me and motivated me in equal measure. Thanks, in particular, to Charles Sabine for allowing me to use his photographs to illustrate TRACK-HD assessments.

Sarah Tabrizi is an inspirational researcher and clinician. Her unending energy and enthusiasm for HD research is truly infectious and without her supervision, faith and support this thesis would not have been completed. Thanks also to Simon Mead and Nicholas Wood, my secondary supervisors. The Tabrizi research group at UCL, Institute of Neurology have provided intellectual stimulation, practical support and above all friendship. In particular, I would like to thank; Ed Wild for leaving large shoes for me to fill; the other members of TRACK-HD London site team, Joy Read and Miranda Say, who were the perfect fit. Thanks also to Gail Owen, the TRACK-HD co-investigators, Ulrike Träger, Ralph Andre, Jon Beck and Gary Adamson for their practical support. I would like to thank our collaborators; Paola Piccini and Marios Politis, Maria Björkqvist, Anna Magnusson, Peter Holmans, Lesley Jones and Denise Harold for their advice and support. I would like to give special thanks to the European Huntington’s Disease Network for allowing me access to data and samples collected as part of the REGISTRY study. It has been a privilege to become part of the global HD community; a research community who truly thrive on collaboration and open discourse; a strategy that has lead tantalisingly close to the prospect of treatments for HD.

I am extremely grateful to the organisation that has funded this research. The Cure Huntington’s Disease Initiative (CHDI), Inc. who funded both the experimental work and my research post.

Finally, I would like to thank my long-suffering family, in particular my husband, James, for their constant encouragement, enormous patience and love.
ABSTRACT

Huntington’s Disease is a progressive, adult onset, neurodegenerative disease. It is inherited in an autosomal dominant fashion and is caused by a trinucleotide repeat expansion in huntingtin, which encodes the protein huntingtin. The length of the expanded trinucleotide repeats accounts for some, but not all of the age of onset of the condition. Despite the monogenic basis of Huntington’s disease, the clinical features display marked variability within families and between those who carry the same length expansion. The variability in age of onset and in clinical features is likely to be due to a number of environmental and other genetic factors. Identification of these factors may lead to novel therapeutic approaches.

A number of potential disease modifying agents have been elucidated and are approaching clinical trials. Robust ‘biomarkers’ of disease onset and progression are essential for developing a framework for future clinical trials that have the ability to judge the efficacy of any therapeutic intervention. In this thesis I will present work arising from TRACK-HD, an international observational biomarker study of Huntington’s Disease which has identified a panel of biomarkers for use in future clinical trials.

A number of subs-studies arising from TRACK-HD are also presented here. Firstly, a study investigating the use of Positron Emission Tomography and peripheral immune markers as biomarkers of disease progression followed by a candidate genetic modifier study focusing on immune pathways as genetic modifiers of age of onset in Huntington’s Disease.
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<tr>
<td>3T</td>
<td>3 Tesla</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ASO</td>
<td>Antisense oligonucleotide</td>
</tr>
<tr>
<td>BCA</td>
<td>Bias Corrected and Accelerated</td>
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<tr>
<td>BDNF</td>
<td>Brain Derived Neurotropic factor</td>
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<tr>
<td>BDI</td>
<td>Beck Depression Inventory</td>
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<tr>
<td>BSI</td>
<td>Boundary shift integral</td>
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<tr>
<td>BPND</td>
<td>Parametric Binding potential</td>
</tr>
<tr>
<td>BRAINS</td>
<td>Brain Research: Analysis of Images, Networks, and Systems</td>
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<tr>
<td>CAG</td>
<td>Cytosine-adenine-guanine</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>eCRF</td>
<td>Electronic Clinical Record Form</td>
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<td>EHDN</td>
<td>European Huntington’s Disease Network</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ES</td>
<td>Effect Size</td>
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<td>FA</td>
<td>Flip Angle</td>
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<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<td>FOV</td>
<td>Field of View</td>
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<tr>
<td>GLS</td>
<td>Generalised Least Squares</td>
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<td>GM</td>
<td>Grey Matter</td>
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<td>GWAS</td>
<td>Genomewide Association Study</td>
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<td>HADS</td>
<td>Hospital Anxiety and Depression Scale</td>
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<td>HAP-1</td>
<td>Huntingtin associated protein-1</td>
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<td>HD</td>
<td>Huntington’s Disease</td>
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<tr>
<td>HD1</td>
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<tr>
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<td>HD stage 2</td>
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<td>HDA</td>
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<td>HDAC</td>
<td>Histone deacetylase</td>
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<td>HTT</td>
<td>Huntingtin gene</td>
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<td>Htt</td>
<td>Huntingtin protein</td>
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<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>ICV</td>
<td>Intracranial Volume</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>iPSC</td>
<td>induced Pluripotent Stem Cells</td>
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<td>JHD</td>
<td>Juvenile Huntington’s Disease</td>
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<tr>
<td>KMO</td>
<td>Kynurenine 3-monoxygenase</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>mHtt</td>
<td>Mutant Huntingtin protein</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>MPRAGE</td>
<td>Magnetization-prepared rapid gradient echo</td>
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<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
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<tr>
<td>mTOR</td>
<td>mammalian Target Of Rapamycin</td>
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<tr>
<td>NRF1</td>
<td>Nuclear Respiratory Factor 1</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NRS1</td>
<td>Nuclear Respiratory Factor 1</td>
</tr>
<tr>
<td>PBA-s</td>
<td>Problems Based Assessment (short)</td>
</tr>
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<td>PD</td>
<td>Parkinson’s Disease</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PGC-1α</td>
<td>Peroxisome proliferator-activated receptor γ coactivator 1α</td>
</tr>
<tr>
<td>PI</td>
<td>Principle Investigator</td>
</tr>
<tr>
<td>PK</td>
<td>$^{11}$C-(R)-PK11195</td>
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<td>preHD-A</td>
<td>Premanifest HD further from predicted diagnosis</td>
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<td>preHD-B</td>
<td>Premanifest HD nearer to predicted diagnosis</td>
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<tr>
<td>QoL</td>
<td>Quality of Life Index</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SBAC</td>
<td>Scientific and Bioethics Advisory Committee</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<td>SEM</td>
<td>Standard Error of the Mean</td>
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<td>SF-36</td>
<td>Short Form 36</td>
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<td>siRNA</td>
<td>small interfering RNA</td>
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<td>Sirtuin 1</td>
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<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<tr>
<td>SPM</td>
<td>Stastical Parametric Mapping</td>
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<tr>
<td>TAC</td>
<td>Time Activity Curves</td>
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<td>TE</td>
<td>Echo Time</td>
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<td>TR</td>
<td>Repitition Time</td>
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<tr>
<td>TAC</td>
<td>Time Activity Curves</td>
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<tr>
<td>TFAM</td>
<td>mitochondrial transcriptional factor A</td>
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<td>TFC</td>
<td>Total Functional Capacity</td>
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1 INTRODUCTION

In my experience, one of the most striking and challenging aspects of working with families with Huntington’s Disease (HD) has been the unpredictability of the disease. Between individuals with the same Cytosine-Adenosine-Guanine (CAG) repeat expansion, within families and even between siblings and twins; there is a huge variability in the stories that we hear; from family history, prodromal symptoms, onset of definite clinical features, symptoms and progression. I have found this remarkable considering that HD is a condition caused by a single aberration in a single gene.

It has been 20 years since the discovery of the genetic mechanism underlying HD. In the intervening period much has been learned about the natural history of the disease and pathogenic mechanisms. Our understanding of HD is not only crucial to the development of treatments that may help HD families but also our ability to carry out predictive genetic testing years before onset of disease means that HD can serve as a model for the study of common pathways of neurodegeneration giving insights into more common neurodegenerative disorders, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). Drug discovery programmes have identified potential disease modifying therapies of HD and so clinical trials, long anticipated by family members, are becoming a reality.

For such trials to be informative and for the dream of disease modifying therapies for HD to become a reality, we still require a better understanding of the variability that exists in HD; to better understand the factors that influence onset and progression of HD.

The main aim of this thesis is to explore the variability in the age of onset and rate of progression in HD with the aim of identifying robust biomarkers of disease onset and progression. This variability has been explored through the multinational prospective observational biomarker study of premanifest and early stage HD; TRACK-HD. I have also further investigated the innate immune system dysfunction that exists in HD to investigate its potential as a biomarker of disease progression using both Positron Emission Tomography (PET) imaging and peripheral immune markers of inflammation.
In addition, I have examined variation in certain genes related to the immune system to investigate them as potential candidate genetic modifiers of age of onset.

This introductory chapter will set out the epidemiology, clinical and pathological features of HD before reviewing the current state of the search for potential genetic modifiers, biomarkers and potential therapeutic interventions and then finally setting out the aims of this thesis.

1.1 Huntington’s Disease

Charles Waters first described hereditary involuntary movements in certain afflicted individuals in New York State in the 1840’s (Waters 1842). However, it was following a lecture and article entitled ‘On Chorea’ by a twenty-two year old medical student, George Sumner Huntington, that the condition became known as Huntington’s disease. (Huntington 1872)

“… is confined to certain and fortunately a few families, and has been transmitted to them, an heirloom from generations away back in the dim past. It is spoken of by those in whose veins the seeds of the disease are known to exist, with a kind of horror, and not at all alluded to except through dire necessity, when it is mentioned as “that disorder”. It is attended generally by all the symptoms of common chorea, only in an aggravated degree, hardly ever manifesting itself until adult or middle life, and then coming on gradually but surely, increasing by degrees, and often occupying years in its development, until the hapless sufferer is but a quivering wreck of his former self”… “There are three marked peculiarities in this disease: 1. Its hereditary nature. 2. A tendency to insanity and suicide. 3. Its manifesting itself as a grave disease only in adult life…”

His description remains largely true today. HD remains a progressive, autosomal dominantly inherited, adult onset, neurodegenerative disorder characterised by a movement disorder, neuropsychiatric symptoms and cognitive decline.
1.1.1 Epidemiology

HD is the third most common inherited neurological disorder in the United Kingdom (UK), after type 1 neurofibromatosis and Charcot-Marie-Tooth Disease (MacMillan and Harper 1991). The prevalence of HD in European populations has previously been estimated to be between 4 and 8 per 100,000 (Harper 1992; Pringsheim, Wiltshire et al. 2012). Lower incidence and prevalence has been reported in Asian studies compared to Europe, North America and Australia (Pringsheim, Wiltshire et al. 2012). Pockets of high prevalence are also known to exist, in particular in Venezuela and Tasmania (Young, Shoulson et al. 1986; Pridmore 1990).

It is generally held that these figures are an underestimate. A number of the studies were carried out before genetic testing for HD was available so it is likely that some patients with HD went undiagnosed or were misdiagnosed. It is also possible that the stigma that has long surrounded HD patients and their families has prevented accurate collection of prevalence data (Rawlins 2010). Aside from these ascertainment biases, increased life expectancy due to improved care of HD patients and an ageing population would also suggest an increase in prevalence.

New UK prevalence studies are on-going following reports that the Huntington’s Disease Association of England and Wales (HDA) cares for over six thousand individuals with symptomatic HD which translates to a prevalence figure of 12.4 per 100,000. The HDA, however, does not provide services in all areas of England and Wales; and there are an unknown number of patients with the disease who have never been referred to the Association. This prevalence estimate of 12.4 per 100,000 of the population is, therefore, also an underestimate but by how much is uncertain. A recent population study in British Columbia, Canada, also suggests a much higher prevalence than previously thought with figures in the region of 14.3 per 100,000 affected with HD (Hayden 2012). Figure 1 illustrates the increasing prevalence of HD.
Prevalence figures do not account for the numbers at risk of developing HD. Due to the dominant inheritance pattern, there is a 50% chance for each child of an affected parent of inheriting the expanded HD allele. As the disease generally remains asymptomatic for many years and only a minority of those at risk undergo predictive testing (Morrison 2010) many more individuals then are counted are at risk of developing the disease and in addition, some mutation carriers remain unaware of their at-risk status due to the presence of intermediate and reduced penetrance alleles in the general population. In a recent, as yet unpublished study from British Columbia, Canada, this ‘at risk’ figure is estimated to be in the region of 1/1000 (Hayden 2012).

Whatever the estimates of prevalence, the impact of HD on families cannot be overestimated. Those at risk; often raised in chaotic households due to the effects of disease and having witnessed the decline of at least one loved one, live their lives with the knowledge that they are at risk of the same condition. Even more agonizing is the grip of HD on the uncounted spouses who not only care for their partners but also are left with the knowledge that their children may face the same devastating future.

Figure 1  Increasing prevalence estimates of HD in Europe/North America/Oceania

Data from Pringsheim, Wiltshire et al, 2012 and adapted to include new prevalence figures from Rawlins, 2010 and Hayden, 2012
1.1.2 Clinical Characteristics

The clinical features of HD are often described as a triad of movement disorder, cognitive dysfunction and neuropsychiatric or behavioural disturbance. The nature and the severity of each of these features can be hugely variable.

In most research studies, the diagnostic confidence score (Table 1), which forms the final part of the Unified Huntington Disease Rating Scale (UHDRS) motor component (1996), is used as the basis for the diagnosis of HD. A trained specialist completes the diagnostic confidence assessment following examination of a patient using the 124-point motor score (see Appendix 1). The final assessment of the UHDRS motor component is the Diagnostic Confidence Score (DCS) as shown in Table 1 with a score of 4 being required for the formal diagnosis of HD.

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (no abnormalities)</td>
<td>0</td>
</tr>
<tr>
<td>Non-specific motor abnormalities (less than 50 % confidence)</td>
<td>1</td>
</tr>
<tr>
<td>Motor abnormalities that may be signs of HD (50 - 89 % confidence)</td>
<td>2</td>
</tr>
<tr>
<td>Motor abnormalities that are likely signs of HD (90 - 98 % confidence)</td>
<td>3</td>
</tr>
<tr>
<td>Motor abnormalities that are unequivocal signs of HD (≥ 99 % confidence)</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1 UHDRS diagnostic confidence score

Therefore, the diagnosis of HD is made solely on the basis of characteristic motor signs in an individual despite the fact that many patients develop cognitive and/or psychiatric symptoms during the prodromal or premanifest period, often many years before any motor signs are seen, and it is generally the non-motor symptoms that have the greatest impact on a patient and their family and contribute most to the patient’s loss of independence.

In a study of 960 patients diagnosed as having undergone ‘motor onset’ only 55.7% were felt to have had motor abnormalities as their earliest disease symptom or sign. Psychiatric, cognitive and multifactorial presentations were almost as common as shown in Figure 2 (Marder, Zhao et al. 2000).
1.1.2.1 Motor Symptoms

Chorea becomes a feature of HD in over 90% of patients. It is defined as a hyperkinetic movement disorder characterised by excessive spontaneous movements that are irregularly timed, randomly distributed and abrupt. Choreic movements in HD are continuously present, cannot be voluntarily suppressed by the patient and worsen during stress (Kremer 2002). While chorea is often the most obvious feature to onlookers, the movements are often not noticed by the patients themselves (Snowden, Craufurd et al. 1998).

As the disease advances, chorea will tend to decline and non-choreic movement disorders; dystonia, rigidity and bradykinesia become more marked. In Juvenile Huntington’s Disease (JHD) and 10% of adult onset cases (known as the Westphal variant) chorea is minimal and a rigid phenotype predominates (Louis, Anderson et al. 2000).

Nonspecific impairment of voluntary motor function is also a common motor feature with impairment of fine motor control presenting as clumsiness in common daily activities (Kremer 2002).
Oculomotor disturbances are amongst the earliest of the motor signs and are present in the vast majority of affected individuals. Delayed initiation of voluntary saccades, slowing of saccades, impaired pursuit with saccadic intrusions and an inability to suppress blinking during saccades are common oculomotor features (Lasker and Zee 1997; Blekher, Johnson et al. 2006).

Gait disturbances can be observed even early in the illness and include particular difficulties with tandem walking. The gait disturbances can be worsened by loss of postural reflexes. Trips and falls can cause significant morbidity as the disease advances.

Most patients display speech abnormalities with initially a mild dysarthria which progresses and in the late stages of the disease mutism is not uncommon. Dysphagia can also occur and is a major cause of morbidity and mortality in later stage HD.

1.1.2.2 Cognitive symptoms

Cognitive and behavioural changes place the greatest burden on HD families and are most highly associated with functional decline. They are a universal feature of HD and frequently emerge early in the clinical course or even several years before overt neurological signs (Craufurd 2002; Johnson, Stout et al. 2007; Solomon, Stout et al. 2007; Paulsen, Langbehn et al. 2008). The cognitive impairment is often thought of as a frontal-subcortical dementia as opposed to the “cortical” phenotype of AD, which is characterized predominantly by memory loss. Cognitive impairment in HD includes slowing of thought process and deterioration of executive function. Typically patients report difficulty with multitasking, concentration and short-term memory. Thinking style becomes more concrete and less efficient with particular impairment of planning and problem solving. People with HD are often impulsive and develop psychomotor perseveration and have difficulty with organisation of time, thoughts and activities. Visuospatial perception and construction can also deteriorate (Craufurd 2002).

A diagnosis of dementia in HD should include evidence of impairment in at least two areas of cognition (e.g. attention, speed of processing, executive functions, visuospatial abilities, memory) but without a requirement of memory impairment (Peavy, Jacobson et al. 2010).
1.1.2.3 Psychiatric and Behavioural Symptoms

Psychiatric manifestations of HD can be disabling and unpredictable and are nearly universal (Paulsen, Ready et al. 2001). However, as opposed to the cognitive features, there are somewhat effective symptomatic treatments available. It is, therefore, important to recognise the psychiatric symptoms in HD so that symptomatic treatment can be offered.

Depression is the most common psychiatric symptom. The prevalence of depression in HD is up to 50% (compared with 4% in the general population) and can predate the diagnosis of manifest HD by up to two decades (Folstein, Abbott et al. 1983; Pflanz, Besson et al. 1991; Craufurd, Thompson et al. 2001). Other studies have shown that individuals at risk of HD have an increased risk of depression compared with their spouses and that increased risk was the same whether that individual was a gene carrier or not (Shiwach and Norbury 1994). The high prevalence of depression in HD is not solely due to the effects of living with an incurable degenerative disease but has its basis in neurodegeneration, though there is no clear relationship between affective symptoms and the severity of motor and cognitive changes (Mindham, Steele et al. 1985; Craufurd, Thompson et al. 2001).

As George Huntington, himself, recognised when he wrote; ‘that form of insanity which leads to suicide’ (Huntington 1872), the frequency of suicide is greater in patients with HD and their families compared with the general population (Di Maio, Squitieri et al. 1993). Suicidal ideation is highest in gene carriers nearing the threshold of being diagnosed with manifest disease and in those who are beginning to lose their functional ability and independence. Risk factors for suicide in HD include depression and impulsivity (Craufurd 2002).

Agitation, irritability, apathy and anxiety are also frequent features of HD and can be highly challenging behaviours for family members to manage (Paulsen, Ready et al. 2001). Other reported psychiatric symptoms include obsessive-compulsive symptoms and psychosis (Craufurd 2002).

The assessment of psychiatric phenomena in HD is a challenge. Generic scales, such as the Beck Depression Inventory (BDI) (Beck, Steer et al. 1996) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith 1983) lack questions about important behavioural manifestations of HD, such as irritability and apathy, or ask
questions that may be confounded by non-behavioural features common in HD, such as weight loss. The Problem Behaviours Assessment (PBA) for HD (Craufurd, Thompson et al. 2001) was developed to evaluate the behavioural features of HD based on theoretical features of interest from publications of disorders of the frontal lobes, symptoms mentioned during clinical consultations and features from the literature in HD. It encompassed a 40-point questionnaire, which was thorough and evidence-based, but use was limited due to the time taken to administer the assessment. The PBA-s, a shortened version of the assessment, has been developed by the Behavioural Phenotype Working Group of the European Huntington’s Disease Network (EHDN) and combines the UHDRS behavioural assessment and the longer PBA in the form of a structured interview which can be administered in 15-30 minutes.

1.1.2.4 Advanced disease

The average duration of symptomatic HD is between 15 and 20 years (Kremer 2002) though it may be longer than this today, with improved care and medical management of symptoms. End-stage disease is characterised by profound disability with severe rigidity, akinesia and an inability to communicate. Cachexia due to a combination of a catabolic state and swallowing difficulties can be a profound challenge and death in is most commonly due to cardiac failure, aspiration pneumonia or malnutrition (Lanska, Lavine et al. 1988).

1.1.2.5 Peripheral Features

HD is not just a disease of the brain. Patients with HD have a number of non-neurological features as shown in Figure 3 and several observations have shown that these may not be due to neurological dysfunction or secondary to a general disease process.

Huntingtin (htt) protein is expressed widely (Li, Schilling et al. 1993) and is thought to be ubiquitously expressed. Dysfunction of peripheral cells occurs even when these cells are isolated (Panov, Lund et al. 2005; Almeida, Sarmento-Ribeiro et al. 2008; Bjorkqvist, Wild et al. 2008; Chaturvedi, Adhihetty et al. 2009).
**Weight Loss**

Unintended weight loss is the most common non-neurological abnormality in HD. Several studies have indicated that this is not secondary to hyperactivity or anorexia but results from an increased metabolic rate (Myers, Sax et al. 1991; van der Burg, Bjorkqvist et al. 2009). Patients with a higher body mass index at onset of symptoms tend to have a slower rate of disease progression (Myers, Sax et al. 1991). Clearly, weight loss is compounded by dysphagia in the latter stages of the disease.

![Figure 3 Peripheral pathology in patients with Huntington’s Disease. Reproduced from (van der Burg, Bjorkqvist et al. 2009) with permission from the Elselvier publishing group.](image)

**Skeletal Muscle**

Skeletal-muscle wasting is a hallmark of HD. Skeletal muscles undergo substantial wasting, despite the muscles being active as a result of dyskinesias. Myocytes are
affected in HD, possibly as a direct effect of mutant huntingtin (mHtt), and muscles undergo atrophy as a result.

The muscle wasting is possibly due to defects caused by the presence of mHtt forming inclusion bodies in myocytes (Orth, Cooper et al. 2003), through transcriptional dysregulation (Luthi-Carter, Hanson et al. 2002; Strand, Aragaki et al. 2005), or through effects on mitochondrial function (Ciammola, Sassone et al. 2006; Turner, Cooper et al. 2007).

Cardiac Failure

Cardiac failure occurs in about 30% of patients with HD (compared with only 2% in age-matched controls) and is a leading cause of death in these patients (Lanska, Lavine et al. 1988) The mechanism underlying cardiac failure in HD is not known but may be the same as those involved in the skeletal muscle dysfunction.

Inflammatory Dysfunction in HD

Blood plasma samples of patients with HD show signs of immune activation, such as increased concentrations of interleukins (IL); IL-6 and IL-8 (Dalrymple, Wild et al. 2007; Bjorkqvist, Wild et al. 2008). This immune activation may be in reaction to cellular pathology, such as huntingtin protein aggregation, oxidative damage or necrotic cell death. However, evidence that the immune activation is present many years before the onset of symptoms (Bjorkqvist, Wild et al. 2008), suggests that inflammatory changes in HD are not secondary to brain pathology or a response to a general disease process. Altered inflammatory signalling in the periphery might contribute to several features of HD, including weight loss and muscle wasting and targeting immune activation may provide a therapeutic target. Section 1.3 of this Introduction and Chapter 4 of this thesis will focus further on the immune system in HD.

Endocrine Dysfunction

Widespread endocrine dysfunction has been described in HD including Hyperactivity of the hypothalamic-pituitary-adrenal axis (Aziz, Pijl et al. 2009), autonomic dysfunction (Aziz, Anguelova et al. 2010) and dysfunction of thyrotropic and lactotropic axes (Aziz, Pijl et al. 2010). Dysfunction of these axes may contribute to signs and symptoms in HD patients such as sleep disturbance and unintended weight loss.
Osteoporosis might also be part of the HD phenotype and may correlate with CAG repeats length (van der Burg, Bjorkqvist et al. 2009). The reason for decreased bone mineral density in this disorder is not known, though it may be secondary to neuroleptic treatment or might result from immobility rather than being a direct effect of the disorder itself.

1.1.3 Genetics of Huntington’s Disease

1.1.3.1 Molecular Genetics

The gene for HD, huntingtin (HTT, previously known as interesting transcript 15, IT15), was the first disease-associated gene to be molecularly mapped to a human chromosome, 4p16.3, in 1983 (Gusella, Wexler et al. 1983). Ten years later, following an intense and international collaborative effort, HTT and the nature of the HD-associated mutation were identified (The Huntington's Disease Research Collaborative Research Group 1993). They identified a Cytosine-Adeonsine-Guanine (CAG) repeat expansion on one allele of the first coding exon of HTT in affected members from all 75 HD families examined. Analysis of individuals with HD showed that they always had 40 or more CAG repeats; in fact, the largest number of CAG repeats in this first study was 100 (The Huntington's Disease Collaborative Research Group 1993). Non-HD controls showed CAG repeats from 6 to 35 in the same region.

Figure 4 represents our understanding of the CAG repeat expansion related to HD pathogenesis. HD is fully penetrant within a normal lifespan in individuals with a CAG repeat length of 40 or more. Expansions of between 36 and 39 confer an increased risk of developing HD but with reduced penetrance. CAG repeats of 70 and above invariably cause Juvenile Huntington’s Disease (JHD) with repeat of greater than 60 seen in approximately 50% of cases (Quarrell, O'Donovan et al. 2012). However, consistent with findings in adult cases, CAG repeat size does not always correlate with age of onset in JHD. The largest repeat that has been reported had approximately 250 repeats although repeats of 80 and above are extremely rare (Nicolas, Devys et al. 2011).
Intermediate alleles lie within the CAG repeat range 27-35. Non-pathogenic alleles within this high normal range can expand into the pathogenic range so while individuals with an intermediate allele will usually not develop HD, there remains an unknown risk for their children and future generations to become affected. 30% of individuals with onset at an age greater than 60 do not have a family history of HD and these new cases are likely due to expansion from an intermediate allele (Hayden 2012). The frequency of intermediate alleles in the population of British Columbia, Canada, has recently been estimated by analysing the CAG sizes of 2000 general population samples. This showed a frequency of intermediate alleles to be 5.78%; approximately 1/17 people (Hayden 2012). These alleles can be unstable during transmission and a mutation rate of approximately 10% in each generation has been suggested (Semaka, Collins et al. 2010). More pronounced instability has been observed in paternal transmissions. In particular, JHD is paternally inherited in 80-90% of cases (Trottier, Biancalana et al. 1994; Nicolas, Devys et al. 2011). Instability of CAG repeat in somatic tissues has been reported and tissue specific somatic expansion may be associated with the age of onset (Telenius, Kremer et al. 1994; Kennedy, Evans et al. 2003; Swami, Hendricks et al. 2009).
1.1.3.2 CAG repeat length and age of onset

There is a significant inverse relationship between the CAG repeat length and the likely age at which disease signs will appear. CAG length has been reported to account for up to 73% of the variation in the age of onset (Andrew, Goldberg et al. 1993; Duyao, Ambrose et al. 1993; Snell, MacMillan et al. 1993). As shown in Figure 5 repeat lengths of 40 and above are associated with over 90% lifetime probability of motor onset, assuming a life expectancy of 80 years (Langbehn, Brinkman et al. 2004). With higher the CAG repeat lengths, the probability curve is shifted to the left meaning an earlier age of onset is observed. However, the effect is not linear. The curves are steeper for higher repeats, and therefore the age range is more narrow. For example, for an individual with 40 repeats, the probability of onset increases from 10% to 90% over a period of 27 years but for someone with 56 repeats, that probability range spans just 15 years.

Figure 5 Cumulative probability of motor onset for a given age, by CAG repeat length

(Reproduced from Langbehn et al. 2004. With permission from Jon Wiley and Sons publishing group.)
1.1.3.3 CAG repeat and rate of progression

In contrast to the clear relationship between CAG repeat length and age at onset, there has been conflicting evidence for a similar correlation with rate of disease progression. Some studies report an association between CAG repeat length and rate of progression (Illariohskhin, Igarashi et al. 1994; Brandt, Bylsma et al. 1996; Mahant, McCusker et al. 2003; Rosenblatt, Liang et al. 2006; Ravina, Romer et al. 2008) and some studies report no association (Ashizawa, Wong et al. 1994; Kieburtz, MacDonald et al. 1994; Claes, Van Zand et al. 1995; Marder, Zhao et al. 2000; Snowden, Craufurd et al. 2001). In a recent study of 569 subjects, CAG repeat length did correlate with rate of progression when controlling for age at onset (Rosenblatt, Kumar et al. 2012). However, the CAG repeat lengths in this most recent study varied between 36 and 109 and therefore it may be that, where correlations exist, they arise largely from inclusion of atypical juvenile cases with very large repeat lengths.

1.1.3.4 Predictive genetic testing

Discovery of a linked genetic marker for HD in 1983 introduced the possibility for presymptomatic diagnosis of at-risk individuals in HD families where there was DNA available from affected members (Gusella, Wexler et al. 1983). Following the cloning of the HTT gene in 1993 (The Huntington's Disease Collaborative Research Group 1993), a direct test became available based on measurement of the CAG repeat length. This test is now used for differential diagnosis, confirmation of suspected HD in the absence of a clear family history, prenatal diagnosis and predictive testing. Despite early surveys that suggested a high amount of interest, only a small number (approximately 14%) of individuals at risk of HD choose to actually pursue genetic testing (Tibben 2007).

Predictive testing for HD may remove the uncertainty of whether HD should be anticipated but no certainty can be given as to when or how the disease may manifest. The discovery of an intermediate allele may also complicate genetic testing. Those who do undergo testing generally do so usually to reduce uncertainty and to assist in making career and family choices. Predictive testing is not without risk; anxiety and stress may remain elevated following a positive result and there is a risk of suicide, though this occurs less frequently than was expected before predictive testing was instituted. Anxiety may also follow a negative result in the form of survivor guilt and highlights
the importance of adequate pre- and post-test counselling (Almqvist, Bloch et al. 1999; Broadstock, Michie et al. 2000).

Current predictive counselling guidelines have been designed to inform those at risk of the implications of test results for themselves and relatives, to identify sources of subsequent support, to discuss opportunities for research and to discuss options for family planning prior to undertaking genetic testing to ensure that individuals are as well prepared as possible for the potentially devastating news (Macleod, Tibben et al. 2012).

Predictive testing in HD has made research studies involving pre-symptomatic HD gene carriers possible. These have advanced our understanding of the natural history and neurobiology of the disease. In the future, the availability of predictive testing may mean that disease-modifying therapies could be administered prior to the onset of symptoms. In addition, our experience of the testing process has served as a model for predictive testing in other late-onset disorders.

1.1.4 Pathogenesis

1.1.4.1 Macroscopic & Microscopic Pathology

At post-mortem, end-stage HD brains typically weigh approximately 10-20% less than age matched controls (Gutekunst 2002), as shown in Figure 6.

HD is characterized by degeneration of the medium spiny GABAergic projection neurons in the caudate and putamen with the dopaminergic and nigrostriatal pathways being relatively preserved, although there remains significant generalised cortical degeneration (Vonsattel, Myers et al. 1985). Huntingtin (Htt) aggregates, consisting of N-terminal fragments of the mutant huntingtin protein (mHtt) from large dense protein inclusions in the nucleus of affected cells but also in the cytoplasm, neurites and terminals (Bates 2003).
Figure 6 Gross and microscopic neuropathology in Huntington’s disease.

Coronal sections from formaldehyde-fixed cerebral hemispheres at the level of the head of the caudate nucleus (CN) and putamen (Put) from an age-matched and normal control (A) and a 62-year old female with HD (B). Note the marked gross atrophy of the neostriatum (CN and Put) and enlarged lateral ventricle (lv) in panel B. The corresponding haematoxylin and eosin staining of the caudate nucleus shows significant neuronal loss and astrogliosis in HD (D), in comparison with the normal control (C).

Reproduced from (Ryu, Rosas et al. 2005) with permission from the Elsevier Publishing group.
1.1.4.2 Molecular and cellular pathology

Our knowledge of the cellular biology of Htt has exponentially increased since the discovery of the gene and through the use of multiple animal and cell models. Though the precise mechanisms remain incompletely understood many functions of Htt are now known. It is a large protein (350 kiloDaltons) and is ubiquitously expressed within and outside the nervous system (Li, Schilling et al. 1993). It is found in several different subcellular compartments and several Htt-interacting proteins have been identified; many of them binding to the N-terminus of the protein (Li and Li 2004). The functions of Htt include endocytosis, vesicular transport, cell signalling, apoptosis and transcriptional regulation (Ross and Tabrizi 2011). Htt seems to act as a scaffolding protein and once bound with its interacting proteins, allows them to transfer information between cellular compartments (Harjes and Wanker 2003). Post translational modifications also play a major role in Htt protein function (Ehrnhoefer, Sutton et al. 2011).

![Figure 7 Postulated Intracellular pathogenesis of Huntington’s Disease](image)

*Mutant Htt (mHtt) (shown as a blue helical structure) with an expanded polyglutamine repeat (shown in red) undergoes a conformational change and interferes with cellular trafficking, especially of Brain Derived Natriuretic Factor (BDNF): mHtt is cleared at...*
several points to generate toxic fragments with abnormal compact β confirmation. Pathogenic species can be monomeric or, more likely (and as shown) form small oligomers. Toxic effects in the cytoplasm include inhibition of chaperones, proteasomes and autophagy, which can cause accumulation of abnormally, folded proteins and other cellular constituents. There may be direct interactions between mHtt and mitochondria. Other interactions between Htt and cellular proteins in the cytoplasm are still poorly understood. Pathognomonic inclusion bodies are found in the nucleus (and small inclusions are also found in cytoplasmic regions). However, inclusions are not the primary pathogenic species. A major action of mHtt is interference with gene transcription, in part via PGC1α, leading to decreased transcription of BDNF and nuclear-encoded mitochondrial proteins. ROS=reactive oxygen species.

(Reproduced from (Ross and Tabrizi 2011) with permission from the Elsevier publishing group)

The CAG repeat expansion gives rise to an expanded polyglutamine stretch in mHtt and it is most likely that HD is due to a toxic gain of function from the abnormal conformation that arises. This theory is supported by a dominant inheritance pattern, the presence of abnormal aggregated proteins in cells and the findings from biochemical, cellular and mouse model studies. HTT RNA might also have toxic properties. However, loss of function of Htt might also contribute to disease pathogenesis as normal expression of Htt is essential for early development and targeted knockouts are embryonic lethal (Duyao, Auerbach et al. 1995; Nasir, Floresco et al. 1995; White, Auerbach et al. 1997).

mHtt has been implicated in the disruption of multiple cellular processes, including protein clearance, protein-protein interaction, mitochondrial function, axonal trafficking, gene transcription and posttranslational modification (Ross and Tabrizi 2011).

Many of these mechanisms have been implicated as potential therapeutic targets.

1.1.5 Therapeutic Approaches

Access to symptomatic treatments and expert multidisciplinary care are key as there are currently no treatments that can delay disease progression. However, a concerted effort by HD families and the HD research community have led to an explosion of interventional human studies of both symptomatic therapies and potentially disease modifying agents, with 65 such studies listed in North America of which 15 are open to recruitment at the time of writing (clinicaltrials.gov as of May 2013).
1.1.5.1 Symptomatic Therapies

There is a general lack of evidence base for the symptomatic treatment of HD. A multidisciplinary approach to management is recommended and physiotherapy, speech and language therapy as well as psychological interventions and behavioural therapies play a vital role.

Tetrabenazine remains the only drug licensed for treatment of chorea in HD though exacerbation of depression is a potential adverse effect (Frank and Jankovic 2010). However antipsychotic drugs, such as olanzapine, are the preferred treatment amongst experts in Europe (Burgunder, Guttman et al. 2011). Pridopidine, a novel dopaminergic stabiliser, looked promising in preliminary trials, however in a recent phase III study, it failed to show significant changes to movements though it was found to be safe and well tolerated (de Yebebes, Landwehrmeyer et al. 2011; Squitieri, Landwehrmeyer et al. 2013).

Selective serotonin reuptake inhibitors, such as citalopram, are suggested for the treatment of depression, irritability and obsessive-compulsive symptoms (Videnovic 2013). No drug treatments are currently available for cognitive symptoms and many of the troublesome behavioural symptoms, such as apathy.

1.1.5.2 Disease Modifying Therapies

There are now a number of promising disease modifying therapies in the pipeline and approaching human clinical trials. Research is continuing to identify novel therapeutic approaches as it is generally held that several parallel approaches will be necessary to optimise therapeutic benefit. A few promising approaches, that are in varying stages of development, are discussed here.

Gene silencing

Reduction of the amounts of mHtt in the brain by preventing protein translation can be achieved via targeted small interfering RNA (siRNA), short hairpin RNA (shRNA) or antisense oligonucleotides (ASO). Use of siRNAs and ASOs is safe, can decrease Htt expression and improve symptoms in mouse and primate models of HD (Sah and Aronin 2011). Selective reduction of mHtt without affecting levels of wild-type Htt is also now possible and may avoid the possibility of harmful effects from indiscriminate knockdown. Implantable infusion systems are used in trials for Parkinson’s Disease to
deliver glia-cell derived neurotrophic factor and could be used in HD (Patel, Bunnage et al. 2005; Lang, Gill et al. 2006). Significant questions regarding distribution and safety have recently been partially addressed following a 6-month non-human primate safety trial and academic-industry collaborations are bringing the possibility of human trials in the near future closer (Grondin, Kaytor et al. 2012).

**Stem cell transplantation**

In one early study of foetal stem cell transplantation, three of five patients maintained or slightly improved motor and cognitive function after transplantation but the improvement was not sustained (Bachoud-Levi, Gaura et al. 2006). There are major drawbacks in terms of technical, ethical and legislative concerns surrounding the use of foetal stem cells and so readily renewable sources for transplants are being investigated. Inducible pluripotent stem cells (iPSCs) isolated and engineered from the patients themselves, are being considered for use, thereby avoiding activation of the immune system. This strategy involves genetically reprogramming fibroblasts to iPSCs (Takahashi, Okita et al. 2007; Park, Arora et al. 2008), before correcting the CAG repeat expansion, differentiating the cells into medium spiny neurons and transplanting the cells into the brain of the patient. So far, several human HD iPSC lines have been established (HD iPSC Consortium 2012). It has been shown that iPSCs can reverse mHTT-driven transcription changes and can differentiate into striatal neurons *in vitro* and *in vivo* after correction of the CAG repeat in mice models of HD, demonstrating the potential of this approach for therapy (An, Zhang et al. 2012).

**Clearance of mutant huntingtin**

The two major pathways for clearance of misfolded proteins are the ubiquitin-proteasome system and autophagy. Autophagy enhancers could improve the removal of mHtt from cells. The enzyme mTOR (‘mammalian target of rapamycin’) down regulates autophagy and mTOR inhibition with rapamycin reduces aggregate formation and improves motor deficits in fly and mouse models of HD (Ravikumar and Rubinsztein 2004). However, the stimulation of protein clearance systems are not specific and therefore lead to increased degradation of all proteins, increasing the likelihood of unwanted side-effects.
Targeting transcriptional dysregulation

Studies have shown Histone Deacetylase (HDAC) inhibitors to be neuroprotective in various HD models (Kazantsev and Thompson 2008). Although the precise mechanism is not known, it could include chromatin remodelling and amelioration of transcriptional dysregulation, by enhancing the availability of gene promoter sites (Kazantsev and Thompson 2008). Selective clearance of mHtt may also be facilitated by hyperacetylation of the mutant protein with HDAC inhibitors (Jeong, Then et al. 2009). HDAC inhibitors are already used in cancer but work is underway to identify less toxic, selective HDAC inhibitors for use in HD (Thomas, Coppola et al. 2008).

The Sirtuins are a highly conserved family of primarily NAD-dependent deacetylase proteins. Sirtuins have been implicated in ageing and there is evidence that they are involved in metabolic control and transcriptional regulation (Finkel, Deng et al. 2009; Jeong, Cohen et al. 2012; Jiang, Wang et al. 2012). Sirtuin 1 (SIRT1) has been shown to protect against mutant huntingtin neurotoxicity in three different mouse models of HD (Jeong, Cohen et al. 2012; Jiang, Wang et al. 2012) and Selisistat (SEN0014196), a selective inhibitor of SIRT1 has entered Phase II trials in Europe following completion of phase I studies in 2010 (Westerberg, Diamanti et al. 2012).

Improvement of neuronal function

One way to improve neuronal function and arrest neuronal loss is to block excitotoxicity caused by over activation of the N-methyl-D-aspartate (NMDA) receptor by glutamate or kynurenine metabolites. Glutamate receptor antagonists such as riluzole and memantine have shown promise in HD mouse models, but have shown little effect in clinical settings (Mestre et al., 2009; Milnerwood et al., 2010). Modulation of the kynurenine 3-monooxygenase (KMO) pathway to reduce the levels of the NMDA receptor agonists 3-hydroxykynurenine and quinolinic acid, whilst increasing the levels of the neuroprotective NMDA receptor antagonist, kynurenic acid, has shown beneficial effects in yeast, fly and mouse models of HD (Campesan et al., 2011; Giorgini et al., 2005; Zwilling et al., 2011). Another possible way to improve neuronal function in HD is to increase the level of the neurotrophin BDNF, which is depleted in the HD brain (Gauthier et al., 2004; Zuccato et
Indeed, overexpression of the BDNF gene in the YAC128 HD mouse model prevented neuronal loss and motor abnormalities (Xie et al., 2010). Adenoviral administration of BDNF into the striatum of R6/2 mice delayed motor impairments and prolonged survival (Cho et al., 2007), demonstrating the promise of this strategy.

1.2 Biomarkers

Though a number of potential therapeutic strategies are now approaching clinical trials, in order to accurately assess the effectiveness of disease-modifying therapies, it is necessary to identify biomarkers that will reliably detect subtle changes of disease progression.

The Total Functional Capacity (TFC) Scale (Shoulson and Fahn 1979) is used crudely to ‘stage’ the progression of HD and was possibly the first biomarker for the disease (see Appendix A and also Table 2). The scale reflects the progression of the disease, in particular the psychosocial and functional effects on the patient and their family. Points are assigned according to the individual’s ability to work, to manage money, to perform household chores, to perform activities of daily living, and to live at home or in supervised care.

<table>
<thead>
<tr>
<th>Stage</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early HD</td>
<td>1 11-13</td>
</tr>
<tr>
<td></td>
<td>2 7-10</td>
</tr>
<tr>
<td>Moderate HD</td>
<td>3 3-6</td>
</tr>
<tr>
<td></td>
<td>4 1-2</td>
</tr>
<tr>
<td>Advanced HD</td>
<td>5 0</td>
</tr>
</tbody>
</table>

Table 2 Staging HD using Total Functional Capacity

The TFC (see Appendix A and Table 2) was integrated with motor, behavioural and functional components in 1996 to form the Unified Huntington Disease Rating Scale (UHDRS) (HSG 1996) which is now widely used, both clinically and in observational and interventional studies of HD. However, the UHDRS may not be sensitive to change in early stages of disease and may be limited by inter-rater and intra-rater variability, and floor and ceiling effects (HSG 1996; de Boo, Tibben et al. 1998; Reilmann, Bohlen...
et al. 2011) and it is clear that more robust markers of disease progression are necessary. An ideal biomarker candidate should be readily quantifiable, be user-dependant and should correlate with disease progression. A candidate biomarker should also have low variability in the control population and not be affected by unrelated comorbidities. Due to the variability in HD, a combination of different biomarkers may be required for tracking progression and assessing potential therapies. Until recently, biomarker studies were limited by cross-sectional design and limited numbers. The advent of PREDICT-HD (Paulsen, Hayden et al. 2006) and, the focus of much of this thesis, TRACK-HD (Tabrizi, Langbehn et al. 2009) have allowed the investigation of biomarkers in high quality, multi-site, longitudinal observational studies.

1.2.1 Neuroimaging

Neuroimaging techniques, such as magnetic resonance imaging (MRI), enable high quality images of brain structure and function to be obtained and advanced image analysis techniques allow robust measurement of differences between subjects and change within individuals. Neuroimaging techniques used in HD include structural, functional and metabolic imaging measures.

1.2.1.1 Structural MRI

As described earlier, post-mortem studies have shown that the whole brains of HD patients are 10-20% lighter at post-mortem (Vonsattel, Myers et al. 1985). The boundary shift integral (BSI) is a semi automated method by which change in brain volume can be calculated from registered pairs of scans. Using this technique, Henley et al, demonstrated that whole brain atrophy was significantly faster in early HD than in controls, over a 6-month period (Henley, Frost et al. 2006). Whole brain atrophy, as measured by BSI continued over 2 years in the early HD group but significant differences were not present in the premanifest group (Wild, Henley et al. 2010). Other whole-brain-based methods used to study the pattern of neurodegeneration and neuro-anatomical correlates of HD include voxel based morphometry (VBM) and measures of cortical thickness. Cross-sectional studies of both of these identified brain regions differentially affected between different disease groups and controls (Kassubek, Juengling et al. 2004; Rosas, Hevelone et al. 2005; Nopoulos, Aylward et al. 2010). Widespread white matter (WM) changes have been noted in premanifest HD (Paulsen,
and have been shown to progress over 2 years using VBM (Hobbs, Henley et al. 2010).

Region of interest (ROI) structural imaging including measures of caudate nucleus and putamen atrophy may provide more specific biomarkers. In HD, striatal atrophy correlates with age of onset, CAG repeat length and motor dysfunction and has been reported in individuals 15-20 years from predicted disease onset with rapid, almost linear, striatal tissue loss over time (Harris, Codori et al. 1999; Aylward, Sparks et al. 2004; Paulsen, Langbehn et al. 2008). Longitudinal studies have been shown to confirm striatal measures and WM change measures as potential biomarkers (Aylward, Nopoulos et al. 2011).

Diffusion tensor imaging (DTI), an MRI technique that provides information on neuronal fibre orientation and on patterns of WM connectivity, is also being investigated. DTI has been used to differentiate premanifest individuals from controls and to measure WM degeneration longitudinally (Rosas, Tuch et al. 2006; Weaver, Richards et al. 2009). Altered WM connections of the sensorimotor cortex have also been described in both manifest and premanifest HD and correlate with phenotypic deterioration and estimates of probability to disease onset (Dumas, van den Bogaard et al. 2012).

1.2.1.2 Functional imaging, metabolic and molecular imaging

Neurodegeneration in HD is likely to be preceded by substantial neuronal dysfunction. Therefore, techniques such as functional MRI (fMRI), magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), which are used to measure functional and metabolic changes in brain tissue, might enable identification of neuronal dysfunction prior to macroscopic tissue loss. These techniques may allow monitoring of very early disease as compared to structural imaging techniques and may measure processes that could be reversed by therapeutic interventions. However, these techniques have limitations as biomarkers for clinical trials in HD. In particular, they are costly, the equipment and expertise required are less widespread than conventional MRI techniques and scanning times are typically longer; hence the limited number of large longitudinal studies.
Functional MRI

Functional MRI uses changes of blood-oxygen-level dependent contrast during neuronal activity to identify brain regions active during performance of experimental tasks. fMRI studies have demonstrated functional abnormalities in premanifest and early HD cross-sectionally (Paulsen, Zimbelman et al. 2004; Wolf, Vasic et al. 2007; Zimbelman, Paulsen et al. 2007; Saft, Schuttke et al. 2008; Kloppel, Draganski et al. 2009; Gray, Egan et al. 2013) but the only longitudinal study to date failed to show change over a 2-year period (Wolf, Sambataro et al. 2011).

Magnetic Resonance Spectroscopy

MRS is a magnetic resonance technique that is used to measure metabolite concentrations within brain regions. Lactate levels have been shown to be elevated in the striatum in premanifest and early HD but this has not been reproduced across all studies. MRS has identified cross-sectional changes in N-acetylaspartate (a putative marker of neuronal viability) and creatine, which correlate with declining motor function (Hoang, Bluml et al. 1998; Sanchez-Pernaute, Garcia-Segura et al. 1999; Sturrock, Laule et al. 2010). It has also been used in a trial of creatine therapy to demonstrate that the compound entered the brain (i.e. as a pharmacodynamic biomarker) (Hersch, Gevorkian et al. 2006).

Positron Emission Tomography

PET uses positron emission by radioisotopes to identify molecular variations in the brain in vivo. Post-mortem studies in HD have shown a 50% loss of dopamine receptors. Dopamine receptors have been widely studied using the PET ligands $^{11}$C-raclopride (which binds to D$_2$ receptors) and $^{11}$C-SCH23390 (which binds to D$_1$). PET studies have shown a dramatic reduction of D$_2$ and D$_1$ binding sites in manifest premanifest HD gene carriers and have demonstrated correlation with the duration of motor manifestations (Turjanski, Weeks et al. 1995; Ginovart, Lundin et al. 1997; van Oostrom, Maguire et al. 2005). Longitudinal studies have shown consistent decreases in D$_2$ binding in both manifest and premanifest disease, with faster rates of loss in those approaching motor onset, suggesting that $^{11}$C-raclopride binding may detect both progression and incipient motor onset (Andrews, Weeks et al. 1999). Microglial PET imaging is discussed in more detail in section 1.3.1.
A means of quantifying wild-type and mutant huntingtin in the brain would be a valuable tool for demonstrating central nervous system (CNS) target engagement of huntingtin-lowering therapies in the brain. Work is under-way to develop and test PET ligands that will enable visualisation of huntingtin load, as a predictive tool and a means of assessing response to therapy.

1.2.2 Quantitative Clinical Measures

Clinical rating scales, such as the UHDRS are the present standard assessment of progression in HD. As already described, the UHDRS motor component (Appendix A) (HSG 1996) is based on examination of these key motor features of HD and forms a 124-point motor scale. Though extensively validated, these scales are somewhat subjective and inter- and intra-rater variability remains (HSG 1996; Hogarth, Kayson et al. 2005; Klempir J. 2006). In addition, hyperkinetic movement disorders (dystonia and chorea) contribute 48 points whilst impairment of voluntary movements contributes only 32 and bradykinesia 16; even though the latter two categories are each independently more important than chorea in contributing functional disability (Shoulson 1981; Thompson, Berardelli et al. 1988). There are now a number of promising automated techniques aimed at detecting motor abnormalities earlier in premanifest HD and increasing the reliability of motor measures in manifest disease. These have been developed to largely to reflect components of the UHDRS motor rating scale.

1.2.2.1 Oculomotor

Observations of oculomotor abnormalities form the first 3 assessments of the UHDRS motor scale and are an established and consistent feature of HD as described earlier in this chapter. Computerised quantitative eye movement measurement has demonstrated a number of reproducible oculomotor abnormalities in HD that may function as biomarkers. Several studies have demonstrated delays in voluntary saccade initiation which correlated with disease stage in manifest HD, and estimated pathological disease burden in premanifest HD (Golding, Danchaivijitr et al. 2006; Hicks, Robert et al. 2008). Portable ‘saccadometer’ devices have made possible the incorporation of oculomotor assessments into large-scale biomarker studies such as TRACK-HD.
1.2.2.2 Tapping

Finger tapping and dysdiodochokinesis each form one section of the UHDRS motor examination. Tapping tasks can be easily converted to automated measurement and analysis. Several studies have reported reduced tapping rates in manifest HD patients as compared to controls, but not in premanifest individuals; UHDRS motor scores and duration of the disease were highly correlated with the tapping results (Paulsen, Hayden et al. 2006; Saft, Andrich et al. 2006; Paulsen, Langbehn et al. 2008; Biglan, Ross et al. 2009). Longitudinal studies have shown a significant decline in tapping rate over a period of 3 years in manifest HD patients, and a strong correlation between UHDRS scores and the motor tests (Andrich, Saft et al. 2007). In addition, the variability of finger tapping correlated with an index of the probability of motor onset (Hinton, Paulsen et al. 2007).

1.2.2.3 Grip force

Assessment of grip force is a test of non-repetitive voluntary movement encompassing sensorimotor integration and motor persistence. In a cross-sectional study, automated measurement (Figure 8) revealed a greater variability in force during static gripping in HD patients compared with controls that correlated with TFC and cognitive scores (Gordon, Quinn et al. 2000). Longitudinally, grip force variability increased in 100% of subjects over 3 years (Reilmann, Kirsten et al. 2001). Grip force variability has also been shown to be increased in premanifest HD and correlates to disease burden score and UHDRS motor score (Reilmann, Bohlen et al. 2010).

![Figure 8 Grip force apparatus and sample recordings](image)

The force transducer above used for assessing grip force may also be adapted for use in tongue force and tapping tasks.
1.2.2.4 Tongue force

Observation of tongue protrusion forms one component of the UHDRS motor examination. Objective measurement of tongue force variability ‘glossomotography’ is able to distinguish between controls, premanifest and symptomatic HD groups and showed correlation with the UHDRS motor score and disease burden score, cross-sectionally (Reilmann, Bohlen et al. 2010). On the basis of this, the assessment has been incorporated into TRACK-HD for longitudinal study.

1.2.2.5 Gait Analysis

Gait and tandem walking are both observed as part of the UHDRS motor assessment. Easy to use instrumented carpet devices have made the possibility of automated gait analysis possible in HD. Cross-sectionally, both premanifest subjects (with no impairment of gait or balance as measured by UHDRS) and HD patients showed decreased gait velocity and stride length (Rao, Muratori et al. 2008). Clinical assessments of gait have not been found to be as effective as carpet devices in detecting gait abnormalities (Rao, Louis et al. 2009).

1.2.2.6 Cognitive Measures

There have been numerous studies of cognitive tasks as potential biomarkers in HD and a full discussion is beyond the scope of this thesis. Cognitive tasks are, undoubtedly, important as they may relate to functional impairment in HD. However, they may be subject to practical limitations such as difficulty in standardisation across languages, practice effects and the confounding influences of psychiatric and motor symptoms of HD. PREDICT-HD has studied the largest reported cohort with prodromal Huntington's disease to identify biological predictors of disease onset and progression (Paulsen, Hayden et al. 2006). Data from the full PREDICT-HD standardised cognitive battery of 51 tasks have shown that tasks of psychomotor processing, emotion recognition, and working memory are the most sensitive in distinguishing individuals according to time to predicted disease onset (Stout, Paulsen et al. 2011). These tasks have formed the basis of cognitive assessment design for subsequent studies, such as TRACK-HD.
1.2.3 Biofluids

Markers that can be quantified in biofluids, such as blood, urine or CSF, would be ideal biomarkers due to the opportunity for rapid bulk processing of specimens, the availability of reliable assays, and because several analyses could be carried out on a single sample. Both candidate approaches and unbiased ‘omic’ approaches have been employed in HD. In particular, markers of endocrine function and metabolism, oxidative stress, immune response and neurochemicals have been investigated (reviewed in (Weir, Sturrock et al. 2011)). To date, no robust, reproducible, longitudinal biochemical biomarkers of HD have been identified.

Accurately measuring levels of mHtt and Htt in biosamples will be essential for testing therapeutics aimed a reducing mHtt levels including gene-silencing treatments and those targeting clearance of mHtt. Until recently, the detection of Htt was limited by the insensitivity of assays, and a lack of suitable antibodies. Novel antibodies and a fluorescence-enhance antibody assay using time-resolved Förset resonance energy transfer (TR-FRET) have been developed to quantify concentrations of soluble mHTT in brain, plasma and CSF samples(Weiss, Trager et al. 2012)

As with the search for genetic modifiers of disease onset and progression, large longitudinal ‘biobanks’ of well-characterised samples are essential for the development of robust biofluid biomarkers and may be provided by the PREDICT-HD and TRACK-HD cohorts.

1.3 Inflammation and Immune dysfunction

Neurodegenerative disorders; including Parkinson’s Disease and Alzheimer’s Disease as well as HD, are associated with a variety of inflammatory responses. Inflammation in neurodegenerative disorders can result from a number of causes: protein aggregates, accumulation of other abnormally modified cellular constituents, molecules released from or associated with injured neurones or synapses, and dysregulation of inflammatory control mechanisms, as shown in Figure 9 (for review see (Wyss-Coray and Mucke 2002). It is less clear whether the resulting inflammatory responses are protective or if they contribute to the disease process.
Figure 9 Schematic representation of the Inflammatory Response to CNS Degeneration.

Pathogenic triggers, such as accumulation of abnormal proteins in the cells or extracellular spaces, elicit cellular stress responses and can result in the progressive dysfunction and degeneration of neurons. Interactions indicated by arrows involve a large number of soluble factors. Cytokines and other mediators of the innate immune response are released by astrocytes and microglia to orchestrate defence mechanisms and initiate the removal or sequestration of the pathogenic triggers. A selection of molecules and receptors involved in the recognition of abnormal proteins and degenerating cells is illustrated.

Abnormal proteins and degenerating neurons are tagged by complement proteins such as C3b or Clq or by antibodies for recognition and phagocytosis by glial cells. Degenerating neurons may also be phagocytosed if they display intercellular adhesion molecule-3 (ICAM-3), phosphatidyl serine (PS) or oxidised lipids on their cell surface. Receptors on glial cells recognise these tags and initiate inflammatory responses. MHC molecules might display abnormal proteins with novel antigenic epitopes to lymphocytes, resulting in acquired immune responses. ClqRp, Clq receptor for phagocytosis, CR3, complement receptor 3; FcR Fc receptor; PSR phosphatidyl serine receptor; SR scavenger receptor.

Reproduced from (Wyss-Coray and Mucke 2002) with permission from the Elsevier publishing group.

Triggers of inflammatory reactions can engage the immune system at multiple levels. Initially they recruit the innate immune system and subsequently the acquired immune system may also become involved. The responses of the central nervous system (CNS)
cells are more closely related to the innate than the acquired immune system (Wyss-Coray and Mucke 2002). The innate immune system consists of phagocytes, natural killer cells and molecules that mediate local inflammatory responses or support acquired immune responses. Components of the innate immune system in the CNS include microglia and astrocytes (see Figure 9) which are strongly activated in most neurodegenerative diseases and produce a variety of inflammatory mediators and other injury response factors, including Tumour Necrosis Factor α (TNFα) and IL-1β (Wyss-Coray and Mucke 2002; Czirr and Wyss-Coray 2012).

1.3.1 Central immune dysfunction in HD

Increased complement production by microglia and complement activation of neurones, myelin and astrocytes have been demonstrated in the striatum of HD patients (Singhrao, Neal et al. 1999) and post-mortem studies of HD brains have shown an accumulation of activated microglia and reactive gliosis in regions affected by HD, such as the stratum and cortex (Vonsattel, Myers et al. 1985; Sapp, Kegel et al. 2001). These findings are supported by in-vivo PET imaging studies, using $^{11}$C-(R)-PK11195 (PK) which bind to 18kDA translocator protein (TSPO) (formerly known as the peripheral benzodiazepine receptor) as a surrogate marker of microglial activation. Microglial activation correlates with D$_2$ receptor loss, as measured by $^{11}$C-Raclopride PET, and with disease severity, as measured by the UHDRS total motor score (Pavese, Gerhard et al. 2006). The same group demonstrated early microglial activation in premanifest gene carriers in the striatum and cortex (Tai, Pavese et al. 2007).

1.3.2 Peripheral immune dysfunction in HD

In addition to dysfunction of CNS immune cells, there is evidence of dysfunction in the peripheral immune system in HD. Proteomic profiling of HD plasma samples identified several proteins which were significantly up-regulated in HD patient plasma compared with controls and showed correlation with disease progression (Dalrymple, Wild et al. 2007). The abnormal proteins included IL-6, complement proteins (C7 and C9) and clusterin, which are involved in the regulation of the innate immune system. Widespread activation of the innate immune system is detectable in HD plasma throughout the course of disease. Abnormal IL-6 levels in plasma are detectable, on average, sixteen years before predicted onset of clinical signs and other pro-
inflammatory cytokines (such as IL-8 and TNFα) are increased significantly in HD patient samples compared to controls (Björkqvist, Wild et al. 2008). Chemokine levels are also elevated in plasma from HD patients compared with controls (Wild, Magnusson et al. 2011). Peripheral human monocytes express mHtt and are hyper-reactive in terms of IL-6 production upon Lipopolysaccharide (LPS) stimulation in vivo. LPS is a component of bacterial cell walls, which acts as an endotoxin eliciting a strong immune response. The same hyperstimulation effect was found after stimulation of macrophages and microglia from different HD mouse models, suggesting that mHtt triggers a cell autonomous innate immune activation in the CNS in parallel with the periphery (Björkqvist, Wild et al. 2008).

Figure 10 Immune activation, induced by mHtt, in both peripheral and central immune cells in HD.

A cell-autonomous effect of the mutant protein may be responsible for the innate immune response in HD. The NFκB signalling pathway that triggers IL-6 release is known to be up-regulated by mutant huntingtin (Khoshnan, Ko et al. 2004) and microglia-derived toxicity can influence disease progression (Khoshnan, Ko et al. 2004; Giorgini, Guidetti et al. 2005). This figure shows that the innate immune response detectable in plasma very early in the disease is strongly linked to disease progression and recapitulated in HD striatum, that human monocytes express mHtt and that monocytes, macrophages and microglia overexpress IL-6 when stimulation.

© Björkqvist et al., 2008.


The pathway responsible for this immune activation is not yet certain. One candidate pathway is the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)
pathway. The NFκB signalling pathway is one pathway which triggers IL-6 release. It has previously been implicated in HD pathogenesis as overexpression of mHTT exon 1 in in vitro cell models of HD has been shown to activate this pathway (Khoshnan, Ko et al. 2004).

1.3.3 The immune system as a modifier of disease progression

It is not clear whether activation of the immune system is protective or detrimental to disease progression in HD. Several recent studies have suggested the peripheral immune system can act as a modifier of HD neuropathology. Bone marrow transplantation of wild-type cells into lethally irradiated HD mouse models normalises abnormal peripheral levels of cytokines and chemokines, and partially suppresses motor and neuropathological defects (Kwan, Magnusson et al. 2012). Excitotoxicity in HD has been linked to metabolites of the Kynurenin Monooxygenase (KMO) pathway. Oral administration of a small-molecule prodrug of KMO extends the lifespan and decreases microglial activation in a mouse model of HD. As the drug cannot cross the blood brain barrier, the neuroprotective effect is secondary to inhibition of KMO in peripheral immune cells (Zwilling, Huang et al. 2011). Studies in an HD fly model have shown similar results (Campesan, Green et al. 2011). The cannabinoid receptor 2 (CB2) is expressed on central and peripheral immune cells and is critical in regulating the production of pro-inflammatory cytokines during inflammation by dampening the NFκB signalling cascade. Knockdown of CB2 has been shown to accelerate HD progression in mouse models and treatment with CB2 agonists extended lifespan (Bouchard, Truong et al. 2012).

Together these studies provide strong evidence that the immune system plays a disease-modifying role in HD neuropathogenesis, but the exact mechanism has not yet been established.

1.4 Genetic Modifiers

Even though all individuals with HD have the same mechanism of mutation, namely an expanded CAG repeat length, and the CAG length shows an inverse correlation with age at neurologic onset, two individuals with the same CAG repeat length are unlikely to present with symptoms at exactly the same age (see Figure 11). Two individuals
with the same CAG repeat length are also unlikely to display identical psychiatric, cognitive or peripheral phenotypes; symptoms are unlikely to progress at the same rate and duration of disease is equally unlikely to be precisely the same. The age at onset, rate of progression and precise disease manifestations are, therefore, clearly modifiable by other factors. These factors are likely to be a combination of environmental, experiential and genetic factors.

A gene is a disease modifier if altering its structure or expression alters the manifestation of phenotypes associated with the primary disease mutation, in this case, the HD CAG expansion. Modifier genes have become increasingly recognised as an important source of phenotypic variation in Mendelian disorders. (Slavotinek and Biesecker 2003).

The ultimate aim of identifying genetic modifiers of HD is that they could be used to target particular biochemical pathways for development and validation of therapeutic interventions.

Additionally, once robust genetic modifiers of a particular phenotype are known, they could be incorporated into the design of clinical trials involving that phenotype. This would increase the power of clinical trials to detect an effect of an intervention by controlling for the effect of the genetic background of the participant.

The HD-MAPS study was the first to explore the heritability of the variation in age at motor onset. (Djousse, Knowlton et al. 2003; Li, Hayden et al. 2003). It found that while the CAG repeat length accounted for 67% of the variability in the age at motor onset, the heritability of the residual variance was high ($h=0.56$). This was confirmed in large HD pedigrees from Venezuela (Wexler, Lorimer et al. 2004), where approximately 40% of the variance remaining in onset age was attributable to genes other than the HD gene and 60% was environmental. Other phenotypes have not yet been examined but it is likely that genetic modifiers will play some role in the variability of each disease feature.
Figure 11 Inverse correlation of age at neurologic onset and HD CAG repeat length.

The plot shows data points from 1200 HD subjects of known age at neurologic onset. For each individual, the measured CAG repeat length in blood DNA (x-axis) is plotted against the age at neurologic onset (y-axis). The line represents the best-fit simple logarithmic regression to the data. The CAG repeat length accounts for approximately 67% of the overall variation in age at neurologic onset, and the remaining variation shows a heritability of approximately 0.56. Reproduced from (Arning, Kraus et al. 2005) under the BioMed Central copyright and licence agreement.

1.4.1 Candidate gene approach to identifying genetic modifiers

Examining ‘candidate genes’; genes connected to pathways and processes thought to be involved in HD pathogenesis has been the most utilised approach to identifying genetic modifiers in HD. These genes are examined for genetic variation, usually in the form of single nucleotide polymorphisms (SNP) in humans, and variants linked to the gene of interest are chosen for genotyping in Deoxyribose Nucleic Acid (DNA) from manifest HD individuals to test for an effect of genotype on age at motor onset. There are several approaches to identifying potential genetic modifiers and these are listed in Table 3.
Classes of genes that may include genetic modifiers of HD

- Genes known to influence normal ageing
- Genes encoding proteins known to interact with huntingtin
- Genes encoding proteins involved in pathways suggested to be important in HD pathogenesis
- Genes encoding proteins with glutamine tracts (glutamine tracts bind to each other)
- Genes predisposing to DNA repeat instability, particularly repeat expansion

Table 3 Classes of genes that may include genetic modifiers of HD

A number of candidate genes have been explored but the results have been mixed and no outstanding candidate has yet been reproducibly identified.

The normal \(HTT\) allele has been extensively studied as a genetic modifier of age of onset in HD. Whether or not the normal allele acts as a genetic modifier is a crucial question as it would have implications for the development of nonselective gene therapies in HD and also the potential for using wild-type huntingtin as a therapeutic protein. In some, but not all, studies conducted soon after the identification of the \(HTT\) gene, it was suggested that the number of CAG repeats in the nonexpanded allele might influence the timing of disease onset (Farrer, Cupples et al. 1993; Snell, MacMillan et al. 1993; Djousse, Knowlton et al. 2003). More recently, the debate has been reopened as an interaction between the expanded and normal allele was reported to modify age at onset based upon motor signs, cognitive change and behavioural manifestations (Aziz, van Roon-Mom et al. 2011). Longer normal alleles (e.g. 30 CAG repeats) delayed age at onset of subjects with longer expanded CAG alleles. A subsequent replication study initially found a similar effect but when the data were analysed further, they found that a single outlier with a mutant allele of 120 CAGs and a normal allele of 11 CAGs was driving this effect (Lee, Ramos et al. 2012). Excluding this outlier removed the modifier effect of the unexpanded allele.

\(GRIK2\) (Glutamate receptor, inotropic kainate 2), encodes the GluR6 subunit of the predominant excitatory neurotransmitter receptor family in the human brain and was considered an attractive candidate as an HD modifier due to its potential role in excitotoxic cell death (Naze, Vuillaume et al. 2002). \(GRIK2\) was the earliest reported genetic modifier and for some time remained the most promising. It showed an effect in multiple studies where a 16 TAA repeat allele at the 3’ untranslated region (UTR)
TAA trinucleotide repeat appeared to be associated with an earlier motor onset in HD. These studies were small with the largest including fewer than 300 HD subjects (Holbert, Denghien et al. 2001; Naze, Vuillaume et al. 2002; Chattopadhyay, Ghosh et al. 2003; Cannella, Gellera et al. 2004; Metzger, Bauer et al. 2006). In a recently published study, a large sample of 2911 HD subjects with known age at onset were tested for a potential modifier effect of GRIK2 using a variety of statistical approaches and showed no evidence of a modifier effect on motor, psychiatric or cognitive age at onset (Arning, Saft et al. 2007).

Another candidate genetic modifier, which has been intensively studied, is PPARGC1A, which encodes peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α). It is a transcriptional regulator of adaptive thermogenesis, mitochondrial respiration and oxidative stress (Rodgers, Lerin et al. 2008). PGC-1α knock down produces an HD-like phenotype in mice (Lin, Wu et al. 2004; Leone, Lehman et al. 2005). Additionally, mutant, but not wild type, huntingtin has been shown to down-regulate the expression of PGC-1α and its target genes leading to mitochondrial dysfunction and neurodegeneration in mouse models of HD (Cui, Jeong et al. 2006; Weydt, Pineda et al. 2006). Several studies have reported an association of one particular polymorphism in PPARGC1A with a later age of onset of HD symptoms (Taherzadeh-Fard, Saft et al. 2009; Weydt, Soyal et al. 2009; Che, Metzger et al. 2011) and an additional study reported that polymorphisms in PGC-1α downstream target genes, namely nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) may also influence the age of onset in HD. (Taherzadeh-Fard, Saft et al. 2011). A recent replication study of 1727 HD subjects of European origin showed a significant effect in the entire cohort (Ramos, Latourelle et al. 2012). However, cases of Southern European origin had an increased minor allele frequency of the SNP consistent with it being an ancestry-tagging SNP. When the sample was controlled for ancestry, PPARGC1A was no longer significantly associated with age at onset. Interestingly, the Southern European cases, despite similar mean CAG allele size, had a significantly older mean age of onset, suggesting population differences in the genetic or environmental factors which certainly merits further investigation.

Brain-derived neurotrophic factor (BDNF) is a protein thought to be important for the maintenance of striatal neurons (Nagahara and Tuszynski 2011). There is evidence that huntingtin may play an important role in regulating BDNF expression (Zuccato and Cattaneo 2007). A functional polymorphism in BDNF is associated with a number of
altered phenotypes in mood disorders (Fan and Sklar 2008; Rybakowski 2008) and several studies have shown it to be a modifier of PD pathogenesis (Parsian, Sinha et al. 2004; Karamohamed, Latourelle et al. 2005), though a recent meta-analysis showed no detectable association (Dai, Wang et al. 2013). A number of studies have shown that this functional polymorphism does not modify age at neurologic onset in HD (Di Maria, Marasco et al. 2006; Kishikawa, Li et al. 2006; Mai, Akkad et al. 2006). BDNF continues to be studied as a potential therapeutic intervention in neurodegenerative disease including HD (Nagahara and Tuszynski 2011) but it is not yet certain whether it may play a critical role in the events that occur after motor onset or in development of other disease features in HD.

Other potential candidate genes chosen for their involvement in processes thought to be related to HD pathogenesis have been studied and found to have some effect on age of onset. GRIN2A and GRIN2B (glutamergic transmission) (Arning, Kraus et al. 2005; Arning, Saft et al. 2007); UCHL1 (protein degradation) (Naze, Vuillaume et al. 2002; Metzger, Bauer et al. 2006); TCERG1 and TP53 (gene transcription) (Holbert, Denghien et al. 2001; Chattopadhyay, Baksi et al. 2005); DFFB, MAP3K5, MAP2K6 (stress response/apoptosis); APOE (lipoprotein metabolism); HAP1 (neuronal trafficking) (Metzger, Rong et al. 2008); and MTHFR (folate metabolism)(Brune, Andrich et al. 2004). In some cases, gender stratification implied a sex-specific effect (APOE, GRIN2A, GRIN2B, MAP2K6) (Kehoe, Krawczak et al. 1999; Hansen, Saft et al. 2005; Arning, Saft et al. 2007; Arning, Monte et al. 2008). In many cases, initial positive reports have been followed by negative studies of the same polymorphisms (for example, GRN2B, UCHL1, TP53, DFFB, APOE, MTHFR) (Naze, Vuillaume et al. 2002; Saft, Andrich et al. 2004; Andresen, Gayan et al. 2007). In some cases they have not yet been confirmed (HAP1, MAP3K5, MAP2K6) or they have shown very small effects and require further confirmation in a larger sample size (TCERG1, GRIN2A) (Andresen, Gayan et al. 2007; Saft, Epplen et al. 2011). More recently a SNP in the Kalirin gene (KALRN) was investigated. Kalirin is a protein involved in spinal plasticity and interacts with huntingtin-associated protein (HAP-1). The results did not reveal an association between the analysed SNP and the age at onset in HD (Tsai, Metzger et al. 2012).
1.4.2 Genomewide Association Studies

Initial studies used genetic linkage to search for chromosome regions associated with alteration of age at neurologic onset from that expected based upon the CAG repeat length in the individuals tested. A large international collaborative study, HD-MAPS, performed this analysis in sibling pairs and small families and identified a number of regions of interest (Li, Hayden et al. 2003). A follow-up study with additional samples achieved a genome-wide significance score for a region of 6q (Li, Hayden et al. 2006). A study investigating sibships by the US-Venezuela Collaborative Group identified genome-wide linkage to 2p and several other possible regions including 6q (Gayan, Brocklebank et al. 2008). The regions implicated are large and have not yet yielded specific genes responsible for the effect.

Advances in the understanding of human genetic variation and in technologies for investigating that variation in a large number of individuals have made GWAS using densely spaced SNPs and copy number probes in HD possible. These studies are ongoing, using large sample sizes of several thousand HD subjects. GWAS studies should be able clarify the candidate genes already suggested, narrow known linkage peaks and identify new polymorphisms that exhibit association with age at neurologic onset. They should also be able to investigate the variation of phenotypes other than neurologic onset.

There are a number of potential pitfalls, however. Due to the large number of SNPs being investigated, sample size is a huge consideration for any GWAS. In HD the major genetic modifier is already known; namely the CAG repeat length. Using a GWAS approach, genetic variants that are rare, or likely to exert a small effect are unlikely to be detected. A third major challenge facing GWAS in HD is that of identifying an independent replication cohort of suitable size and power to follow up any genes identified.

1.5 Aims of this thesis

This introduction has provided a review of the aetiology, epidemiology and pathogenic processes involved in HD as well as the current state of research relating to emerging therapies, biomarkers and genetic modifiers.
The remaining chapters of this thesis will present my own research relating to developing biomarkers and identifying genetic modifiers of HD. Chapter 2 will outline the general methods relating this thesis. Methods specific to particular studies will be described in the relevant chapters.

The main aims of this thesis are;

1. To identify robust clinical and imaging biomarkers of HD onset and progression in HD patients in the longitudinal observational TRACK-HD study. (Chapter 3)

2. To investigate whether central and peripheral markers of immune activation may provide biomarkers of disease onset and progression in premanifest HD. (Chapter 4)

3. To investigate genes involved in the immune system as potential genetic modifiers of disease onset and progression in HD patients. (Chapter 5 and Chapter 6)
2 GENERAL METHODS

Four studies make up this thesis

The TRACK-HD Study
A study characterising central and peripheral immune activation in HD
A study to identify genetic modifiers of age of onset in HD
A study examining the importance of accurately estimating the age of onset in HD.

This chapter will outline the general methods relating to this thesis. However, the studies and data presented in this thesis are wide ranging and methods specific to particular studies are presented in the relevant chapters.

2.1 Consent and ethics

All studies mentioned in this thesis were carried out at approved research institutions. All experiments were carried out in accordance with the declaration of Helsinki. All human subjects gave informed, written consent to participate.

The author’s own work was all carried out at University College London’s (UCL) Institute of Neurology in conjunction with the National Hospital for Neurology and Neurosurgery, UCL Hospitals NHS Trust.

Human subjects at UCL, Institute of Neurology were enrolled into at least one of the following studies: UCLH/04/N008 (Identification of biomarkers that can be used to track the progression of Huntington’s disease), UCLH/07/H0717/47 (TRACK-HD) and UCLH/10A/VSE04/7 (The European Huntington’s Disease Network REGISTRY v3.0).

All these studies were approved by the London – Queen Square National Research Ethics Committee (formerly known as the National Hospital for Neurology and Neurosurgery and Institute of Neurology Research Ethics Committee and then Central London Research Ethics Committee 3).

The PET imaging studies to assess neuronal dysfunction and microglial activation in TRACK-HD premanifest subjects (TRACK-PET study) had additional ethical approval from the Hammersmith and Queen Charlotte’s and Chelsea Hospital Research Ethics Committee (Ethics No: 10/H0707/18).
2.2 Overall principles of subject selection

2.2.1 TRACK-HD

The TRACK-HD study and data collected as part of it forms the basis of much of this thesis. Subjects with the HD gene expansion were recruited from the National Hospital for Neurology and Neurosurgery, London, the Department of Medical Genetics at University of British Columbia, Vancouver, the Department of Genetics and Cytogenetics at the Hôpital de la Salpêtrière-Université Pierre and Marie Curie and the Department of Neurology at Leiden University Medical Centre. Recruitment targets were 90 per centre, including 30 control, 30 premanifest HD and 30 early HD. Premanifest gene carriers required a ‘disease burden score’ >250 (see section 2.6.3.1) approximating to less than 15 predicted years to onset based on Langbehn et al’s conditional onset probability calculations (Langbehn, Brinkman et al. 2004) (see section 2.6.3.2) and a total motor score of ≤5 in the UHDRS motor assessment (see appendix 1) indicating lack of significant motor signs.

Individuals in the premanifest group were divided at the group median for predicted years to diagnosis (10·8 years) into preHD-A (further from predicted diagnosis age) and preHD-B (nearer to predicted diagnosis age) on the basis of the survival analysis formula described by Langbehn as explained further in section 2.36.3.2 (Langbehn, Brinkman et al. 2004). Patients with early HD were divided into two subgroups—HD stage 1 (HD1) and HD stage 2 (HD2)—on the basis of their score on the total functional capacity scale (see Table 2). Controls were age-matched and gender-matched to individuals in the combined preHD and HD groups and were selected from the spouses or partners of individuals with premanifest or early HD or were gene-negative siblings, to ensure consistency of environments with HD gene carriers.

TRACK-HD subjects were assessed at baseline, 12 months, and 24 months. Full details of the participant demographics are shown in Chapter 3.

2.2.2 TRACK-HD PET imaging and peripheral inflammatory markers sub-study

Samples and data from subjects recruited to the ‘Identification of biomarkers that can be used to track the progression of Huntington’s disease’ study at UCL Institute of
Neurology were used in the initial part of this study examining the activity of monocytes in a large cohort of HD gene carriers. Subsequently, 12 Premanifest gene carriers enrolled in TRACK-HD London Site were also recruited to the TRACK-HD PET imaging and inflammatory markers sub-study. They were chosen to be representative of the premanifest cohort and also due to their willingness to undergo further imaging and blood sample donation. The study was run in parallel with the 24-month time point of TRACK-HD and corresponded to visit 3. Further details of the subjects enrolled in this study are presented in Table 10.

2.2.3 Genetic Modifiers and Age of Onset Study

Samples from subjects recruited in TRACK-HD, who had a recorded age of onset at the 12 month time point (visit 2), were analysed as part of the Genetic Modifiers of Age of Onset in Huntington Disease and The Importance of Accurately Recording Age of Onset in Huntington’s Disease studies.

In addition to those samples analysed from TRACK-HD, samples were also requested from European Huntington’s Disease Networks (EHDN) REGISTRY study. The EHDN (http://www.euro-hd.net) was established in 2004 and is a collaborative network of HD researchers, clinicians and HD family members. REGISTRY is a multicentre, prospective observational study with annual follow-up visits. The participants are manifest and premanifest HD gene carriers, individuals at risk of HD, gene-negative family members and controls (no family history of HD). At August 2010, REGISTRY included 6476 participants from 136 study sites in 16 countries (Orth, Handley et al. 2011). Of these, 663 were pre-manifest (with a diagnostic confidence score of <4 on the UHDRS motor scale as shown in Table 1) and 375 spouse or companion controls. Data from at least two visits was available for 3473 participants.

The EHDN is committed to facilitating HD research. Data and biomaterials collected in the REGISTRY database are available to investigators via application to the Scientific and Bioethics Advisory Committee (SBAC). For inclusion in the ‘Genetic Modifiers of Age of Onset in Huntington Disease’ and ‘The Importance of Accurately Recording Age of Onset in Huntington’s Disease’ studies a proposal was submitted requesting access to samples for which the information in Table 4 was available.
<table>
<thead>
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<th><strong>eCRF category</strong></th>
<th><strong>Information Requested</strong></th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>Date of Birth</td>
<td>Mandatory</td>
</tr>
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<td>Sex</td>
<td>Mandatory</td>
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<td>Ethnicity</td>
<td>Mandatory</td>
</tr>
<tr>
<td>HD diagnosed</td>
<td>Mandatory</td>
</tr>
<tr>
<td>Symptoms first noted by subject</td>
<td></td>
</tr>
<tr>
<td>Symptoms first noted by family</td>
<td></td>
</tr>
<tr>
<td>Rater's estimate of onset</td>
<td>Mandatory</td>
</tr>
<tr>
<td>Predominant Symptom noted at onset</td>
<td></td>
</tr>
<tr>
<td>CAG repeat length large</td>
<td></td>
</tr>
<tr>
<td>CAG repeat length; small</td>
<td></td>
</tr>
<tr>
<td>Total Motor Score (all visits)</td>
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</tr>
<tr>
<td>Diagnostic Confidence Interval</td>
<td>Mandatory</td>
</tr>
<tr>
<td>Total Functional Capacity</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Information requested from the REGISTRY database

### 2.3 Data Collection

For both TRACK-HD and REGISTRY, data are entered online using an electronic web-based data capture system. Electronic case report forms (eCRF) have been translated into several languages allowing data to be collected in the local language. Entries for medication are coded according to the Anatomical Therapeutic Chemical classification (http://www.whocc.no.atcddd), and co-morbidities are coded according to ICD-10 (W.H.O. 1992). To ensure the highest quality data, entries onto the web portal are subject to automatic plausibility checks. Study site investigators are annually trained, assessed and certified to reduce inter- and intra-rater variability. Following data entry, monitors fluent in the language of the contributing study site monitored data online and on-site.

For the ‘Identification of biomarkers that can be used to track the progression of Huntington’s disease’ study, biosamples were collected from participants and clinical and demographic data recorded in a secure database housed at UCL, Institute of
2.4  Clinical assessments

2.4.1 TRACK-HD

The study was planned as a longitudinal study with three annual time-points. Full enrolment at each time point was completed over an eight month time period. In most cases, data were collected in a single visit including demographic information, motor tests, quantitative motor tests, cognitive tasks, neuropsychiatric interviews and questionnaire measures, functional/quality of life measures and 3Tesla (3T) brain magnetic resonance imaging (MRI). A small team of study site investigators at each site assessed subjects. Raters underwent training to perform assessments to decrease inter- and intra-rater variability. Training was updated at each time point and raters remained the same, wherever possible. Further details of the clinical assessments used in TRACK-HD are given in Chapter 3.3.

2.4.2 REGISTRY

At each study site, clinicians with longstanding experience in HD take a careful history and examine patients clinically; motor, psychiatric and cognitive signs are scored using the UHDRS (HSG 1996). Additional self-rating scales of mood, quality of life and health economics are available for some subjects (see Figure 12). Disease stage is derived from the TFC scores.
2.5 Biosample Collection

All blood samples were collected from the antecubital fossa.

2.5.1 CAG genotyping

To overcome known discrepancies in reporting CAG repeat lengths (Quarrell, Handley et al. 2012) all HD gene carriers included in this thesis underwent CAG repeat sizing carried out by Biorep® Technologies Inc., (Milan, Italy). DNA was extracted using standard techniques using an NA3000 automated DNA extractor (AutoGen, MA). Repeat lengths were analysed by fluorescent PCR amplifying the triplet repeat region followed by size fractionation and fragment sizing using an Applied Biosystems 3730XL genetic analyser and GeneMapper software (Applied Biosystems, CA).
2.6 Statistical methods

Due to the differences between the studies presented in this thesis, this section is limited to topics of relevance to all studies, or those applying to many aspects of one study. Specific analyses are discussed separately in the statistical methods sections of the following chapters.

Data were collated in Microsoft Excel 2007 (Microsoft Inc.). Statistical analysis was carried out using Stata Statistical Software: Release 10. (StataCorp. 2007. College Station, TX: StataCorp LP.) and GraphPad Prism software version 6.0a for Mac OS X, (GraphPad Software, Inc., San Diego California USA, www.graphpad.com) and PLINK (Purcell, Neale et al. 2007).

2.6.1 Tests for normality of data

Linear regression modelling and other parametric statistics rely (among other requirements) on the data in question having a normal (Gaussian) distribution. Formal statistical testing for whether data are normally distributed can be performed, but such tests can fail to detect non-normality in small data sets and in larger data sets may falsely infer that a small or localised deviation from the normal distribution is ‘not significant’. Instead, visual comparison of the distribution of the data was used to establish normality or otherwise, by inspection of simple histograms and comparisons of data quantiles against those of a normal distribution with equivalent parameters.

2.6.2 Handling of non-normal data

Three options were available for the statistical analysis of data that were not normally distributed. Non-parametric tests of hypothesis can be used. Simple mathematical adjustment of data can be attempted to transform the data into a normal distribution (e.g. log-transformation) but such transformation is not always possible. Finally, the data can be resampled using a bootstrapping technique. This involves repeatedly constructing a new sample set based on random resampling a subset of subjects within a sample to produce a new data set; some subjects may be sampled more than once and others may be omitted. This is repeated many times (typically 2-3000). It enables the use of the same parametric statistical models as were applied to normally distributed
data, including covariates, without having to apply specific mathematical transformations. In this thesis, all three methods were employed. The specific test is mentioned where appropriate.

2.6.3 Modelling predictors of disease course

2.6.3.1 Disease Burden Score

The burden of pathology score has been used in HD biomarker studies to assess the relation among variables of interest and the estimated burden of disease (Golding, Danchaivijitr et al. 2006; Kloppel, Draganski et al. 2008). Penney and colleagues (Penney, Vonsattel et al. 1997) showed that the degree of post-mortem striatal pathology was predicted by age at death and the length of the CAG repeat. On the basis of this observation, Sanchez-Pernaute (Sanchez-Pernaute, Garcia-Segura et al. 1999), who was studying striatal MRI abnormalities in vivo, found a similar relation between age and the length of the CAG repeat.

The burden of pathology score was proposed as an index of disease burden on the basis of these findings. The score is calculated from a formula; \((age \times [CAG - 35.5])\), (Penney, Vonsattel et al. 1997) and functions are calculated as a simple estimate of an individual's lifetime exposure to mutant huntingtin, at any age, before and after motor onset. Other authors have reported that effect sizes could be estimated for different stages of preHD (Paulsen, Hayden et al. 2006; Paulsen, Langbehn et al. 2008). The disease-burden score was used in TRACK-HD to optimise the recruitment of individuals with premanifest HD to compile a cohort with the best chance of showing detectible changes in one or more outcome measures over the course of the study. A more broad-ranging preHD sample would result in smaller differences between the preHD groups and controls and would have required a larger sample size to detect group differences, a possibility that is prohibited by the limited time and funds available for the study.

There are a number of limitations to using the disease burden score as a predictor of disease course in analyses, as has been pointed out by others (Rosenblatt, Margolis et al. 1998). First, it is derived from cross-sectional post-mortem data, with its inevitable bias towards end-stage brain changes (which can only be avoided by limiting study to subjects who have died prematurely from causes unrelated to HD). The validity of
using such data to make inferences about the progression of pathology in vivo is limited. Second, the study of Penney et al. was based on quantification of caudate atrophy, which, based on MRI measurements, is certainly selectively increased early in the disease course but does not appear to increase linearly throughout the disease as Penney and colleagues propose (Aylward, Sparks et al. 2004), nor is there any reason to believe that the trajectory of caudate atrophy is capable of acting per se as a universal marker of pathology.

2.6.3.2 Conditional onset probability calculation

A more widely accepted measure of the combined contributions of age and CAG is the conditional onset probability calculation of Langbehn and colleagues (Langbehn et al. 2004). Based on a very large cohort of 2913 individuals from 9 countries, it uses a parametric survival model to predict for a given subject the probability $p$ that they will remain disease-free for a specified number of years (conventionally five):

$$p = 1 - \left(1 + e^{\frac{\pi}{\sqrt{3}} \times e^{\frac{-1}{17.71 - 0.327 \times \text{CAG}}}} \times \frac{\sqrt{35.55 + e^{\frac{-1}{17.71 - 0.327 \times \text{CAG}}}}}{\sqrt{35.55 + e^{\frac{\text{age}_{\text{now}} - 21.54}{1 + e^{\frac{\pi}{\sqrt{3}} \times e^{\frac{-1}{17.71 - 0.327 \times \text{CAG}}}}}}}} \times \frac{\sqrt{35.55 + e^{\frac{\text{age}_{\text{onset}} - 21.54}{1 + e^{\frac{\pi}{\sqrt{3}} \times e^{\frac{-1}{17.71 - 0.327 \times \text{CAG}}}}}}}}{\sqrt{35.55 + e^{\frac{\text{age}_{\text{now}} - 21.54}{1 + e^{\frac{\pi}{\sqrt{3}} \times e^{\frac{-1}{17.71 - 0.327 \times \text{CAG}}}}}}}}\right)$$

Where $\text{age}_{\text{now}}$ is the subject’s current age, $\text{age}_{\text{onset}}$ is the projected disease-free age (e.g. $\text{age}_{\text{now}} + 5$) and CAG is the subject’s CAG repeat length.

This can be rearranged to predict, for a given individual, the number of years of disease-free life that must elapse before they reach a conditional onset probability of interest (conventionally 50 or 60%):

$$\text{yearstoonset} = \frac{\sqrt{3}}{\pi} \times \ln \left(\frac{1 + e^{\frac{-1}{17.72 - 0.327 \times \text{CAG}}}}{1 - p} \times \sqrt{35.55 + e^{\frac{17.72 - 0.327 \times \text{CAG}}{1 + e^{\frac{-1}{17.72 - 0.327 \times \text{CAG}}}}}} + 21.54 + e^{\frac{-1.956 - 0.146 \times \text{CAG}}{1 + e^{\frac{-1}{17.72 - 0.327 \times \text{CAG}}}}} - \text{age}_{\text{now}}\right)$$

Where $\text{age}_{\text{now}}$ is the subject’s current age, CAG is the subject’s CAG repeat length and $p$ is the conditional onset probability of interest.
Conditional onset probabilities calculated using this method have several advantages. First, they are based on genuine population data from a large sample using the most widely agreed-upon event in premanifest HD: diagnosis of motor onset. Second, they make no assumptions of linearity of progression but, rather, attempt to model the non-linearity of onset probability. Third, unlike models that simply use an individual’s CAG repeat length to predict their probability of onset at a given age, this conditional model makes use of the additional clinical information that the individual has survived to $age_{now}$ without developing motor signs, to modify the onset probability. For example, an individual with a repeat length of 44 would be predicted, at birth, to have a 77% probability of onset by the age of 50 years; but if the same individual remained disease-free at 45, their conditional probability of onset by the age of 50 would be reduced to 53%, because of the information about their genotype-phenotype concordance encoded in 45 years’ disease-free survival.

Because of these advantages, the Langbehn et al. model is widely used in the study of premanifest gene carriers (Paulsen, Hayden et al. 2006). However, though the modelling of the interaction between CAG and age is considerably more sophisticated than in the Penney et al. formula, because the model was developed in premanifest subjects, and moreover carries the assumption of a lack of motor signs at $age_{now}$, it would be meaningless if applied to subjects with manifest disease and cannot be used to carry out analyses across both groups.

The chapters following will present the experimental work related to this thesis. As mentioned at the start of this chapter, the methods specific to each study will be presented in the relevant chapters.

Chapter 3 presents the longitudinal observational biomarker study; TRACK-HD

Chapter 4 presents a study characterising central and peripheral immune activation in HD

Chapter 5 presents a study to identify genetic modifiers of age of onset in HD and

Chapter 6 presents a study examining the importance of accurately estimating the age of onset in HD.

The final conclusions arising from this thesis will be presented in Chapter 7.
3 TRACK-HD

3.1 Introduction

Onset of HD is defined as the unambiguous presence of an otherwise unexplained movement disorder. This diagnostic practice, as already discussed, disregards the fact that many patients show cognitive or behavioural disturbances many years before onset of motor symptoms. In addition, sub-clinical changes are known to precede the onset of overt clinical manifestations as discussed in Chapter 1.2.

Though the first trials of potential disease modifying interventions are likely to be carried out in early symptomatic patients, the optimal point at which to introduce disease modifying agents in the hope of delaying symptom onset or slowing the rate of disease progression is likely to be in the premanifest stage before the onset of rapid neuronal degeneration and emergence of clinical symptoms. In order to trial potential treatments, it is, therefore, necessary to identify sensitive and stable markers of change in both premanifest and early HD patients. Current clinical rating scales lack sensitivity, display floor or ceiling effects particularly in premanifest subjects, and require long observation periods to unequivocally demonstrate change. Improvements in the precision of objective measurement of disease progression in premanifest and early stage HD could lead to biomarkers better able to assess progression and measure the effects of therapeutic interventions.

The genetic predictability of HD affords a unique opportunity to examine the pattern of signs and symptoms and neurobiological changes as they emerge. The aim of TRACK-HD was to provide the essential methodological advances required to optimise disease-modifying clinical trials. The study was designed using similar principles to a clinical trial with rigorous quality assurance and quality control and blinded data analysis.

3.2 Contributions and Collaborations

Sarah Tabrizi was global Principal Investigator (PI) of TRACK-HD based at UCL, Institute of Neurology, London, UK. Along with the other members of the TRACK-HD
steering committee, she was responsible for the study concept and design. Blair Leavitt was the PI for the University of British Columbia, Vancouver, Canada; Alexandra Durr was the PI for the Université Pierre and Marie Curie, Paris, France; Raymund Roos was the PI for the Universiteit Leiden, Netherlands. The members of the steering committee and the full list of site staff are listed in Appendix B.

I was the clinical research fellow at UCL, Institute of Neurology, London UK. Miranda Say (clinical research psychologist) and Joy Read (clinical research assistant) were the other clinical site staff in London. Together we recruited all the subjects for the London site and performed all assessments. As the clinical research fellow, it was my responsibility was to collect all clinical data and I also collected and processed all biosamples. I administered the oculomotor and quantitative motor assessments and was trained in the administration of the cognitive and neuropsychiatric assessments to act as a backup rater. I worked closely with Gail Owen, Clinical Trial Manager, on revisions to the Biosamples Standard Operating Procedure (SOP) and submission of protocol revisions for approval by the local research ethics committee. The statistical analysis was carried out by a dedicated team of statisticians under the supervision of Douglas Langbehn (University of Iowa) and Chris Frost (London School of Hygiene and Tropical Medicine). The study was funded by the CHDI Foundation.

3.3 Methods

TRACK-HD was a multi-centre, multi-national and multi-lingual observational biomarker study of pre-manifest and early HD. TRACK-HD was designed using similar principles to a clinical trial with rigorous quality assurance, quality control and blinded, pre-defined primary data analysis using a range of novel neuroimaging and clinical assessment tools to identify sensitive clinical and biological markers of HD disease progression. The protocol was also designed to be flexible, allowing any promising potential clinical tests to be incorporated during the course of the study.

TRACK-HD was designed as a prospective study for which each participant was enrolled for 24 months with assessments at baseline, 1 year and 2 years.

The analyses from the entire cohort are presented. However, only a subset of the data collected is included in this thesis, focussing on the assessments to which I contributed a significant amount data; the clinical, imaging, motor, oculomotor and quantitative motor assessments.
3.3.1 Measures

At each time point, full enrolment and testing was completed over an 8-month period. In most cases, data were collected in a single visit and included demographic information, the UHDRS-99, 3T brain MRI, quantitative oculomotor and motor tests, cognitive tasks, neuropsychiatric interview and questionnaires, functional/quality of life measures and biosample collection. Methods for the measures presented in this thesis are described below.

3.3.1.1 UHDRS-99

The UHDRS (HSG 1996; Marder, Zhao et al. 2000) assesses four major clinical domains of impairment: (1) motor, (2) cognitive, (3) neuropsychiatric, and (4) functional capacity. In devising this scale, items were selected that were likely to be sensitive to measure progression in the early stages of the illness. The UHDRS-99 has undergone extensive testing of reliability and consistency and has been shown to have a good inter-rater reliability for the total motor score (HSG 1996; Marder, Zhao et al. 2000). The UHDRS has been widely used in HD clinical trials ((Tabrizi, Blamire et al. 2005; Hersch, Gevorkian et al. 2006; Kieburtz, McDermott et al. 2010).

Motor Assessment:

The UHDRS motor examination is the gold standard for HD (see Appendix A). The total motor score (TMS) measures a range of motor features characteristically impaired in HD including gait, tongue protrusion, ocular function and postural stability. Higher scores indicate more severe motor impairment than lower scores. The raters in both TRACK-HD and REGISTRY must be certified by the EHDN UHDRS-TMS online certification (www.euro-hd.net). This requires successful rating of three sample patients, filmed during UHDRS-TMS application, within a range defined as acceptable by experts in the field (as determined by a task force of the EHDN Motor working group).

UHDRS Total Function Capacity:

The goal of the UHDRS TFC (see Appendix A) is to obtain a clinician’s assessment of the participants’ capacity to perform in each of the five functional domains including
occupation, finances, domestic chores, activities of daily living and care level. The TFC (see Appendix A) is a clinical rated 14-unit scale (range 0-13) with higher scores indicating better functioning than lower scores. It is sensitive in manifest HD and is a standard in the field for diagnosed HD.

**UHDRS Functional Checklist and Independence Scale:**

The UHDRS Functional Checklist and Independence Scale (see Appendix A) have a relatively greater focus on more basic activities of daily living (i.e. toileting, ambulation) compared to the TFC.

### 3.3.1.2 TRACK-HD Imaging

MRI data were acquired using standardized T1-weighted and T2-weighted protocols developed for the TRACK-HD study. This involved a 3D magnetization-prepared rapid gradient echo (MPRAGE) acquisition sequence on a 3T Siemens (London and Paris) or a 3T Phillips (Leiden and Vancouver) whole body scanner with the following imaging parameters (Siemens/Phils): Repetition time (TR) = 2200ms/7.7ms, Echo Time (TE) = 2.2ms/3.5ms, Flip Angle (FA) = 10°/8°, Field of View (FOV) = 28cm/24cm, matrix size 256x256/224x224, yielding 208/164 sagittal slices to cover the entire brain with a slice thickness of 1.0 mm with no inter-slice gap.

During the study, each incoming image was checked for quality. This consisted of checking the images for complete brain coverage, wrap, missing data, motion artefact, noise, inhomogeneity, flow, susceptibility, and other artefacts as well as ensuring the imaging protocol parameters had not changed from baseline to follow-up. All scans were rated on overall quality with regards to the overall image quality and the quality of deep grey structures, such as the caudate, which are to be delineated. Two T1-weighted MPRAGE scans were acquired during each scanning session to better ensure that one high quality scan was available for analysis. The quality of the images was compared during the quality control process, and a further grade was given to determine whether the T1 images were of comparable quality or if one T1 weighted image was of higher quality.

Rigorous quality control was performed on all image datasets (IXICO Ltd, London, UK) and image data were archived at Laboratory of Neuroimaging at University of
California, Los Angeles. Four image analysis techniques were applied at three image analysis specialty sites: 1) semi-automated measurements of intracranial volume (ICV) and whole-brain volumes were performed using MIDAS (Freeborough, Fox et al. 1997). 2) automated segmentation of intracranial volume (ICV), whole brain, caudate and putamen tissue volume were performed on collected images using BRAINS (Brain Research: Analysis of Images, Networks, and Systems, Iowa City, Iowa, USA (Magnotta, Harris et al. 2002); 3) cortical thickness analysis was performed using the Freesurfer method of Fischl et al (Fischl, Liu et al. 2001); and 4) voxel-based morphometry (VBM) analysis was performed using SPM5 (Statistical Parametric Mapping 5) (www.fil.ion.ac.uk/spm).

3.3.1.3 Quantitative Motor Assessments

A pre-calibrated and temperature controlled force transducer (Mini-40, ATI Industrial Automation, NC, USA) was used for all force transducer based assessments (see Figures 13-15). The force transducer had a circular plane contact surface measuring 40 mm in diameter. It could be easily attached to the different setups used for tongue and grip force assessment and tapping in a modular fashion. All data was sampled at 400 Hz, stored and analysed on a flexible laboratory computer system (WINSC/WINZOOM, University of Umeå, Sweden). All sites were equipped with identical systems and software. All data evaluation was performed blinded in the motor laboratory at the University of Munster using automated software.

Tongue Force Measurement

Isometric tongue protrusion force was measured with subjects seated upright on a chair in front of a table with their chin resting on a height-adjustable base as shown in Figure 13. The force transducer was mounted 2 cm in front of their lips. A disposable plastic cover covered the surface of the transducer. A monitor was placed 30 cm in front of the subject’s eyes presenting feedback about the tongue force exerted. Following a cueing tone subjects were instructed to open their mouth widely, protrude their tongue and generate an isometric force with their tongue matching a target force level presented as a straight line on the monitor. After 20 seconds a second cueing tone marked the end of each trial and subjects were instructed to retract their tongue. Following three test trials, four trials were recorded for each of the two target force levels “low” 0.25 N (=Newton)
and “high” 0.5 N. Variability of tongue protrusion forces (expressed as coefficient of variation) was calculated during a 15 second period prior to the second cueing tone (Reilmann, Bohlen et al. 2010).

Figure 13  Photograph illustrating the isometric tongue protrusion force assessment.

Isometric Grip force and involuntary movements measurement

Assessment of isometric grip forces and involuntary choreatic movements (orientation-index and position-index) were performed as previously described (Reilmann, Bohlen et al. 2010; Reilmann, Bohlen et al. 2011) and as shown in Figure 14. Subjects were seated upright on a height-adjustable chair with their right shoulder in front of the grip instrument and both elbows positioned at about 90° of flexion and forearms resting on a table. Following an auditory cue, subjects extended the forearms, grasped the instrument between the thumb and index finger with their right (dominant) hands, and lifted it next to a marker approximately 10 cm high to obtain standardized movements. They were instructed to hold it as stable as possible for 35 seconds; a second auditory cue signalled the end of the trial. The object was replaced on the table surface and released. The force transducer measured the grip (normal) and load (tangential) force
components (0.025N resolution) of the thumb and index finger. An electromagnetic sensor (Polhemus, VT) continuously measured the position (x, y, z; 0.75-mm resolution) and orientation (roll, pitch, yaw; 0.025° resolution) of the instrument. Five trials were conducted with both a 250g and 500g object with the dominant and non-dominant hand.

![Image](image1.png)

**Figure 14** Photograph illustrating the isometric grip force and involuntary choreatic movements assessment.

**Tapping tasks**

Force transducer based tapping was measured with the subject placing their non-dominant hand palm down on a support surface located on the table in front of the force transducer such that they could comfortably tap on the transducer surface with their index finger as shown in Figure 15. A cueing tone was presented at 1.8 Hz and subjects were instructed to tap at the same rate as the cue and to continue tapping at this rate when the cuing tone stopped. Five trials were performed. The subject continued to tap for 10 seconds after the cuing tone ceased. Variability of tap deviation, from the pre-
defined 1.8 Hz frequency, was calculated during this period (Hinton, Paulsen et al. 2007).

![Photograph illustrating the force transducer based tapping assessment.](image)

**Gait Assessment**

Gait analysis was measured by asking subjects to walk barefoot on an automated gait analysis carpet (Platinum GAITRite, 4.27 m length, GAITRite, Havertown, Pennsylvania, USA) as shown in Figure 16. Six walks were recorded at normal speed. Subjects started their walks 3m in front of the carpet to ensure assessment of gait in regular motion. Variability (expressed as coefficient of variation) of stride length was calculated by the software provided by GAITRite. Due to practical considerations, Gait assessments were removed from the protocol after the baseline assessments so cross-sectional data only are presented here.
3.3.1.4 Oculomotor Assessments

Eye-tracking and stimuli presentation were carried out using a Saccadometer Advanced (Ober Consulting): a head-mounted, infrared system that measured horizontal eye position based on the averaged reflectance off the inner canthi of both eyes as shown in Figure 17A. Acquisition rate was 1KHz at 12 bit resolution. Horizontal linearity was assured for ±15°. Four head mounted lasers projected the stimuli directly on a wall ~2
metres in front of the subject, and consisted of a red and green central cue and two red targets at ±10° as shown in Figure 17B. Subjects were presented with a random, centrally cued mixed pro/anti saccade paradigm (Hicks, Robert et al. 2008). Testing was carried out in normal lighting conditions and saccade latency, direction, amplitude and velocity were recorded.

Figure 17  Photograph illustrating the oculomotor assessment.
A) Set-up of the Saccadometer and B) Projection of the head mounted lasers

The first task was a prosaccade task in which a variable length central fixation (500 – 2000 ms) was followed by a 200 ms gap and then replaced by a central green cue and a red target on either the left or right. The subject was asked to look quickly at the target until it disappeared and then return their gaze to the centre when the fixation reappeared. Subjects performed one block of 40 trials; the data serving as a baseline for saccade latency and velocity.

The second task was a centrally cued mixed pro/anti saccade task with the same temporal parameters as above. After the fixation and gap, a green or red central cue would appear, along with a target on either the left or right. Subjects were asked to perform a prosaccade to target at the green cue, and an antisaccade away from target at the red cue, with the instruction to behave “quickly and accurately”. Both central cue colour, and target position were varied randomly. Subjects performed three blocks of 70 trials, with an inter-block break and reinstruction of at least one minute.
The prosaccade, and the first mixed pro/anti saccade tasks were preceded by an experimenter led, laser guided instruction set where each condition was projected onto the wall. Subjects were asked to demonstrate their comprehension of the rules before commencing the task. Data was downloaded from the Saccadometer using LatencyMeter (Ober Consulting) and checked for quality. Saccades with latencies less than 70ms and amplitudes less than two degrees were removed from the analysis. The first four trials from each block were also removed to reduce the effect of learning.

3.3.2 Statistical Analysis

The statistical analyses for TRACK-HD were complex and therefore carried out by an expert team of statisticians lead by Douglas Langbehn and Christopher Frost.

3.3.2.1 Baseline Cross-sectional Study

The five HD subgroups defined in TRACK-HD (control, preHD-A, preHD-B, HD1, HD2) were the predictor variables of interest in the primary statistical analyses. The potential confounders, controlled for in all formal analyses, were age, gender, study site, and education level (as a proxy for premorbid intelligence). For the imaging variables, education level was not controlled for but intracranial volume was controlled for. Age is a complicated factor to control for as HD measures show a non-ignorable relationship to age in control subjects and this “normal” aging relationship also is presumed present in preHD subjects who are not experiencing any notable HD impairment. Nonetheless, among HD subjects, aging is also inevitably associated with HD progression. The intent in the models used was to control for the latter consideration via division of subjects into the above-defined HD subgroups. As a secondary check, potential age-by-HD-group interactions were checked in all formal analyses and none were found among the results reported. Study site was included among confounders to help control unappreciated systematic differences or measurement biases related to site. Adjusted differences among HD subgroups and controls were estimated using linear models. All outcome measures were continuous or suitably quasi-continuous for this approach. HD subgroup (including control) membership was treated as a categorical variable, as was study site and gender. Primary comparisons of interest were formed by linear contrasts of subgroup membership. Age and education level were modelled as linear effects. The default estimation method was ordinary least squares; however,
iterative weighted least squares (WLS) estimation, with weighting by HD subgroup, was used when residual variance showed notable systematic heteroscedasticity among these groups (Draper 1998). Additionally, simple outcome transforms (inverse, log, square root) were used when these substantially improved linearity and residual normality.

TRACK-HD was designed to quantify clinically meaningful longitudinal changes over a two-year period. Sample size was determined on the basis of projected uncertainty in the resultant sample size recommendations for future clinical trials. The sample size provides ample power to detect meaningful cross-sectional difference among the HD subgroups.

3.3.2.2 24-Month Longitudinal Study

**Group Differences in 24-month change**

Each outcome was analysed separately using a generalised least squares (GLS) regression model, since GLS allows variances to differ between participant groups and also for correlations among measurements from the same participant. Participant data were only included if data from at least two of the three timepoints (baseline, 12 and 24 months) were available for a given assessment.

Non-imaging outcomes were analysed using models for absolute measures at baseline, 12 and 24 months. Many of these assessments are subject to practice effects, which would be expected to be greatest from the first to second exposure. Thus both time from baseline and an indicator variable were included; allowing for an additional practice effect between the baseline and first follow-up. To allow the linear effect of time, practice effect and average performance over all three time periods to vary according to HD status, GLS models were used with group-specific intercepts, slopes and practice effect indicators. Neuroimaging outcomes were measured directly as change between two timepoints from registered scan pairs. The response variables were either change between 0-12 and 0-24 months (whole brain, ventricular and caudate volume) or change between 0-12 and 12-24 months (WM and GM), expressed as a percentage of baseline volume.

All analyses were controlled for age, sex and study site as well as their interactions with time and practice effects (or acceleration effects). Models for non-imaging outcomes
were additionally corrected for educational level (an ordered categorical variable treated as a continuous covariate) and its interactions with time and practice effects. So as not to constrain the residual variances for different timepoints or the covariances between pairs of timepoints, unstructured covariance matrices were specified that were allowed to differ according to HD group. Marginal means at baseline, 12- and 24-month follow-up in each subgroup were computed and displayed graphically for each outcome.

Effect Sizes

Effect sizes (ES) are unit-free measures, allowing case-control differences to be compared between different variables. Standardised ES for differences in the rate of change over 24 months for each assessment were calculated as the estimated adjusted difference in longitudinal change in each gene-expanded group relative to controls, standardized by the residual standard deviation (SD) of change in the gene-expanded group. We report bias-corrected and accelerated (BCA) bootstrap 95% confidence intervals (CI) based on 2000 replications (Carpenter and Bithell 2000). (This method is an adjustment that accounts, at least partially, for non-normality in the underlying statistic and typically leads to more accurate CIs.) For selected outcomes, we applied the 24-month ES described above to calculate estimates of the sample sizes necessary to detect 50% and 20% differences in longitudinal change between placebo and treatment groups with 90% power using the standard formula based on two-group z tests (Julious 2009).

Progression analysis

The associations of each change measurement with HD progression, as defined by traditional clinical measures, were measured. For the early manifest groups, partial Pearson correlations between each variable’s rate of change and the change in the UHDRS total motor score (TMS) and total functional capacity (TFC) scores are reported. Partial correlations were corrected for age, gender, study site and education level. In several instances, the estimates were sensitive to the influence of outlying, though not necessarily invalid, measures. In these cases, we also report the rank-based (Spearman) partial correlations of residuals after least-squares regression of the affected variables on the demographic covariates.
In the preHD subjects, TMS and TFC have poor metric properties if treated as continuous, often showing no decline and highly skewed overall change distributions. Consequently we identified an objectively defined milestone, which we considered suggestive of individuals approaching clinical onset. The criterion was defined as change in one or more of three variables: a new-onset UHDRS Diagnostic Confidence Score (DCS) of 4 (the criteria for clinical diagnosis of early HD (HSG 1996)), any net 24-month decline in TFC, or an increase in TMS of 5 or more. We termed this group of individuals ‘progressors’, although recognising that the remainder may also have shown disease progression below the specified threshold. We compared change rates in the progressors versus non-progressors using an analysis of variance (ANOVA) model, adjusting for the demographic variables above and incorporated unequal within-group variances via iteratively reweighted least squares.

3.4 Results

3.4.1 Baseline Cross-sectional Study

3.4.1.1 Participants
Three hundred and eighty-five potential participants were screened, and 381 were found to meet inclusion criteria and enrolled in the study. Of these, 13 were subsequently excluded because they were unable to undergo MRI, one due to presence of stage 3 disease, and one due to participant-initiated withdrawal of consent. Thus, the final sample included 366 participants, of whom 123 were controls, with the remaining 243 people in the HD (n=123) and preHD groups (n=120). Demographic data and pathological disease burden scores are summarised in Table 5. The preHD group was significantly younger than the HD group, as expected. Groups were gender-matched by design, and controls were age-matched to the combined preHD and HD distribution. Education levels were not significantly different across groups except for the HD2 group, which had lower education levels. Table 6 shows adjusted between- and within-group differences with all p values for the imaging and motor assessments reported in this thesis.
Table 5 Demographic characteristics of the TRACK-HD Cohort at baseline

<table>
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<tr>
<th>Characteristic</th>
<th>Controls (N=123)</th>
<th>PreHD Group</th>
<th>HD-Group</th>
<th>Combined (N=120)</th>
<th>HD1 (N=77)</th>
<th>HD2 (N=46)</th>
<th>Combined (N=123)</th>
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<td>mean (SD)</td>
<td>46.1 (10.2)</td>
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<td>40.8 (8.9)</td>
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<td>Female N (%)</td>
<td>68 (55.3)</td>
<td>33 (53.2)</td>
<td>33 (56.9)</td>
<td>66 (55.0)</td>
<td>46 (59.7)</td>
<td>21 (45.7)</td>
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<td>Male N (%)</td>
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<td>25 (43.1)</td>
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<td>333.1 (30.0)</td>
<td>294.8 (47.7)</td>
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<td>Neuroimaging Measures</td>
<td>PreHD vs Controls</td>
<td>HD vs Controls</td>
<td>PreHD vs HD</td>
<td>PreHD-A vs Controls</td>
<td>PreHD-B vs PreHD-A</td>
<td>HD1 vs PreHD-B</td>
<td>HD2 vs HD1</td>
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<td>-------------------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Whole-brain volume (% of total intracranial volume)</td>
<td>-1.84 (-2.70 to -0.98)</td>
<td>-5.94 (-6.79 to -5.09)</td>
<td>-4.09 (-4.99 to -3.20)</td>
<td>-0.68 (-1.71 to 0.35)</td>
<td>-2.41 (-3.6 to -1.21)</td>
<td>-2.23 (-3.4 to -1.06)</td>
<td>-1.61 (-2.87 to -0.34)</td>
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<td>&lt;0.0001</td>
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<td><strong>Quantitative Motor and Oculomotor Measures</strong></td>
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<tr>
<td>Antisaccade error rate (%)</td>
<td>3.45 (-1.97 to 8.87)</td>
<td>26.78 (21.44 to 32.13)</td>
<td>23.34 (17.72 to 28.96)</td>
<td>-0.50 (-7.02 to 6.02)</td>
<td>8.17 (0.62 to 15.71)</td>
<td>14.16 (6.84 to 21.48)</td>
<td>13.64 (5.61 to 21.67)</td>
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<tr>
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<td>0.21</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.88</td>
<td>0.34</td>
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<td>Tongue force heavy (log CV)</td>
<td>0.30 (0.17 to 0.42)</td>
<td>1.10 (0.97 to 1.22)</td>
<td>0.80 (0.67 to 0.93)</td>
<td>0.19 (0.03 to 0.34)</td>
<td>0.23 (0.05 to 0.40)</td>
<td>0.64 (0.46 to 0.81)</td>
<td>0.13 (-0.06 to 0.32)</td>
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<td>&lt;0.0001</td>
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<td>0.18</td>
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<tr>
<td>Self-paced tapping precision, non-dominant hand* (1/sec)</td>
<td>-9.84 (-12.92 to -6.77)</td>
<td>-27.53 (-30.43 to -24.64)</td>
<td>-17.69 (-20.43 to -14.95)</td>
<td>-5.64 (-9.34 to -1.94)</td>
<td>-8.54 (-12.43 to -4.65)</td>
<td>-11.10 (-14.66 to -7.55)</td>
<td>-6.04 (-9.57 to -2.51)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
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<tr>
<td>Gaitrite stride length normal speed (log mean CV)</td>
<td>0.12 (0.04 to 0.21)</td>
<td>0.36 (0.28 to 0.44)</td>
<td>0.24 (0.15 to 0.32)</td>
<td>0.08 (-0.02 to 0.18)</td>
<td>0.09 (-0.03 to 0.21)</td>
<td>0.11 (-0.003 to 0.22)</td>
<td>0.22 (0.1 to 0.34)</td>
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<tr>
<td></td>
<td>0.0041</td>
<td>&lt;0.0001</td>
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<td>0.12</td>
<td>0.13</td>
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</tbody>
</table>

All analyses by ordinary least squares unless otherwise noted. (See statistical methods section.)
* Weighted Least Squares (WLS) analysis.

**Table 6 Results: TRACK-HD cross-sectional results.**

Adjusted between and within group differences (estimates, 95% CIs and p values). All scores are adjusted for age, gender, study site and education level with the exception of whole-brain volume which is adjusted for age, gender, study site and ICV.
3.4.1.2 Neuroimaging assessments

Simple visual inspection of representative MR images showed shrinkage of the caudate nuclei and expansion of the CSF spaces, both in preHD and early HD compared with controls (Figure 18A). Semi-automated volumetric analysis demonstrated that intracranial volume (ICV) did not differ significantly between the groups. Semi-automated whole-brain measures (as a percentage of ICV) showed a stepwise decline across the groups, with significantly reduced volumes in the preHD-B and HD groups compared with controls (Figure 18Bi). The magnitude of these volume reductions compared with controls were 0.02%, 3%, 6% and 10% in the preHD-A, preHD-B, HD1 and HD2 groups respectively. Figure 18Bii demonstrates the association between disease burden and whole-brain volume.

Cortical thinning increased in magnitude from the premanifest to the HD groups (Figure 18C). PreHD-A showed localised thinning in the posterior frontal region. In the preHD-B group there was involvement of the occipital, parietal, superior temporal and superior frontal lobes. HD1 and HD2 showed similar patterns with extensive thinning throughout the cortex and relative sparing of the anterior frontal and lateral temporal regions.
Figure 18 Whole Brain and Regional Atrophy

Figure 18A: 3T volumetric MRI scan in a 50 year old healthy control, a 55 year old preHD subject and a 49 year old early HD subject. Brain volumes shown are corrected for ICV.

Figure 18Bi: Box plot showing brain volume as a percentage of ICV in all subject groups.

Figure 18Bii: Scatter plot showing brain volume as a percentage of ICV against disease burden.

Figure 18C: Cortical thinning in subject groups compared with controls. The top panel shows statistical maps corrected using the false discovery rate and magnitude maps are shown below. Results are adjusted for age and gender.
VBM comparisons between each HD subgroup and controls suggested progressive abnormalities in both grey and WM in all groups (Figure 19). In the preHD-A group, grey matter (GM) loss was relatively circumscribed to the putamen, but also beginning to appear in the caudate. This pattern became more apparent in preHD-B, with increasingly widespread GM loss in HD1 and HD2, extending well beyond the neostriatum into the cingulate, pre-central and pre-frontal cortices, as well as into occipital, parietal, and temporal cortices. WM loss in the posterior-frontal regions was also apparent very early in the disease process, with preHD-B showing more widespread involvement than preHD-A, and more involvement still in HD1 and HD2, which appeared similar.

Overall, findings across these four independent quantitative neuroimaging techniques are striking for the consistency of evidence they provide. Brain imaging indicates that abnormalities occur prior to diagnosis, in the absence of overt motor signs, in both grey and WM and involve both cortical and subcortical regions.

Figure 19 Voxel Based Morphometry

*Statistical parametric maps of grey and WM differences in subject groups compared with controls. Age, gender, study site and ICV were adjusted for and results are corrected for multiple comparisons using familywise error at the p<0.05 level.*
3.4.1.3 Oculomotor and quantitative motor assessments

**Oculomotor:**

Antisaccade error rates showed stepwise increases across groups, with more errors in preHD-B than preHD-A, in HD1 than preHD-B, and HD2 compared to HD1 (Figure 20Ai). PreHD-A did not differ from controls. Higher error rates were also associated with higher disease burden scores (Figure 20Aii).

**Tongue force variability:**

Tongue protrusion force variability also showed stepwise increases across groups (Figure 20Bi). Variability was greater in preHD-A compared to controls, in preHD-B compared to preHD-A, and in HD1 compared to preHD-B. Variability did not differ between HD2 and HD1. This pattern of results suggests that variability in tongue protrusion force is an early sign of the disease process but that it may level off as individuals progress beyond the earliest stage of diagnosis. Tongue force variability was also clearly associated with increasing disease burden scores (Figure 20Bii).

**Self-paced tapping:**

Precision of self-paced tapping (defined as 1/SD of the deviation of taps from the training tap rate) was very sensitive, showing differences between all adjacent group pairs (Figure 20Ci). Specifically, precision was lower in preHD-A than controls, preHD-B than preHD-A, HD1 than preHD-B, and HD2 compared to HD1. Thus, self-paced tapping was sensitive from the earliest to the latest timepoints we assessed. Self-paced tapping was also clearly associated with disease burden (Figure 20Cii).

**Gait:**

Gait, measured as the coefficient of variation for stride length at normal walking speed, was less sensitive than the other quantitative motor measures across all groups, as evidenced by small effect sizes (Figure 20Di). Only the HD2 group differed significantly from controls, although when composite groups were compared (HD to controls, preHD to controls, HD to preHD), these comparisons reached significance (Figure 20Dii)
Figure 20 Oculomotor and Quantitative motor Measures

Figure 20A) Antisaccade error rate; Figure 20B) Static tongue force variability; Figure 20C) Self-paced tapping; and Figure 20D) Gait- normal speed stance, all shown as i) box plots across all groups and ii) against disease burden score for all HD gene carriers.
3.4.2 24 month Longitudinal Study

3.4.2.1 Participants

Of the 366 subjects enrolled at baseline, 347 (95%) and 332 (91%) completed 12- and 24-month follow-up assessments, respectively (Table 7). At 24-months, 10 controls (8.1%), 1 preHD-A (1.6%), 5 preHD-B (8.6%), 5 HD1 (6.5%), and 5 HD2 (10.9%) subjects had withdrawn, and a further 8 participants were unable to attend, but remained in the study. All data were analysed according to baseline subgroup. However, at 24 months, 12 (10.8%) of the premanifest group had reached a UHDRS DCS of 4, and 17 (15.3%) of the HD group met the criteria for HD Stage 3 or greater (See Table 2 and Appendix A). All participants who contributed at least two datasets were included in the statistical analysis for each assessment; the maximum numbers for analysis were 116 controls, 62 preHD-A, 55 preHD-B, 73 HD1 and 43 HD2. Details of the cohort at 24 months are shown in Table 7. The lower section of the table gives a breakdown of participants’ disease progression. For example, of the 58 participants who were designated PreB at baseline, 51 attended the 24-month assessment. Of these 51, 28 were still classified as premanifest after 24 months, while 15 had become perimanifest and 8 were diagnosed as having progressed to stage I disease. Thirteen of these preHD-A subjects had advanced to preHD-B but the “transition” from preHD-A to –B, as opposed to progression from HD1 to HD2, does not have its basis in clinical change as the calculation is merely based on CAG repeat length and age. Progressing from preHD-A to -B means only that, due to ageing, a subject is likely to be within 10·8 years to diagnosis but there is no implication that anything has changed clinically.

3.4.2.2 Imaging

Over 24 months, the preHD-B, HD1 and HD2 groups all showed statistically significantly greater mean annual change in whole-brain volume loss and ventricular expansion relative to controls (Figure 21A and B) and significantly elevated atrophy in the caudate and putamen was evident in all subgroups (Figure 21 C and D).
Figure 21 Longitudinal changes in brain volume

Mean values at baseline, 12 months and 24 months for (A) whole-brain volume, (B) ventricular volume, (C) caudate volume and (D) putamen volume. Significant change differences relative to controls over 0-12, 12-24 and 0-24 months are represented by 'p<0.05, **p<0.01, and ***p<0.001.

Figure 22 A and B show statistical parametric maps of WM and GM loss for each subgroup, indicating regions where atrophy rates differed significantly from controls. Elevated rates of WM loss were evident from the very earliest disease stage: preHD-A subjects showed loss around the striatum, corpus callosum and in the posterior WM tract; all other groups showed extensive WM loss throughout the brain. In the preHD-A group GM loss was limited to the striatum, but the preHD-B group additionally showed early occipital involvement. In early HD GM loss was widespread. Quantification
showed significantly higher rates of GM atrophy in preHD-B, HD1 and HD2 compared with controls over 24 months and WM atrophy rates were elevated in all gene-expanded groups (Figure 22).

Figure 22 Longitudinal changes in grey and WM.
Parametric maps showing regions with statistically significant atrophy in (A) WM and (B) GM over 24 months, relative to controls. Results were adjusted for age, sex, study site, and scan interval and are corrected for multiple comparisons with family wise-error at the p<0.05 level. Corresponding longitudinal plots show mean values at baseline, 12 months and 24 months. Significant change differences relative to controls over 0-12, 12-24 and 0-24 months are represented by *p<0.05, **p<0.01, and ***p<0.001.

3.4.2.3 Motor

UHDRS

Mean annual change in TMS was greater in all subgroups relative to controls (Figure 23A). Compared to the preHD groups, the mean TMS change was larger, but variability was also substantially greater in HD1 and HD2. In these two groups there was also a significant decrease in TFC relative to controls (Figure 23B).

Figure 23 Longitudinal changes in the UHDRS.
Mean values at baseline, 12 months, and 24 months for (A) UHDRS total motor score and (B) UHDRS total functional capacity. Significant change differences relative to controls over 0-12, 12-24 and 0-24 months are represented by *p<0.05, **p<0.01 and ***p<0.001
### Summary of participant numbers

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Pre HD Group</th>
<th>HD Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre A</td>
<td>Pre B</td>
<td>All PreHD</td>
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<tr>
<td>Participants at baseline (N)</td>
<td>123</td>
<td>62</td>
<td>58</td>
<td><strong>120</strong></td>
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<tr>
<td>Participants attending at 12 months (N) (and total N still enrolled)</td>
<td>116 (117)</td>
<td>62 (62)</td>
<td>55 (56)</td>
<td><strong>117</strong> (118)</td>
</tr>
<tr>
<td>Participants attending at 24 months (N) (and total N still enrolled)</td>
<td>110 (113)</td>
<td>60 (61)</td>
<td>51 (53)</td>
<td><strong>111</strong> (114)</td>
</tr>
</tbody>
</table>

### Demographics for participants with 24-month follow-up

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Pre HD Group</th>
<th>HD Group</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td><strong>46·2</strong> (10·2)</td>
<td>41·2 (8·7)</td>
<td>40·8 (9·2)</td>
<td><strong>41·0</strong> (8·9)</td>
</tr>
<tr>
<td>Female N (%)</td>
<td>62 (56·3)</td>
<td>33 (55·0)</td>
<td>28 (54·9)</td>
<td><strong>61</strong> (55·0)</td>
</tr>
<tr>
<td>Size of CAG repeat length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>NA</td>
<td>42·1 (1·8)</td>
<td>44·1 (2·4)</td>
<td><strong>43·0</strong> (2·3)</td>
</tr>
<tr>
<td>Disease burden score at baseline</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>NA</td>
<td>259·1 (30·1)</td>
<td>331·9 (29·8)</td>
<td><strong>292·6</strong> (47·3)</td>
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</tbody>
</table>

### Disease status at 24-month follow-up

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Pre HD Group</th>
<th>HD Group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PreHD-A (TMS ≤5, DCS &lt;4)</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td><strong>50</strong></td>
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<tr>
<td>PreHD-B (TMS ≤5, DCS &lt;4)</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td><strong>28</strong></td>
</tr>
<tr>
<td>Perimanifest (△TMS ≥5, △ TFC ≥1, DCS &lt;4)</td>
<td>-</td>
<td>6</td>
<td>15</td>
<td><strong>21</strong></td>
</tr>
<tr>
<td>Stage I (TFC 11-13, DCS=4)</td>
<td>-</td>
<td>3</td>
<td>8</td>
<td><strong>11</strong></td>
</tr>
<tr>
<td>Stage II (TFC 7-10, DCS=4)</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td><strong>1</strong></td>
</tr>
<tr>
<td>Stage III (TFC 3-6, DCS=4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stage IV (TFC 2-1, DCS=4)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 7 Demographic characteristics of the TRACK-HD participants and summary of disease progression at 24 months
All analyses by ordinary least squares unless otherwise noted. (See statistical methods section.)

Weighted Least Squares (WLS) analysis.

Table 6 Results: TRACK-HD cross-sectional results.

* Adjusted between and within group differences (estimates, 95% CIs and p values). All scores are adjusted for age, gender, study site and education level with the exception of whole-brain volume which is adjusted for age, gender, study site and ICV.
Quantitative Motor

Over 24 months, statistically significantly greater change in the early HD groups relative to controls was observed in the chorea orientation and chorea position indices, grip force variability, speeded tapping tap duration variability and mean inter-tap interval (Figure 25). The latter was also significantly elevated in both premanifest groups compared with controls.

Oculomotor

Over 24 months, the amplitude of the primary pro-saccade became increasingly variable in the HD2 group compared with controls (Figure 24).

Figure 24 Longitudinal change in oculomotor measures

i) Boxplot showing 0-12 and 0-24 month change and ii) longitudinal plots showing mean values and significant change differences relative to control at baseline, 12- and 24- months for prosaccade amplitude variability (s). Significant change differences relative to controls over 0-12, 12-24 and 0-24 months are represented by *p<0.05, **p<0.01, and ***p<0.001
Figure 25 Longitudinal change in quantitative motor measures

Mean values at baseline, 12 months and 24 months for (A) chorea position index, (B) grip force variability, (C) tongue force variability, 1.5N target force, (D) speeded tapping tap duration variability, non-dominant hand; and (E) speeded-tapping mean inter-tap interval, non-dominant hand. Significant change differences relative to controls over 0-12, 12-24 and 0-24 months are represented by *p<0.05, **p<0.01, and ***p<0.001
As control and premanifest HD groups are not expected to show any change in UHDRS functional capacity, this outcome was modelled and effect sizes are presented for symptomatic HD groups only. When calculating effect sizes, change over 24-months in HD groups was compared to zero expected change rather than estimated change in controls.

Table 8 (Part 1) Standardised effect sizes (95% CI) for differences in 24-month change compared to controls.
Table 8 (part 2) Standardised effect sizes (95% CI) for differences in 24-month change compared to controls.

All estimates are adjusted for age, gender, education level and study site with the exception of the neuroimaging measures which are adjusted for age, gender and study site only.
3.4.2.4 Effect Sizes

Table 8 contains the TRACK-HD assessments proposed as potential outcome measures for future clinical trials in early HD. The table shows longitudinal 24-month effect sizes, presenting differences for each subgroup compared with controls, including bootstrapped 95% confidence intervals (CI).

In terms of effect sizes for the combined early HD group, the imaging variables provided the largest effects, ranging from 1·01 (95% CI: 0·82 to 1·24) for GM atrophy to 2·04 (95% CI: 1·68 to 2·48) for caudate atrophy. Effect sizes for the quantitative motor variables ranged from 0·33 (95% CI: 0·08 to 0·60) for tongue force variability to 0·68 (95% CI: 0·39 to 1·00) for speeded tapping tap duration variability, whilst the UHDRS TMS and TFC variables provided effect sizes of 0·81 (95% CI: 0·63 to 1·04) and 0·85 (95% CI: 0·62 to 1·09) respectively. Effect sizes for the oculomotor assessments were relatively small at 0·41 (95% CI: 0·16 to 0·66).

3.4.2.5 Progression analysis

Table compares the rate of decline in the 33 preHD progressors with the remaining 78 non-progressors. All imaging measures showed higher rates of atrophy in progressors, with the exception of the putamen, which had borderline statistical significance (p=0·07). Rates of deterioration in the chorea orientation index, speeded tapping tap duration variability and grip force variability were also greater.

In the early HD group there was only modest partial correlation between changes in TMS and TFC (Pearson: -0·192, p=0·039, Spearman: -0·145, p=0·121). Most structural imaging showed similar, statistically significant associations with both worsening TFC and TMS (Table ). The exception was ventricular volume change which did not have a significant partial correlation with either measure. Putamen change was preferentially associated with TMS rather than TFC and conversely caudate change associated with TFC alone. The quantitative motor tasks showed fewer significant associations with TMS and none with TFC. Grip force variability and speeded-tapping inter-tap interval were both significantly associated with TMS change.
<table>
<thead>
<tr>
<th>24-Month Change Outcome Variable</th>
<th>Progressor N=33</th>
<th>Non-Progressors N=78</th>
<th>Progressor vs Non-Progressors (Adj Mean Diff)</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroimaging Measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-brain atrophy</td>
<td>1.083 (0.703)</td>
<td>0.604 (0.788)</td>
<td>0.480</td>
<td>0·108 to 0·771</td>
<td>0·010</td>
</tr>
<tr>
<td>Ventricular atrophy (ml)</td>
<td>11.335 (7.692)</td>
<td>7.260 (8.289)</td>
<td>4.075</td>
<td>0·404 to 7·653</td>
<td>0·030</td>
</tr>
<tr>
<td>Caudate atrophy</td>
<td>5.589 (2.231)</td>
<td>3.560 (2.407)</td>
<td>2.029</td>
<td>1·336 to 3·259</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>Putamen atrophy (%)</td>
<td>7.174 (5.148)</td>
<td>5.339 (3.620)</td>
<td>1.835</td>
<td>-0·135 to 3·784</td>
<td>0·067</td>
</tr>
<tr>
<td>White matter atrophy (%)</td>
<td>2.619 (0.574)</td>
<td>1.860 (0.637)</td>
<td>0·759</td>
<td>0·375 to 0·874</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>Grey matter atrophy (%)</td>
<td>0.399 (0.452)</td>
<td>0.216 (0.349)</td>
<td>0·207</td>
<td>0·020 to 0·394</td>
<td>0·031</td>
</tr>
<tr>
<td>Quantitative Motor Measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorea position index (log cm/s)</td>
<td>0.134 (0.379)</td>
<td>0.015 (0.357)</td>
<td>0·119</td>
<td>-0·035 to 0·261</td>
<td>0·132</td>
</tr>
<tr>
<td>Chorea orientation index (log °/s)</td>
<td>0.157 (0.348)</td>
<td>-0·071 (0.379)</td>
<td>0·235</td>
<td>0·089 to 0·381</td>
<td>0·002</td>
</tr>
<tr>
<td>Grip force variability, 250g, non-dominant hand (log CV)</td>
<td>0.132 (0.527)</td>
<td>-0·164 (0.529)</td>
<td>0·316</td>
<td>0·086 to 0·547</td>
<td>0·008</td>
</tr>
<tr>
<td>Tongue force variability, 1·5 N target force (log CV)</td>
<td>-0·179 (0.366)</td>
<td>-0·211 (0.381)</td>
<td>0·038</td>
<td>-0·123 to 0·199</td>
<td>0·642</td>
</tr>
<tr>
<td>Speeded tapping tap duration variability, non-dominant hand (log s)</td>
<td>0.127 (0.337)</td>
<td>-0·044 (0.377)</td>
<td>0·190</td>
<td>0·037 to 0·343</td>
<td>0·016</td>
</tr>
<tr>
<td>Speeded tapping mean inter-tap interval, non-dominant hand (s)</td>
<td>0.008 (0.028)</td>
<td>0.008 (0.021)</td>
<td>0·001</td>
<td>-0·010 to 0·012</td>
<td>0·845</td>
</tr>
</tbody>
</table>

Table 9 Comparisons of 24-month change in premanifest HD progressors and non-progressors.

All estimates are adjusted for age, gender, educational level and study site.
<table>
<thead>
<tr>
<th>24-Month Change Outcome Variable</th>
<th>UHDRS functional capacity (TFC) (change in score)</th>
<th>UHDRS motor assessment (TMS) (change in score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Partial Correlation</td>
<td>p-value</td>
</tr>
<tr>
<td>Neuroimaging Measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-brain atrophy (% of baseline volume)</td>
<td>0.305</td>
<td>0.003</td>
</tr>
<tr>
<td>Ventricular expansion (ml)</td>
<td>-0.108</td>
<td>0.294</td>
</tr>
<tr>
<td>Caudate atrophy (% of baseline volume)</td>
<td>0.260</td>
<td>0.011</td>
</tr>
<tr>
<td>Putamen atrophy (% of baseline volume)</td>
<td>0.017</td>
<td>0.874</td>
</tr>
<tr>
<td>Grey matter atrophy (% baseline)</td>
<td>0.362</td>
<td>0.0003</td>
</tr>
<tr>
<td>White matter atrophy (% baseline)</td>
<td>0.250</td>
<td>0.015</td>
</tr>
<tr>
<td>Quantitative Motor Measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorea position index (log cm/s)</td>
<td>-0.053</td>
<td>0.582</td>
</tr>
<tr>
<td>Chorea orientation index (log °/s)</td>
<td>0.052</td>
<td>0.592</td>
</tr>
<tr>
<td>Grip force variability, 250g, non-dominant hand (log CV)</td>
<td>-0.071</td>
<td>0.462</td>
</tr>
<tr>
<td>Tongue force variability, 1.5 N target force (log CV)</td>
<td>0.148</td>
<td>0.139</td>
</tr>
<tr>
<td>Speeded tapping tap duration variability, non-dominant hand (log s)</td>
<td>0.073</td>
<td>0.456</td>
</tr>
<tr>
<td>Speeded tapping mean inter-tap interval, non-dominant hand (s)</td>
<td>-0.034</td>
<td>0.727</td>
</tr>
</tbody>
</table>

Table 10 Partial correlations of change rates between TRACK-HD measures and the UHDRS TFC and TMS in early HD

All values adjusted for age, gender, educational level and study site.

3.5 Conclusions

3.5.1 Baseline Cross-Sectional Study

The cross-sectional TRACK-HD study collected data from rigorous assessments that included novel multi-site applications of 3T MRI, quantitative motor, cognitive, and neuropsychiatric methods. The findings have shown an increasing separation of the disease course of preclinical HD from healthy individuals at an early stage, in a cohort of premanifest HD gene carriers with no or minimum motor signs, and continuing into stage 2 disease. The results from the TRACK-HD cross-sectional data analyses build on what has been learned from PREDICT-HD (Paulsen, Hayden et al. 2006; Paulsen, Langbehn et al. 2008) and other studies to show that preHD findings can be detected,
even in the absence of early clinical motor signs, and that some measures are sensitive to disease effects across a broad range of stages. The observations support the hypothesis that neuronal dysfunction occurs many years before the development of the motor signs that are diagnostic of HD.

Using advanced imaging techniques structural changes in many brain regions were detected. Even in the premanifest stages, atrophy is not confined to the striatum; whole-brain volume loss is seen in the preHD-B group, who are up to 11 years from predicted age of onset, as well as cortical thinning, particularly in the posterior regions of the brain, with whole-brain volumes being reduced by 0.8%, even in those furthest from onset. Atrophy becomes increasingly widespread as the disease progresses through stages 1 and 2, which is in agreement with other reports (Rosas, Liu et al. 2002; Paulsen, Magnotta et al. 2006; Henley, Wild et al. 2009). In accordance with previous studies (Aylward, Sparks et al. 2004; Ciarmiello, Cannella et al. 2006) Voxel-based morphometry and volumetric measures showed volume reductions in the caudate and putamen in the preHD-A group, who are up to about 16 years from expected disease onset. There were no significant differences in the volume of the caudate or putamen between HD stage 1 and 2 showing that the rate of volume loss seems to slow early in the disease. Striking WM loss was evident before motor onset, which is consistent with other reports (Ciarmiello, Cannella et al. 2006) and suggests that a loss of connectivity might underlie many of the early clinical decrements. These findings support the reduction in functional connectivity in premanifest individuals that has been reported previously (Wolf, Vasic et al. 2007).

The results show that there are subtle deficits in motor coordination in individuals more than a decade before a diagnosis of HD. Variability in tongue force is a useful measure in the premanifest stage of HD, converting a clinical impression (unsteady tongue) into a quantitative measure. The variability in the timing of voluntary finger taps was significantly different between all sub-groups, showing that impairment of voluntary movements is a feature of the disease, even in premanifest HD gene carriers, up to 16 years before the predicted onset. Gait analysis was a less sensitive measure for separating subgroups, although differences between controls, all premanifest, and symptomatic individuals were significant. For this reason, and for a number of practical reasons, gait analysis was not included in the longitudinal analysis. The oculomotor antisaccade error rate also increased proportionally with disease progression.
These results show that individuals who are classified as “premanifest” on the basis of current definitions (HSG 1996; Munoz and Everling 2004) are not “pre-motor” in the sense that they have no reproducible, measurable motor abnormalities. They have also reinforced that the current convention of defining the onset of HD as the onset of the movement disorder does not do justice to the full spectrum of presentations of this heterogeneous condition. The presence of neuronal dysfunction while individuals are still functioning at a high level and in full employment suggests that disease-modifying interventions should be initiated in the premanifest phase; while functional capacity is still within normal range and any deficits might be fully reversible. Finally, the strong association between pathological disease burden scores and most phenotypic features, biological (MRI) and clinical read-outs, suggest that a panel of markers to detect these early changes will enable testing of novel therapeutic drugs in this population, which have been tested in the longitudinal study.

3.5.2 24-month Longitudinal Study

There is clear, widespread change over time in the early HD groups. Whole-brain, GM, WM and striatal atrophy measures proved highly sensitive, with caudate volume loss showing an effect size of 2·18 (95% CI:1·54 to 3·69) for the HD2 group. Variability of speeded tapping tap duration, the most sensitive of the quantitative motor assessments, had an effect size of 1·10 (95% CI: 0·65 to 2·19), larger than that of the most widely used clinical assessments in HD: TMS (0·91; 95% CI: 0·57 to 1·50) and TFC (0·83; 95% CI 0·53 to 1·6). Oculomotor deficits were statistically significant in HD2 only and, in its current form, the oculomotor protocol does not show promise for clinical trial application.

Imaging markers appear to be the most effective in detecting disease-related group differences, over both 12- and 24-month intervals. However, before they are adopted as outcome measures for use in clinical trials, it is vital to establish their relationship to functional measures. Associations between changing structural brain measures and underlying genetic burden risk have been documented (Bechtel, Scahill et al. 2010; Tabrizi, Scahill et al. 2011). The 24-month data has now shown an association between the progression of regional and whole-brain atrophy and advancing clinical progression (as defined by decline in the TFC and TMS). There are, however, significant challenges to using imaging as an outcome measure in clinical trials of HD. Firstly, changes in
imaging outcomes in response to a treatment may not necessarily be accompanied by functional improvements, and secondly, improved clinical function may occur in the absence of observed changes in imaging measures. Therefore a wide range of functional measures in conjunction with imaging measures will be required. It is unlikely, in fact, that any single modality will be sufficient to assess the impact of a treatment.

In contrast to findings in the early HD group, the 24-month data have not revealed robust outcomes to track disease progression during the premanifest stages. Striatal and total WM atrophy appeared to be the most sensitive measures, with significantly greater rates in premanifest than in control subjects, even in the preHD-A group, where the estimated time to manifest HD is greater than 10 years. However, despite this cumulative structural brain loss, there was limited decline in quantitative motor and oculomotor measures. These findings are important because they demonstrate that over a 24-month period, there is limited functional decline in this group, despite striking progressive brain changes. It is likely that functional reorganisation of neural networks accompanies this widespread structural loss, and protects against functional deficits in premanifest HD (Eidelberg and Surmeier 2011). Symptom onset may occur when this functional neural plasticity is no longer able to compensate for the progressive neurodegenerative process.

3.6 Discussion

A major goal of TRACK-HD has been to discover potential outcome measures for therapeutic trials in HD. Using highly standardised data collection across multiple sites, blinded quality control and centralised, independent statistical analysis, assessments from multiple domains have shown detectable disease-related change over 24 months. The study was able to suggest outcome measures suitable for proof-of-concept and future phase II and III studies of potential disease-modifying agents in early HD. These validated measures represent a pool from which a custom-made battery can be constructed, depending on the requirements of the trial.

The results from the cognitive and neuropsychiatric assessments have not been presented here. There was clear evidence of measurable longitudinal cognitive decline in early DH. SDMT, Stroop word reading and the indirect circle tasks had effect sizes of greater than 1.00 in the HD2 group. Neuropsychiatric measures, with the exception of PBA apathy, did not show significant change over the 24-month interval (Tabrizi,
Reilmann et al. 2012). The relative insensitivity of the quality of life scales show the need for a more sensitive measure of HD-specific assessments for assessing response to therapeutic intervention.

TRACK-HD attempted to mimic the recruitment standards and procedures, training methods and site selection for a clinical trial involving a small number of research sites. Large trials will require the inclusion of multiple sites, and considerable resources need to be invested to ensure between-site consistency and good quality control. This will also allow the assessment of site-specific effects and reliability, which are essential for the translation of experimental trial efficacy to clinical effectiveness.

3.7 Suggestions for future study

The potential outcome measures recommended by the TRACK-HD study await validation in clinical trials in early stage disease. The TRACK-HD study was extended based on the findings from the 24 month timepoint and have recently been published (Tabrizi, Seahill et al. 2013). In the preHD-B group several quantitative motor and cognitive tasks shown significantly increased rates of decline at 36 months compared with controls, whereas few had at 24 months. There remained little evidence of reliable change in non-imaging measures in the preHD-A group. Studies focussed on identifying potential biomarkers, using more challenging tasks which may show increased sensitivity, in the premanifest stages of HD have been initiated in the TRACK-ON study (Tabrizi 2012). This study will also investigate the hypothesis that compensatory functional neural networks exist, but are eventually overwhelmed in the preclinical phase of HD neurodegeneration.
4 CHARACTERISING CENTRAL AND PERIPHERAL IMMUNE ACTIVATION IN HD

4.1 Introduction

There is evidence of microglial activation in the pathogenesis of HD (Sapp, Kegel et al. 2001; Giorgini, Guidetti et al. 2005). There is evidence from previous PK PET imaging studies that microglial activation is an early event in HD (Tai, Pavese et al. 2007; Tai, Pavese et al. 2007) and that microglial activation, as measured by PK PET imaging, increases with severity and may therefore provide a biomarker for HD disease progression. However, PET imaging is challenging in HD as it is time-consuming, requires patients to remain still for long periods of time and is expensive, thereby limiting its usefulness in large studies and reducing its utility as a potential outcome measure in clinical trials.

As described in section 1.3.2, peripheral immune dysfunction is also described in HD with increased levels of plasma cytokines detectable in premanifest HD. In addition patient monocytes are hyper-reactive, producing increased levels of IL-6 upon stimulation with LPS in vitro (Bjorkqvist, Wild et al. 2008).

Firstly, the aim of this study was to extend these findings to include other cytokines by examining the activity of monocytes in a large cohort of HD gene carriers. Secondly, by performing PK PET imaging in a sub-group of TRACK-HD premanifest subjects, the aim was to evaluate the use of PK PET as a potential marker of disease onset and progression. Thirdly, by performing PK PET imaging and monocyte stimulation studies, in parallel, in a cohort of premanifest gene carriers, the aim was to evaluate the possibility that peripheral production of pro-inflammatory cytokines may reflect central microglial activation and thus may provide a useful alternative to PET scanning as a biomarker of disease onset and progression.

4.2 Contributions and collaborations

The study was designed jointly by the author with M Politis, S Tabrizi, P Piccini. The author performed subject recruitment, clinical characterisation and blood sample processing. Monocyte stimulation experiments were performed by the author with support from R Andre, A Magnusson and U Träger. PET imaging was performed at the
Centre for Neuroinflammation and Neurodegeneration, Imperial College London and image data processing was performed by M Politis. Statistical analysis and data interpretation was performed by the author with input and advice from M Politis, S Tabrizi and P Piccini.

4.3 Methods

4.3.1 Subject recruitment

4.3.1.1 Monocyte Stimulation Experiments

Samples from 53 HD gene carriers, ranging from premanifest to moderate-stage disease, and control subjects were collected for the initial monocyte stimulation experiments. Subjects were recruited from the ‘Identification of biomarkers that can be used to track the progression of Huntington’s disease’ study as described in section 2.2.2. Subject demographics are shown in Table 9.

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Number</th>
<th>Age (mean ± SD)</th>
<th>CAG (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27</td>
<td>45.4 ± 11.7</td>
<td></td>
</tr>
<tr>
<td>Premanifest HD</td>
<td>17</td>
<td>42.2 ± 9.9</td>
<td>42.8 ± 2.1</td>
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<tr>
<td>Early HD (stage 1&amp;2)</td>
<td>22</td>
<td>49.1 ± 11.7</td>
<td>43.5 ± 2.8</td>
</tr>
<tr>
<td>Moderate HD (stage 3)</td>
<td>14</td>
<td>59.4 ± 7.3</td>
<td>43 ± 1.3</td>
</tr>
</tbody>
</table>

Table 9 Demographic details of subjects participating in the HD monocyte stimulation study

4.3.1.2 TRACK-PET study

12 premanifest subjects already enrolled in TRACK-HD at the London Site were recruited to take part in the TRACK-PET sub-study. The subjects were recruited to be representative of the TRACK-HD premanifest population. The full demographic and clinical details are shown in Table 10. The probability of being diagnosed with HD in the next 5 years was estimated using the algorithm published by Langbehn and colleagues (Langbehn, Brinkman et al. 2004) see section 2.6.3.2.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
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<th>5-year probability of Disease Onset</th>
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<td>M</td>
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<td>0.28</td>
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<td>F</td>
<td>29.6</td>
<td>47</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Mean ± SD 41.14±7.84 43.67±2.42 0.32±0.12

Table 10 Demographics of Premanifest Subjects enrolled into TRACK-PET study

4.3.2 Functional Studies of Human Monocytes

Whole blood was collected in heparin (CP Pharmaceuticals). Leukocytes were isolated by density gradient centrifugation over Histopaque 1077 solution (Sigma-Aldrich). Monocytes were obtained by magnetic sorting to increase yield and minimize handling time. Mononuclear cell suspensions were labelled with anti-CD14 microbeads and sorted through magnetic cell separation columns (Miltenyi Biotec) to at least 95% purity. Monocytes were counted and 5 x 10^5 cells per well were seeded into 24-well culture plates in RPMI culture medium supplemented with 5% FBS, 2mM L-glutamine, and 1% penicillin/streptomycin (Invitrogen). Cells were incubated for 16 hours before stimulation. The medium was then changed to fresh culture medium with or without 10ng/ml IFN-γ and LPS (R&D Systems). After 24 hours, supernatants were harvested from two separate wells for each subject/condition. The cells remaining were lysed in 50mM Tris, pH 8, 150 mmol NaCl, 0.5% sodium deoxycholate, and 0.5% Triton X-100 and assayed for total protein concentration using a protein assay kit according to the manufacturer’s instructions (Bio-Rad Laboratories). Cytokine concentrations were quantified using Meso Scale Discovery (MSD) assays as per the manufacturer’s protocol and analysed on a SECTOR 2400 instrument (MSD).

For the initial monocyte stimulation experiments, multiplex ELISA assays for inflammatory cytokines IL1β, Il-6, IL-8, IFNγ, TNFα, IL-12p70 and IL-10 were carried out using the mesoscale platform (MSD).
For subsequent monocyte stimulation experiments, multiplex ELISA assays for inflammatory cytokines IL1β, IL-6, IL-8, IFNγ, TNFα were performed using the mesoscale platform (MSD).

Cytokine production by LPS-stimulated monocytes was normalised to the cytokine production of non-stimulated cells from the same subject. Cytokine production by non-stimulated monocytes was very low with less than 10pg/ml for all cytokines besides IL-8, for which levels were around 1ng/ml in supernatants from non-stimulated cells. Cytokine production by non-stimulated cells did not differ between control and HD subjects.

4.3.3 $^{11}$C-(R)-PK11195 Imaging

The PK PET scans were carried out within 3 months of TRACK-HD visit. To prevent any possible effect of the ligand on the TRACK-HD volumetric MRI data, the PET scan was always performed after the TRACK-HD visits. Control data for PET scans were taken from existing age and sex matched normal control data.

4.3.3.1 Imaging methods

The PK PET scan was performed using the GE Discovery RX PET/CT scanner that has an axial field of view (FOV) of 15.7 cm. The mean image transaxial resolution over a 10 cm radius FOV (from the centre) is $5.4 \pm 0.5$ mm (2D and 3D modes) and the axial resolution is $5.6 \pm 0.8$ (2D) and $6.2 \pm 0.4$ (3D) (Kemp, Kim et al. 2006).

The mean injected dose for PK was 360 MBq (range: 344-372 MBq) and scanning began 30s before tracer infusion as an intravenous (iv) bolus, generating 18 time frames of tissue data over 60 min. PK tracer was supplied by Hammersmith Imanet plc, London.

Subjects were positioned supine with their transaxial planes parallel to the line intersecting the anterior-posterior commissure (AC–PC) line. Head position was maintained with the help of individualized foam holders and monitored by video. Subjects were scanned at rest in a quiet room with low light. Smoking and consumption of alcohol, coffee and other caffeinated beverages were not allowed for 12 hours before scanning. Eating and drinking were not allowed for 8 hours before PET scanning.

Each subject also received a a T1 Volumetric (TR = 1900, TE = 3.53, TI = 1100, Flip angle 15, 1mm isotropic voxels) and T2-weighted (Axial T2-spin echo: TR = 4540, TE
MRI sequences were also undertaken for purposes of PET co-registration, to facilitate with localising the ROIs and for excluding secondary structural brain pathology. MRIs were performed using a clinical 1.5-Tesla (T) MRI system (Siemens MAGNETOM Avanto, Ehrlangen, De).

4.3.3.2 Imaging Data Analysis

Following reconstruction of the dynamic PK image volume, a summed image volume was created from the entire dynamic dataset. The input function was derived using the SUPERPK software package (Imperial Innovations) (Turkheimer, Edison et al. 2007; Tomasi, Edison et al. 2008; Boellaard 2009; Yaqub, van Berckel et al. 2012).

In brief, the supervised clustering algorithm models the time-activity curves (TAC) of each pixel as the sum of the kinetics of 4 predefined tissues (normal GM and WM, and the 2 sources of specific binding, activated microglia and the vasculature) obtained from a database of control subjects and patients. Additional signals from the skull and extracerebral muscle tissue are excluded by elimination of nonbrain voxels with the use of the coregistered GM and WM components extracted from the individual MRIs. The reference kinetic is obtained by averaging on the whole brain, whereas each pixel TAC is weighted by its normal GM index. The reference TAC is then used as input for a simplified reference region modeling approach (Gunn, Lammertsma et al. 1997) which additionally incorporates the specific (but of no interest) binding of PK to the vasculature (Tomasi, Edison et al. 2008) and is applied to the PET dynamic volume to generate a pixel-by-pixel map of PK binding potential of the specifically bound radioligand relative to the nondisplaceable radioligand in tissue (BP\textsubscript{ND}).

PK parametric images of each subject were coregistered to their respective MRI scans using the mutual information registration algorithm in the SPM8 software package. This same transformation was applied to the thresholded (probability of 0.5) GM and WM masks and to the 83 ROIs generated with the MAPER approach. Multiplication across these co-registered images and masks created BP\textsubscript{ND} maps, the patients’ individual 83 ROIs in which then were sampled with the ANALYZE medical imaging software (version 11, Mayo Foundation).
4.3.4 Clinical assessments

The TRACK-PET study corresponded with the 24-month assessments of TRACK-HD and therefore data from 3 visits were available for analysis. Clinical and imaging variables encompassing a range of domains were chosen for correlation with PK PET imaging data based on 12-month longitudinal performance in the premanifest cohort in TRACK-HD but the number of variables chosen were limited to avoid the pitfalls of multiple testing (Tabrizi, Seahill et al. 2011). Variables used in this analysis were the BSI imaging variable, inter-tap time and tap duration quantitative motor variables (see section 3.3.1 for a full description of these clinical assessments); the Problems Based Assessment–Short (PBA-S) apathy score from the neuropsychiatric assessments and the negative emotion recognition score and indirect circle tracing annulus length from the cognitive assessments (Tabrizi, Seahill et al. 2011).

4.3.5 Statistical Analysis

ANOVA followed by post-hoc Tukey HSD testing was used for the monocyte stimulation experiments. Parametric statistical tests were used for 2 group comparisons (students un-paired t-test); where values were non-normally distributed, non-parametric statistical tests were used (Mann–Whitney U test). To interrogate correlations between PK BPND values and variables of interest, Spearman's correlations were used. All significance levels reported are two-tailed.

4.4 Results

4.4.1 HD monocytes are hyper-reactive after LPS stimulation

CD14+ monocytes were isolated and seeded in vitro, before being primed with IFNγ and stimulated with LPS. In multiplex ELISA assays, stimulated monocytes from HD subjects at each disease stage were found to produce more of the pro-inflammatory cytokines, IL-6 and TNFα, than control cells (see Figure 26). Production of IL1β was also significantly increased in LPS-stimulated premanifest HD monocytes. Levels of the chemokine IL-8, anti-inflammatory IL-10 and pro-inflammatory IL-12 did not differ between stimulated HD and control monocytes.
Cytokine production by LPS-stimulated monocytes was normalised to the cytokine production of non-stimulated cells from the same subject. Cytokine production by non-stimulated monocytes was very low with 10pg/ml for all cytokines besides IL-8, for which levels were around 1ng/ml in supernatants from non-stimulated cells. Cytokine production by non-stimulated cells did not differ between control and HD subjects at each stage.

**Figure 26** Pro-Inflammatory cytokine production by monocytes is elevated in HD patients.

Monocytes were isolated from control and HD patient blood samples using magnetic cell sorting. After stimulation with 10ng/ml IFN and 2 g/ml LPS for 24 h, multiplex ELISA demonstrated that innate immune regulators IL-1, IL-6 and TNF are elevated in culture media of HD patient blood monocytes. Data shows mean concentrations.
corrected to basal condition ± SEM, n= individual biological repeats. Analysis *p<0.05, **p<0.01, ***p<0.001.

4.4.2 TRACK-PET study

4.4.2.1 Whole brain volumes of subjects are no different to controls.

To investigate whether any difference in PK BP$_{ND}$ may be the function of cerebral atrophy, the whole brain volumes subjects enrolled in the TRACK-PET study compared with age and sex matched controls and no significant difference was found (Figure 27).

![Figure 27 Comparison of whole brain volumes from the TRACK-PET cohort](image)

There is no significant difference in the whole brain volumes of premanifest gene carriers enrolled in TRACK-PET compared with age and sex matched TRACK-HD controls. (Mean and SD. p=0.068. Mann Whitney Test)

4.4.3 $^{11}$C-(R)-PK11195 binding is increased in premanifest gene carriers

There were no differences in the whole brain PK BP$_{ND}$ between premanifest subjects and age and sex matched controls.

ROI analyses, focussed on brain regions specifically implicated in disease pathogenesis in HD, revealed cross-sectional differences in PK BP$_{ND}$ between age and sex matched controls in the insula, thalamus, pallidum and striatum (comprising the caudate nucleus, putamen and nucleus accumbens) as shown in Figure 28.
Figure 28 PK binding potentials of premanifest gene carriers enrolled in the TRACK-PET study

Mean PK BP_{ND} per unit volume ± SD  *Consisting of Caudate nucleus, putamen and nucleus accumbens.

4.4.4 $^{11}$C-(R)-PK11195 Binding Potential may be associated with disease progression

To investigate whether PK BP_{ND} correlates with markers of disease progression, the PK BP_{ND} from the 4 ROI were compared with the 5-year probability of disease onset (Langbehn, Brinkman et al. 2004). PK BP_{ND} in the caudate nucleus, but in no other ROI, showed significant correlation with 5-year probability of disease onset (Figure 29).
Figure 29 PK BPND correlates with 5-year probability of disease onset in premanifest HD.

(Spearman Correlation. r=0.64, p=0.028)

To further investigate the potential of PK PET as a biomarker of disease progression. PK BPND of ROI was correlated with measures of disease progression selected from the TRACK-HD study and based on good longitudinal performance. Whilst most measures did not show any significant correlation with PK PET, PK BPND in the corpus striatum was related to differences in tap duration as measured at visit 3 (see Figure 30) and PK BPND in the thalamus was associated with progression of subclinical motor phenotype in premanifest subjects as measured by the mean change in intertap time from baseline to 24 months (see Figure 30). These findings raise the possibility that microglial activation in the thalamus can reflect subclinical progression and therefore act as a marker of disease onset.
Figure 30 PK Binding Potential correlates with quantitative motor impairment

PK BP\textsubscript{ND} per unit volume are plotted. Corpus Striatum consists of Putamen, Caudate Nucleus and Pallidum. PK BP\textsubscript{ND} of the corpus striatum shows correlation with tap duration. PK BP\textsubscript{ND} of the correlates with mean change in intertap time over 24 months.

4.4.5 Central microglial activation in premanifest HD does not correlate with peripheral monocyte stimulation.

In multiplex ELISA assays, stimulated monocytes from premanifest HD subjects participating in the TRACK-PET study were found to produce more of the pro-inflammatory cytokine IL-1\textbeta (Figure 31) than control cells. Mean levels of IL-6, TNF\textalpha and IL-8 were higher in premanifest subjects compared with controls but the differences did not reach statistical significance. There was no significant correlation between PET BP\textsubscript{ND} of any ROI (whole brain, thalamus or corpus striatum; encompassing putamen, caudate and pallidum) and the inflammatory markers tested.
Results from the monocyte stimulations from TRACK-PET premanifest subjects.

Monocytes were isolated from controls and premanifest HD gene carriers using magnetic cell sorting. After stimulation with 10ng/ml IFN and 2 g/ml LPS for 24 h, multiplex ELISA demonstrated that innate immune regulator IL-1β is elevated in the culture media of premanifest HD gene carriers. Data shows mean concentrations corrected to basal condition ± SD.

4.5 Conclusions

Monocytes isolated directly from HD patients and stimulated with LPS produced significantly more IL-6, IL-8 and TNFα compared with controls and in addition monocytes isolated from premanifest HD gene carriers (n=17) were hyper-reactive for IL-1β. This supports previous findings that in premanifest subjects with a mean of 16 years to expected onset, myeloid cells isolated from premanifest HD patients were hyperactive to the same degree as cells isolated from late-stage patients (Bjorkqvist, Wild et al. 2008). This suggests that an early deficit is already present many years before disease onset and this may offer an early marker of HD pathogenesis. Stimulated monocytes from the 12 premanifest subjects from TRACK-HD were hyper-reactive for IL-1β but did not produce significantly more IL-6, IL-8 or TNFα. It may be that this study was insufficiently powered to show a significant difference and, in addition,
cytokine levels were extremely variable in this cohort as shown by the large standard deviations presented in Figure 31.

This study confirmed previous findings of increased microglial activation in premanifest HD gene carriers compared to healthy controls (Pavese, Gerhard et al. 2006; Tai, Pavese et al. 2007; Politis, Pavese et al. 2008; Politis, Pavese et al. 2011). As in previous studies, microglial activation in the striatum has been shown to predict the 5-year disease clinical onset in premanifest HD patients (Tai, Pavese et al. 2007; Politis, Pavese et al. 2011). In this study PK BP\textsubscript{ND} in the thalamus reflected subclinical progression of HD in premanifest gene carriers, with increased binding in those subjects whose performance on one of the TRACK-HD tapping tasks had deteriorated most over the previous 24 months. This finding was not reproduced for the cognitive or neuropsychiatric tasks investigated. This observation suggests that PK PET is a marker of subclinical motor progression and could be helpful in stratifying those that are closer to motor onset in clinical trials of disease modifying agents. In this cohort, monocyte stimulation in the periphery did not reflect central microglial activation and would not be a suitable alternative to PET imaging as a biomarker of disease progression. Cytokines are known to vary diurnally and this pattern may change with disease progression, furthermore cytokine levels can be altered by infections, smoking, diet and a number of other factors challenging their reliability as use as a biomarker. However, the relation between central and peripheral immune reaction is worth exploring in a larger sample of HD gene carriers and by applying corrections for partial volume and vascular effects in the PK PET data.

4.6 Future Directions

Despite a large number of research studies, the exact role of immune activation in chronic neurodegenerative disease remains uncertain. In line with the high plasticity of microglia that allows them to perform numerous CNS functions, they are likely to play a dichromatic role in disease, depending on signals present in their microenvironment and the duration of activation. While early immune activation could present a beneficial response (i.e. removal of CNS threat, promotion of tissue repair and removal of misfolded protein) chronic exposure could induce detrimental effects by promoting neuronal death (i.e. through the sustained release of neurotoxic factors), thus contributing to progression of disease.
PET imaging may be a tool that allows us to track the progression and severity of neuroinflammation in the brain as a useful indicator of active CNS disease. A longitudinal study of PK PET in premanifest HD is essential to assess whether it is truly a marker of disease progression. However, there are limitations associated with the use of PK including a high level of non-specific binding (Petit-Taboue, Baron et al. 1991) and a poor signal to noise ration, which complicates its quantification (Boutin, Chauveau et al. 2007). This has prompted the search for novel PET radioligands of TSPO, such as $^{11}$C-PBR28, $^{11}$C-DAA1106 and $^{18}$F-DPA-714 (Ching, Kuhnast et al. 2012). As yet none of these have shown definite superiority when compared with $^{11}$C-PK11195 but are now being used in studies of neurodegeneration.

Early detection of microglial activation could offer opportunities for pharmacological interventions to limit the potential disruptive effects of chronic microglial activation and could be used as outcome measures in therapeutic trials, particularly for those therapies targeting immune dysfunction in HD; such as KMO inhibitors and CB2 receptor agonists.

Further investigation into the pathways by which hyperstimulation of monocytes and macrophages occur, may lead to novel therapies targeting immune dysfunction. One study, to which the author has contributed, has shown that by lowering total HTT levels in primary human monocytes and macrophages, HD-specific phenotypes, such as increased cytokine production and transcriptional dysregulation can be, at least partially, reversed (Trager 2013). The NFkB pathway and its role in immune dysfunction in HD should also be further investigated; especially since NFkB signaling is not only crucial in innate immune activation but also in neuronal survival (Khoshnan, Ko et al. 2004; Teng and Tang 2010; Trager 2013).
5  **GENETIC MODIFIERS OF AGE OF ONSET IN HUNTINGTON DISEASE**

5.1  **Introduction**

CAG repeat length accounts for 42–73% of the variance in the age of onset in Huntington’s disease (HD) (Andrew, Goldberg et al. 1993; Stine, Pleasant et al. 1993; Brinkman, Mezei et al. 1997). The remainder of the variance is likely due to environmental and other genetic factors (Wexler, Lorimer et al. 2004). Genes that influence age of onset of HD must lie in pathways where they can modulate onset of disease symptoms; and thus finding such genetic variants and identifying the genes associated with them gives a direct insight into molecules and pathways that are important in the manifestation of the disease and may provide therapeutic targets. Several loci may be undetectable by genome-wide association studies due to small effects; however, genetic analysis of candidate genes is an appropriate alternative to identify modifier genes.

The aim of this study was to use age of onset data and DNA samples collected as part of the TRACK-HD and REGISTRY Studies to employ a candidate gene approach to identify genetic modifiers of HD. The genes in question were *CLU*, *PICALM*, *CRI*, and *APOE*, which have all been implicated as modifying genes in Alzheimer’s Disease pathogenesis. These genes were chosen as potential candidate genes given that there are parallels between the neurodegenerative diseases and the processes that occur in AD and HD are likely to involve similar pathways. The genes are described below.

The innate immune system dysfunction is potentially important in the pathogenesis of neurodegenerative diseases as discussed in Section 1.3. *CLU* encodes the protein Clusterin, also known as Apolipoprotein J, and is a key modulator of the innate immune system and the complement cascade and has been implicated in neuroinflammation. There is widespread evidence of immune activation throughout the course of HD (Bjorkqvist, Wild et al. 2008) and clusterin has been shown to be upregulated in peripheral blood in HD subjects (Dalrymple, Wild et al. 2007). It is therefore interesting that 2 large genome-wide association studies have identified variants in the

The CR1 gene encoding the main receptor of the complement C3b protein and has also been associated with increased risk of late-onset AD (Lambert, Heath et al. 2009). The PICALM gene, encoding the PICAL protein (phosphatidylinositol-binding clathrin assembly protein) which is involved in intracellular trafficking of growth factors and neurotransmitters, also showed an association with increased risk of sporadic AD (Harold, Abraham et al. 2009).

The ε4 allele of the apolipoprotein E (APOE) gene has been defined as a critical factor for early onset neurodegeneration in Pick's, Parkinson's, and Alzheimer's disease. A number of HD genetic modifier studies have previously investigated the effects of the APOE on age of onset with conflicting results. APOE ε4 allele appeared to delay onset (Panas, Avramopoulos et al. 1999) and APOE ε2ε3 genotype was associated with an earlier onset in males than females (Kehoe, Krawczak et al. 1999). However, these reports have been followed by a number of negative studies using a larger number of samples (Saft, Andrich et al. 2004; Andresen, Gayan et al. 2007). We feel that APOE merits further study and that the large cohort of REGISTRY samples might resolve the questions of whether or not APOE is a modifier of HD.

5.2 Contributions and Collaborations

The author and Sarah Tabrizi were responsible for the concept, design and data interpretation of the study with input from Peter Holmans and Lesley Jones. The author performed all the laboratory work and data processing under the supervision of Simon Mead and Jon Beck. The author contributed to age of onset and phenotypic data collected in the TRACK-HD and REGISTRY studies as a named co-investigator. The full list of co-investigators for TRACK-HD is given in Appendix B and for REGISTRY is given in Appendix C. Statistical analyses were performed jointly by the author and Denise Harold under the supervision of Peter Holmans.
5.3 Methods

5.3.1 Subjects

Data from two longitudinal, multicentre, observational research studies were analysed; the European Huntington’s Disease Network’s REGISTRY study (Orth, Handley et al. 2011), which collects data in Europe from symptomatic and premanifest HD gene expansion carriers and TRACK-HD (Tabrizi, Langbehn et al. 2009), a prospective observational biomarker study of premanifest and early stage HD at 3 European (UK, France, the Netherlands) and 1 Canadian site.

An application was made to the REGISTRY SBAC for DNA samples and clinical information as specified in Section 2.2.3. A total of 1832 samples were received. From TRACK-HD, samples and clinical information for all premanifest gene carriers and subjects with manifest HD were genotyped.

5.3.2 Genotyping

DNA was extracted using standard techniques by Biorep® Technologies Inc., (Milan, Italy) and CAG repeat sizes were determined as outlined in section 2.5.1. 1 SNP from each candidate gene; CLU (rs7982), CRI (rs1408077), PICALM (rs3851179) and 2 SNPs in APOE (rs429358 and rs7412) were selected based on previous study (Harold, Abraham et al. 2009; Lambert, Heath et al. 2009).

All samples were genotyped using an allelic discrimination assay on the Applied Biosystems 7500 FAST Real Time Polymerase Chain Reaction (PCR) instrument with TaqMan® probes. TaqMan® pre-designed SNP Genotyping Assays were purchased from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). Assays included primers and fluorescently labelled FAM™ and VIC® MGB™ probes for detection of both alleles. Samples were prepared on a MicroAmp™ Optical 96-Well reaction plate using a reaction volume of 25µl per sample comprising 12.5µl 2xTaqMan Universal PCR Master Mix, No AmpErase UNG (PN 4324018), 1.25 20X SNP Genotyping Assay, 10.25µl of DNase-free water and 1µl of DNA at a concentration of 20ng/µl. PCR was carried out with the following conditions; 10 minutes at 94°C followed by 40 cycles of 15 seconds at 92°C and 1 minute at 60°C followed by a hold at 4°C. Allele discrimination was carried out in Applied Biosystems 7500 Fast System Sequence Detection Software v1.3.1 and downloaded to Microsoft® Excel.
5.3.3 Statistics

The relationship between age of onset of Huntington disease and CAG repeat length is curvilinear. To model this relationship, we fitted a simple linear regression predicting the natural log of age of onset of HD from CAG repeat length (Figure 32). To evaluate the effect of genetic markers in candidate genes on age of onset of HD, we included these markers as predictors in the regression model. Age, sex, large CAG repeat length and study region were included as covariates in the model. Analysis was carried out in PLINK (Purcell, Neale et al. 2007).

5.4 Results

838 subjects from REGISTRY and 131 subjects from TRACK-HD were included in the analysis giving a total of 969 cases. The demographic details of the participants are shown in Table 13.

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<tr>
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</tr>
</tbody>
</table>

Table 13 Demographic details of subjects included in genetic modifiers study

Total n=969

The mean age at onset was 44.94 years (SD ± 11.76). There was a significant negative correlation between large CAG repeat length and age of motor onset ($r^2=0.59$, $p,<0.0001$) (see Figure 32). Consistent with other studies, the large CAG repeat length accounted for 59% of the variability in age of onset.
5.4.1 Effects of SNPs on Age at onset

5.4.1.1 Motor onset

For the genotypes at each individual locus we did not find any evidence of an association with motor age of onset as shown in Table 14.

<table>
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<th>Additive p-value</th>
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</tr>
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<td>CLU</td>
<td>rs7982</td>
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</tr>
<tr>
<td>PICALM</td>
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</tr>
<tr>
<td>APOE codon 158</td>
<td>rs429358</td>
<td>0.117</td>
</tr>
<tr>
<td>APOE codon 112</td>
<td>rs7412</td>
<td>0.451</td>
</tr>
<tr>
<td>APOE genotype</td>
<td></td>
<td>0.867</td>
</tr>
</tbody>
</table>

Table 14 P-values from linear regression between variables analysed and age at motor onset
5.5 Conclusions

In this cohort, approximately 60% of the variance in age of onset is attributable to the length of the large CAG repeat. The goal of this study was to investigate whether genetic polymorphisms in other genes; CLU, CRI, PICALM or APOE could account for some of the remaining 40% of variance. None of the polymorphisms investigated in this study revealed an association with age of onset. However, this study does not exclude the possibility that other DNA variants in these genes, or in other genes involved in these pathways, may act as genetic modifiers.

As already discussed in Section 1.4, genetic studies have suggested over 20 loci that may modify age of onset or progression of HD. Many of the specific polymorphisms assessed in multiple studies have failed to replicate. One recent study has exposed genetic ancestry as a critical factor in HD association studies (Ramos, Latourelle et al. 2012). Though only ‘Caucasian’ samples were included in this study, genetic background was not corrected for. Larger samples sizes are better powered to reveal genetic modifiers. To increase the power of this study, two different study populations were included any differences between them may also have affected the analysis. This is discussed further in Chapter 6.

Another complicating factor in the search for genetic modifiers is that age-related instability of the HD allele may increase the severity of disease process. Studies have shown that somatic expansions of the CAG repeat in the brain are associated with an earlier age of disease onset (Veitch, Ennis et al. 2007; Swami, Hendricks et al. 2009). It is challenging to investigate this phenomenon in large-scale genetic modifier studies of peripheral blood or to control for somatic effects in age of onset studies. However, it may be that investigating the processes involved in generating these somatic CAG repeat length differences might itself yield potential modifiers.

5.6 Future Work

Whilst candidate genetic studies are a valuable approach to identifying potential genetic modifiers of age of onset alternative approaches may prove more successful. GWAS studies to investigate common DNA variants that may be associated with age of onset in HD are ongoing but are yet to report. Recent advances in next-generation sequencing technologies now allow cost-effective methods for whole genome sequencing to investigate both rare and common variation. One approach, which may be fruitful, in
HD would be to examine individuals with extremes of phenotype; ie.to carry out whole genome sequencing to examine variants in those whose onset is either much earlier or much later than predicted by their CAG repeat size. This approach is being investigated in the TRACK-HD cohort.

An alternative approach would be to investigate epigenetic changes, including DNA methylation and histone modifications. Epigenetic pathways act as mediators between the environment and the genome and can be activated by various conditions such as stress or exposure to environmental toxins to result in a variety of responses including gene transcription or silencing.

Epigenetic changes are dynamic and unlike genetic mutations, they can be reversed for therapeutic purposes by targeting enzymes or other factors that control or maintain them. Investigation of epigenetic changes as part of the variability in age of onset may be of particular relevance in HD given the interest in HDAC inhibitors and sirtuin inhibitors as potential disease modifying agents.
6 THE IMPORTANCE OF ACCURATELY RECORDING AGE OF ONSET IN HUNTINGTON’S DISEASE

6.1 Introduction

As discussed earlier in this thesis, identifying modifiers of age of onset in HD may help to elucidate pathogenic mechanisms and hence provide therapeutic targets for the disease for which, at present, no disease modifying treatments are available. In addition, understanding of modifying factors may help us to more accurately estimate age of onset, which would be of paramount importance in clinical trials of disease modifying therapies in pre-symptomatic HD gene carriers.

Robust genetic modifiers of other neurodegenerative diseases, such as Alzheimer’s Disease and Parkinson’s Disease have been identified through both candidate gene studies and GWAS (Gasser, Hardy et al. 2011; Bertram and Tanzi 2012). In HD, as already discussed, a number of potential genetic modifiers have been but many have failed to replicate in subsequent studies. Further, higher resolution, genome-wide association studies are planned but none have yet been reported. Genetic modifier studies in HD are complicated by the fact that the expanded CAG repeat already accounts for much of the observed variation in age of onset but also due to the smaller numbers of subjects, differences in the genetic background between subjects and, possibly, variability in the data collected.

Collaborative studies such as the European Huntington’s Disease Network’s REGISTRY study (Orth, Handley et al. 2011) and the Huntington Study Group’s COHORT study (Dorsey 2012) have made large scale genetic modifier identification projects possible by making large sets of comparable data available for study. Smaller, but more rigorously controlled studies, such as TRACK-HD may provide more robust data sets but the power to identify genetic modifiers is likely to be limited by their size. The aim of this study was to investigate whether the ages of onset for a given CAG repeat length are comparable between large scale, clinically less detailed, and smaller scale, more detailed, observational studies of HD.
6.2 Contributions and Collaborations

The author and Sarah Tabrizi were responsible for the concept, design and data interpretation of the study with input from Peter Holmans, Lesley Jones and Doug Langbehn. The author contributed to data collected as part of the TRACK-HD and REGISTRY studies as a co-investigator. The full list of co-investigators for TRACK-HD is given in Appendix B and for REGISTRY is given in Appendix C. The data was collated and processed by the author. Denise Harold carried out the statistical analysis.

6.3 Methods

6.3.1 Subjects

Retrospective data from two ongoing longitudinal, multicentre, observational research studies were analysed: the European Huntington’s Disease Network’s REGISTRY study (Orth, Handley et al. 2011), which collects data in Europe from symptomatic and premanifest HD gene expansion carriers and TRACK-HD (Tabrizi, Langbehn et al. 2009), a prospective observational biomarker study of premanifest and early stage HD at 3 European (UK, France, the Netherlands) and 1 Canadian site.

In both studies age of onset in symptomatic subjects is estimated retrospectively by a ‘rater’ via a combination of questioning the participant and their relatives, and examination of their medical records. Prospective estimation of age of onset occurs during both studies for premanifest gene carriers via a combination of physical examination using the Unified Huntington’s Disease Rating Scale (motor component) and questioning the participants, their relatives and examination of their medical records.

Data from all participants recorded as having symptomatic HD and a DNA sample that had been genotyped for the CAG expansion were requested.

All Caucasian individuals (131) from TRACK-HD with a recorded age at motor onset were included in the analysis.

From the REGISTRY study data, the age of onset was taken to be the earliest age at which symptoms had appeared as determined by the rater. Only Caucasian subjects were included in these analyses. Only those with ‘motor’ symptoms as the prevalent symptom at onset were included in this study. The final number of subjects from REGISTRY included in the analysis was 838.
6.3.2 CAG Genotyping

CAG repeat sizes were determined as outlined in section 2.5.1.

6.3.3 Statistical Analysis

Motor age of onset was used as the dependent variable in an analysis of covariance (ANCOVA), to test for a difference in the mean age of onset between studies after adjusting for the expanded CAG repeat length and gender.

To correct for potential differences in populations between the two studies, we went on to repeat the analysis with Caucasian subjects from the 3 European TRACK-HD sites (94 subjects) and REGISTRY sites from those 3 countries; UK, France and Netherlands (259 subjects).

The analysis was then repeated to perform a comparison between the mean adjusted age of onset of all countries within each study.

6.4 Results

The demographics details of the 2 cohorts are presented in Table 15. The ages of onset for a given CAG repeat length for each cohort are shown in Figure 33. The expanded CAG repeat length accounted for 59% of the age of onset for each study and was not significantly different between studies.

<table>
<thead>
<tr>
<th></th>
<th>REGISTRY</th>
<th>TRACK-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>838</td>
<td>131</td>
</tr>
<tr>
<td>Sex M=412 (49%)</td>
<td>M=69 (53%)</td>
<td></td>
</tr>
<tr>
<td>Mean CAG 44.38 (36-68)</td>
<td>43.58 (39-59)</td>
<td></td>
</tr>
<tr>
<td>Mean adjusted age of onset 45.2</td>
<td>43.2</td>
<td></td>
</tr>
<tr>
<td>Stage at visit 1: PM(^\d) (TFC≥11) 35%</td>
<td>66%</td>
<td></td>
</tr>
<tr>
<td>Stage 2 (TFC 7-10) 33%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Stage 3+ (TFC ≤6) 32%</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>TMS at visit 1 37.37 (SD ± 20.1)</td>
<td>21.34 (SD ± 12.32)</td>
<td></td>
</tr>
<tr>
<td>Age at visit 1 - Age at diagnosis 6.54 (SD ± 5.0)</td>
<td>3.53 (SD ± 3.52)</td>
<td></td>
</tr>
</tbody>
</table>

Table 15 Demographic details of REGISTRY and TRACK-HD subjects include in age of onset study.

\(^\d PM; premanifest\)
The mean adjusted age of onset (when covaried for sex and large CAG repeat length) in REGISTRY was 45.2 years (95%CI 44.7-45.7) and in TRACK-HD 43.2 years (95%CI 42.0-44.5). These are significantly different (p=5x10^{-3}).

![Figure 33 Age of onset in TRACK-HD and REGISTRY Studies.](image)

As population-dependent differences in age of onset of HD have previously been noted in Europe (Ramos, Latourelle et al. 2012), an analysis including only the subjects from geographical areas common to both studies was performed. The mean adjusted age of onset in UK, France and the Netherlands in the REGISTRY study were 47.0 years (95% CI 46.1-47.9) and in TRACK-HD were 43.888 years (95% CI 42.3-45.4). These are also significantly different (p=1x10^{-3}).

From these results it appeared that the mean adjusted age of onset between the UK, the Netherlands and France was almost 2 years later than the whole REGISTRY cohort. To formally test whether this difference in adjusted age of onset was statistically significant, mean adjusted age of onset between the UK, the Netherlands and France and the rest of the REGISTRY study were compared. There was no significant difference.
6.5 Discussion

The mean age of onset of motor symptoms in TRACK-HD is two years earlier than in the REGISTRY study. This difference remains significant when analysis is restricted to matching populations between the 2 studies. The expanded CAG repeat length does not account for this difference.

Taking the initial results, the age of onset in sites from the UK, France and the Netherlands alone were nearly 2 years later than the age of onset in the full REGISTRY cohort. This is consistent with the observation that population stratification may bias analysis of genetic modifier studies (Ramos, Latourelle et al. 2012). However, when this was formally tested, there was no significant difference in age of onset across the REGISTRY study.

The difference in age of onset between TRACK-HD and REGISTRY are likely to represent systematic differences in data collection rather than genetic differences between the populations studied. The TRACK-HD cohort is smaller and more rigorously characterised with the assessments being carried out by a small number of highly trained raters. In addition, as the study enrolled either early stage HD subjects or premanifest HD gene carriers (11% compared to 3.6% in REGISTRY) who may have started to show some symptoms during the course of the study, the age of onset was more recent (on average within 3.5 years of the first visit) and therefore likely to be subject to fewer recollection biases.

If the discrepancy in age of onset between these two observational studies of HD is widely applicable, this is likely to reduce the power of any genetic modifier studies in HD unless it is recognised and dealt with. Controlling any studies for geographical location is also likely to be critical as in HD, in order to obtain the sample size needed for many genome-wide studies, data from many countries is likely to be aggregated: the Registry study has at least 136 study sites from 16 countries contributing (Orth, Handley et al. 2011) and ENROLL aims to have over 200 sites in 30 countries contributing (http://clinicaltrials.gov/ct2/show/NCT01574053).

Our findings may provide part of the explanation as to why genetic modifier studies in HD have not been as successful or reproducible as studies in other, more common neurodegenerative disease. GWAS in other diseases have primarily been aimed at identifying genetic loci influencing presence or absence of disease as a dichotomous trait. In HD the modifier with largest influence on age at onset is already known to be...
the size of the expanded CAG repeat. The previous linkage studies in HD were powered
to find relatively high-risk variants for onset modification and therefore it is most likely
that genetic loci influencing age of onset are of moderate or low risk. Discrepancies in
ages of onset in HD in the order of 2 years are likely to substantially reduce the power
to identify low to moderate risk modifiers.
We have not investigated discrepancies in the onset of symptoms other than motor
symptoms, as the numbers are smaller. In previous versions of REGISTRY, earliest age
of onset were recorded with the predominant symptom. These data are now being re-
defined with the introduction of an ‘HD clinical characteristics’ questionnaire trialled as
part of TRACK-HD, which aims to give ages of onset for major HD symptoms. It is
possible that the variability in different clinical characteristics are due to a variety of
heritable and environmental factors, so these data will be useful in future genetic
analyses.
These results highlight the importance of data collection of the highest quality and of
understanding the differences in data collection within studies. With the advent of the
ENROLL-HD study, a global observational study of HD, whose subjects are likely to
contribute to global genetic modifier studies, it is paramount that data collection is
rigorous and reproducible and that raters are adequately trained. Analyses of these data
must take into account both the genetic differences and systematic differences in data
collection between all the participating sites.
7 CONCLUSIONS

The aims of this thesis were to;

1. Identify robust imaging and clinical biomarkers that could be used reliably in clinical trials through the TRACK-HD study.

2. To further investigate the phenomena of microglial activation and hyperstimulation of the peripheral immune system with the particular aim of developing them as biomarkers of disease onset and progression in premanifest HD.

3. To identify potential genetic modifiers of disease onset and progression.

One of the potential reasons why previous therapeutic trials in HD have failed to show efficacy is the use of measures that, perhaps, are not sensitive to change in HD over the timescale of the studies. TRACK-HD was an ambitious study which has assessed the ability of various outcome measures to track disease progression when administered annually at four highly trained sites in premanifest and early manifest stages of HD. The study was incredibly successful in demonstrating that robust data acquisition over a wide range of disciplines and within the strict time scales required for a pharmaceutical company sponsored clinical trial is possible in HD. In addition, a number of imaging, and clinical measures that would be relatively straightforward to administer in a clinical research facility have successfully been validated for use in trials of early HD.

However, the selection of a battery of outcome measures for any given clinical trial will depend on multiple factors including the phase of trial, disease stage, numbers of subjects and sites, duration, cost, expected primary endpoint and mechanism of action of the putative therapeutic agent. The longitudinal results of TRACK-HD are most relevant for the design of Phase IIb and III clinical trials aimed at slowing the progression of the disease in the early stages of disease. The performance of these outcome measures in trials may be different, as it is likely that assessments will be performed more frequently, that practice effects will be minimized by prior exposure to the battery, and that the comparison will be drug to placebo rather than HD to control. Furthermore, the assessment of outcome measures by how they track disease
progression discounts the potential for signs of disease to actually improve, and this is clearly the aim of future disease-modifying therapies.

Whilst TRACK-HD has been an incredibly successful study, there is still work to be done. The imaging markers have far out-performed the clinical measures tested and there remains potential for the development of more sensitive clinical assessments. In particular, the oculomotor assessments have not performed as well as expected at the outset of the study and it may be that a better understanding of the eye movement abnormalities in HD, analysis of other variables within the oculomotor battery or development of a more sensitive device might create a more robust marker of disease progression. The Gaitrite gait assessment was removed from the TRACK-HD battery after baseline. In the cross-sectional analysis it was able to distinguish between premanifest HD and controls and between HD and controls but was not sensitive within groups. The main consideration for the removal of the Gaitrite device was for practical reasons. It was unwieldy and a challenge to store. There is great scope for the development of a smaller device which is more easily operated, particularly with the widespread availability of wireless technology, sensors which can be attached to the ankles, for example, would be much less cumbersome and generate more natural data.

The cognitive and neuropsychiatric assessments are beyond the scope of this thesis, however, measures with functional relevance will be of paramount importance when data from clinical trials are assessed by drug regulatory authorities and more sensitive tools are still required.

With hindsight it seems unfeasible that measures designed to detect progression in early HD would also be sensitive in the premanifest period. This may reflect the different pathogenic processes with neuronal dysfunction predominating in the premanifest period and neuronal death in manifest HD. Premanifest subjects did show progression, as measured by imaging markers, over a 24 month period but the clinical measures were less clear and even over 36 months TRACK-HD has not been able to define a battery of assessments with effect sizes suitable for use in trials of premanifest HD (Tabrizi, Scahill et al 2013). The TRACK-On study has been designed to address this and to evaluate novel measures aimed particularly at detecting change in the premanifest cohort.

With the aim of developing novel biomarkers of disease onset and progression, particularly in the premanifest stage, I investigated the potential of microglial activation,
as measured by PK PET imaging, and peripheral markers of immune activation in a well-characterised subgroup of premanifest subjects from TRACK-HD. From this study, PK PET seems to be a marker of subclinical motor progression and could be helpful in stratifying those that are closer to motor onset in clinical trials of disease modifying agents. Longitudinal studies are required to assess its potential as a biomarker of disease onset or progression though it may be particularly useful as a marker of ‘target engagement’ in trials of therapies which interact with microglia, in particular, KMO inhibitors.

Of particular value in tracking progression of HD, would be the development of a biomarker from peripheral blood especially because of the ease of obtaining blood samples and the availability of sensitive assays. HD monocytes are hyper-reactive, characterised by increased cytokine production upon LPS stimulation. Despite early promise, in this cohort, monocyte stimulation in the periphery did not reflect central microglial activation and it does not seem that plasma or monocyte derived cytokines would not be a suitable alternative to PK-PET imaging as a biomarker. The variability of peripheral immune markers is disappointing but not unexpected as smoking, diet and exercise are all factors known to alter peripheral cytokine levels. Preliminary data investigating measurement of total and mHTT levels in immune cells has revealed that mHTT levels in monocytes, T cells and B cells differ significantly between HD patients and controls, as well as between premanifest and manifest patients and may provide a more robust marker of progression (Weiss A et al., 2012). Peripheral immune activation does seem to be an early and sustained phenomenon in HD, however, and study of the mechanisms involved may still provide novel therapeutic targets. Further study has revealed the increased cytokine production to be caused by dysregulation of the NFκB pathway (Träger et al 2013). Work from this group has also demonstrated that by lowering HTT levels in primary human HD monocytes and macrophages, HD-specific phenotypes such as increased cytokine production and transcriptional dysregulation can be, at least, partially reversed.

The peripheral immune activation described in HD prompted the study of variability in genes related to the immune system dysfunction and linked to Alzheimer’s Disease pathogenesis that I have presented here. I have not been successful in identifying potential genetic modifiers of age on onset in this thesis. I am not alone and the hunt for
genetic modifiers in HD has not, to date, yielded strong and reproducible candidates. GWAS data analysis is ongoing and may prove more fruitful. However, this thesis has highlighted the importance of accurate and rigorous data acquisition in observational studies which will contribute to future large-scale modifier studies. The collection of age of onset data through existing studies, such as REGISTRY, is retrospective and subject to biases on the part of both the rater and the subject. Data collected prospectively within these studies are likely to be more robust and for this reason, genetic modifiers of rate of progression rather than age of onset may be more readily identifiable. It is still possible that such studies will identify genes which lie within immune pathways as potential modifiers. In addition, further study of epigenetic phenomena in HD, such as the investigation of histone modification and chromatin remodelling is warranted given the relevance of epigenetics to the interaction between genes, the environment and the immune system.

“...I have never known a recovery or even an amelioration of symptoms in this form of chorea; when once it begins it clings to the bitter end. No treatment seems to be of any avail...It seems at least to be one of the incurables....I know nothing of it’s pathology. I have drawn your attention to this form of chorea gentlemen, not that I considered it of any great practical importance to you, but merely as a medical curiosity, and as such it may have some interest.”

(Huntington 1872)

These are the final words from George Huntington’s article on chorea. Though HD remains ‘incurable’ there are now interventions which significantly improve the lives of those affected and we have accumulated a considerable knowledge of it’s pathology. Most importantly, the HD community is edging closer to trials of disease modifying therapies and studies like TRACK-HD and REGISTRY have been instrumental in this.
Manuscripts relating to Chapters 4, 5 and 6 are currently in progress.


### APPENDICES

Appendix A: UHDRS-99

**REGISTRY V3**  
UNIFIED HUNTINGTON’S DISEASE RATING SCALE ’99 - MOTOR ASSESSMENT

<table>
<thead>
<tr>
<th>Study Site:</th>
<th>Subject:</th>
<th>Date info obtained:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Examiner:</th>
<th></th>
</tr>
</thead>
</table>

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

#### General

Motor score:

#### Motor Assessment

**Ocular pursuit:**

- 0 = complete (normal)
- 1 = jerky movement
- 2 = interrupted pursuits/full range
- 3 = incomplete range
- 4 = cannot pursue

**Saccade initiation:**

- 0 = normal
- 1 = increased latency only
- 2 = suppressible blinks or head movements to initiate
- 3 = unsuppressable head movements
- 4 = cannot initiate saccades

**Saccade velocity:**

- 0 = normal
- 1 = mild slowing
- 2 = moderate slowing
- 3 = severely slow, full range
- 4 = incomplete range

**Dysarthria:**

- 0 = normal
- 1 = unclear, no need to repeat
- 2 = must repeat to be understood
- 3 = mostly incomprehensible
- 4 = anarthria

**Tongue protrusion:**

- 0 = can hold tongue fully protruded for 10 sec
- 1 = cannot keep fully protruded for 10 sec
- 2 = cannot keep fully protruded for 5 sec
- 3 = cannot fully protrude tongue
- 4 = cannot protrude tongue beyond lips

**Finger taps:**

- 0 = normal (≥15/5 sec.)
- 1 = mild slowing, reduction in amplitude (11-14/5 sec.)
- 2 = moderately impaired (7-10/5 sec.)
- 3 = severely impaired (3-6/5 sec.)
- 4 = can barely perform task (0-2/5 sec.)

**Pronate/supinate-hands:**

- 0 = normal
- 1 = mild slowing and/or irregular
- 2 = moderate slowing and irregular
- 3 = severe slowing and irregular
- 4 = cannot perform
**Luria:**
- 0 = ≥4 in 10 sec, no cue
- 1 = <4 in 10 sec, no cue
- 2 = ≥4 in 10 sec with cues
- 3 = <4 in 10 sec with cues
- 4 = cannot perform

**Rigidity-arms:**
- 0 = absent
- 1 = slight or present only with activation
- 2 = mild to moderate
- 3 = severe, full range of motion
- 4 = severe with limited range

**Bradykinesia-body:**
- 0 = normal
- 1 = minimally slow (normal)
- 2 = mildly but clearly slow
- 3 = moderately slow, some hesitation
- 4 = markedly slow, long delays in initiation

**Maximal dystonia:**
- 0 = absent
- 1 = slight/intermittent
- 2 = mild/common or moderate/intermittent
- 3 = moderate/common
- 4 = marked/prolonged

**Maximal chorea:**
- 0 = absent
- 1 = slight/intermittent
- 2 = mild/common or moderate/intermittent
- 3 = moderate/common
- 4 = marked/prolonged

**Gait:**
- 0 = normal gait, narrow base
- 1 = wide base and/or slow
- 2 = wide base and walks with difficulty
- 3 = walks only with assistance
- 4 = cannot attempt
### REGISTRY V3

**UNIFIED HUNTINGTON’S DISEASE RATING SCALE ’99 - MOTOR ASSESSMENT**

<table>
<thead>
<tr>
<th>Study Site:</th>
<th>Subject:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examiner:</td>
<td>Date info obtained:</td>
</tr>
</tbody>
</table>

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

<table>
<thead>
<tr>
<th>Tandem walking:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – normal for 10 steps</td>
<td></td>
</tr>
<tr>
<td>1 – 1 to 3 deviations from straight line</td>
<td></td>
</tr>
<tr>
<td>2 – &gt;3 deviations</td>
<td></td>
</tr>
<tr>
<td>3 – cannot complete</td>
<td></td>
</tr>
<tr>
<td>4 – cannot attempt</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Retropulsion pull test:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – normal</td>
<td></td>
</tr>
<tr>
<td>1 – recovers spontaneously</td>
<td></td>
</tr>
<tr>
<td>2 – would fall if not caught</td>
<td></td>
</tr>
<tr>
<td>3 – tends to fall spontaneously</td>
<td></td>
</tr>
<tr>
<td>4 – cannot stand</td>
<td></td>
</tr>
</tbody>
</table>

### Diagnostic Confidence

<table>
<thead>
<tr>
<th>Diagnostic confidence level:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – Normal (no abnormalities)</td>
<td></td>
</tr>
<tr>
<td>1 – non-specific motor abnormalities (less than 50 % confidence)</td>
<td></td>
</tr>
<tr>
<td>2 – motor abnormalities that may be signs of HD (50 - 88 % confidence)</td>
<td></td>
</tr>
<tr>
<td>3 – motor abnormalities that are likely signs of HD (90 - 98 % confidence)</td>
<td></td>
</tr>
<tr>
<td>4 – motor abnormalities that are unequivocal signs of HD (≥ 99 % confidence)</td>
<td></td>
</tr>
</tbody>
</table>

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**136**
General

Functional score:

Functional Capacity

Occupation:
0 = unable  
1 = marginal work only  
2 = reduced capacity for usual job  
3 = normal

Finances:
0 = unable  
1 = major assistance  
2 = slight assistance  
3 = normal

Domestic chores:
0 = unable  
1 = impaired  
2 = normal

ADL:
0 = total care  
1 = gross tasks only  
2 = minimal impairment  
3 = normal

Care level:
0 = full time skilled nursing  
1 = home or chronic care  
2 = home

Information Sources:
Was the information obtained from:
1 = participant only  
2 = participant and family/companion
REGISTRY V3
HUNTINGTON’S DISEASE RATING SCALE ’99 - FUNCTIONAL ASSESSMENT

Study Site:  
Examiner:  
Subject:  
Date info obtained:  

All items must be completed. Use U if information is unavailable. Use N if information is not applicable.

General

Functional Assessment Score:  

Functional Assessment

For the next 25 questions, please use:
1 = yes
0 = no

Could subject engage in gainful employment in his/her accustomed work:  
Could subject engage in any kind of gainful employment?  
Could subject engage in any kind of volunteer or non-gainful work?  
Could subject manage his/her finances (monthly) without any help?  
Could subject shop for groceries without help?  
Could subject handle money as a purchaser in a simple cash (shop) transaction?  
Could subject supervise children without help?  
Could subject operate an automobile safely and independently?  
Could subject do his/her own housework without help?  
Could subject do his/her own laundry (wash/dry) without help?  
Could participant prepare his/her own meals without help?  
Could subject use the telephone without help?  
Could subject take his/her own medications without help?  
Could subject feed himself/herself without help?  
Could subject dress himself/herself without help?  
Could subject bathe himself/herself without help?  
Could subject use public transportation to get places without help?  
Could subject walk to places in his/her neighbourhood without help?  
Could subject walk without falling?  
Could subject walk without help?  
Could subject comb hair without help?  
Could subject transfer between chairs without help?  
Could subject get in and out of bed without help?  
Could subject use toilet/commode without help?  

Adopted from 1999 Huntington Study Group.
Could subject's care still be provided at home?

Information sources:

Was the functional assessment information obtained from:

1 = subject only
2 = subject and family/companion

Independence Scale

Subject's independence in %:

100 = no special care needed
95 = no physical care needed if difficult tasks are avoided
85 = pre-disease level of employment changes or ends; cannot perform household chores to pre-disease level, may need help with finances
75 = self-care maintained for bathing, limited household duties, e.g. cooking and use of knives, driving terminates; unable to manage finances
65 = needs minor assistance in dressing, toileting, bathing; food must be cut for subject
55 = 24-hour supervision appropriate; assistance required for bathing, eating, toileting
45 = chronic care facility needed; limited self feeding, liquid diet
35 = subject provides minimal assistance in own feeding, bathing, toileting
25 = no speech, must be fed
15 = tube fed, total bed care
5

Adopted from 1999 Huntington Study Group.
Appendix B: TRACK-HD co-investigators

<table>
<thead>
<tr>
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REFERENCES


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