

***Clinical Study Protocol*****Huntington's Disease Young Adult Study****PRINCIPAL INVESTIGATOR:****Prof Sarah Tabrizi FRCP PhD FMedSci**

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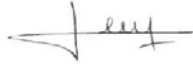
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This Clinical Study Protocol is approved by:



Signature: ..... Date: 04/09/2018

Professor Sarah Tabrizi FRCP PhD FMedSci  
Principal Investigator



Signature: ..... Date: 04/09/2018

On behalf of Sponsor:  
Emily Ikelle, Portfolio Coordinator, Joint Research Office, University College London.

**Protocol Versions**

Version Stage	Versions No	Version Date	Protocol updated & finalised by;	Appendix No detail the reason(s) for the protocol update
Current	Version 6.0	04 <sup>th</sup> September 2018	Prof Sarah Tabrizi	Increase participant number up to 150 recruited
Previous	Version 5.0	11 <sup>th</sup> January 2018	Prof Sarah Tabrizi	Cats and Dogs Visual Processing task (15 mins)
Previous	Version 4.0	1 <sup>st</sup> August 2017	Prof Sarah Tabrizi	Addition of MPM sequence during MRI
Previous	Version 3.0	1 <sup>st</sup> July 2017	Prof Sarah Tabrizi	Learning From Gains and Losses Sample processing time
Previous	Version 2.0	1 <sup>st</sup> Feb 2017	Prof Sarah Tabrizi	Tattoos in MRI Eligibility criteria AMI Questionnaire PIC Selection Participant Categories CAG research genotyping, gene carriers Plasma collection
Previous	Version 1.0	6 <sup>th</sup> May 2016	Prof Sarah Tabrizi	Original

## Synopsis

<b>Study Title:</b> Huntington's Disease Young Adult Study
<b>Short Study Title:</b> HD-YAS
<b>Funding Source:</b> Wellcome Trust (and CHDI Foundation, Inc. for optional CSF and venous blood collection)
<b>Study Location:</b> University College London Institute of Neurology / National Hospital for Neurology & Neurosurgery
<b>Number of Participants planned:</b> 120 (50% healthy controls and 50% pre-manifest gene carriers). Up to 150 participants may be recruited to account for participant drop out, missing data or failed acquisition of data.
<b>Principal Investigator:</b> Professor Sarah Tabrizi FRCP PhD FMedSci Professor of Clinical Neurology, Honorary Consultant Neurologist, Director of UCL Huntington's Disease Centre UCL Institute of Neurology and National Hospital for Neurology and Neurosurgery BOX 104 Queen Square London WC1N 3BG, UK
<b>Study period:</b> Estimated date first subject enrolled: August 2017 Estimated date last subject completed: June 2020

**Objectives:**

## Primary:

We will determine whether young adult gene carriers with a disease burden score (DBS)  $\leq 240$  (Penney et al. 1997) exhibit differences from controls in:

- 1) Regional brain volumes;
- 2) Structural brain connectivity;
- 3) Functional/effective brain connectivity;
- 4) Grey matter and white matter microstructure;
- 5) Myelination;
- 6) Mutant huntingtin levels in the CSF;
- 7) Cognition

in order to determine which measures (or combination of measures) demonstrate the earliest differences.

## Secondary:

Highlight the earliest time points to target intervention, prior to widespread neuronal damage and years before disease onset.

**Study Design:**

Using approaches we have pioneered and validated as part of TRACK-HD in older cohorts of gene carriers, the earliest signs of disease-related brain changes and functional impairment will be characterised in a cohort of young adult Huntington's Disease (HD) gene carriers, with a disease burden score (DBS)  $\leq 240$  (Penney et al. 1997). Using this data the earliest feasible time at which potential therapies can be delivered in future clinical trials will be determined.

We will recruit 120 18-40 year olds (50% healthy controls and 50% pre-manifest gene carriers based on power calculations for our *a priori* primary hypothesis of early striatal volume loss) over a three year period through Enroll-HD, the UK HD Network, HD Association and Youth Organisation, as well as a number of NHS PIC sites (HD outpatient clinics) Up to 150 participants may be recruited to account for participants drop out, missing data, or failed data acquisition. A cross-sectional comparison with one visit per participant will be used to determine any significant differences in a number of established and exploratory measures. The following assessments will be performed:

- Cognitive tasks (CANTAB and EMOTICOM batteries)
- 3T Volumetric MRI
- Resting state functional MRI (rsfMRI)
- Neurite Orientation Dispersion and Density Imaging (NODDI)
- Multi Parametric Mapping (MPM)
- HD core research assessments
- Blood plasma collection for biomarkers

- Clinical review and screening blood sample for CSF collection (optional)
- CSF and fasted blood sample collection. (optional)
- Neuropsychiatric self-report questionnaires

This data will be used to derive the following measures:

#### **Regional brain volumes**

Using optimised segmentation algorithms, brain volumes will be generated from structural MRI to examine group differences between gene carriers and controls.

#### **Structural brain connectivity**

A structural brain network will be generated for each participant using multi-shell constrained spherical deconvolution (Jeurissen et al. 2014). These networks will be weighted using NODDI-specific metrics (Zhang et al. 2012) conferring information on axonal integrity and myelination, with the potential to increase sensitivity to brain network changes in those far from onset. By applying graph theory analysis changes in global network properties can be investigated, such as integration and segregation and regional properties, such as the number or strength of connections to hub and non-hub brain regions (McColgan et al. Brain 2015).

#### **Functional/effective brain connectivity**

Using rsfMRI Dual-Regression Independent Component Analysis (ICA) will be performed to identify brain networks in which functional connectivity differences between controls and gene carriers in early adulthood are most notable. Connectivity measures within these networks will be correlated with clinical measures. Seed-based functional connectivity analyses using regions central to social and emotion cognition processing networks will also be performed, to explore potential differences in functional connectivity which may correlate with early social and emotional deficits.

#### **Grey and white matter microstructure**

Grey matter based spatial statistics (GBSS) and tensor based spatial statistics (TBSS) (Ball et al. 2013; Smith et al. 2006) will be used to interrogate microstructural alterations in grey and white matter respectively, with NODDI-derived neurite density index and orientation dispersion index metrics. In addition, an MPM imaging sequence will be used to assess myelination within the white matter.

#### **Huntingtin levels in CSF**

Using a novel mutant huntingtin (mHTT) assay in collaboration with CHDI and Evotec, the earliest stage at which mHTT is detectable in CSF will be determined, together with how its level predicts progression by clinical and neuroimaging criteria. We will use immunoprecipitation and mass spectrometry to seek evidence of CNS hyper-expansion of the mHTT polyglutamine tract due to somatic mosaicism, one possible driver of selective neuronal vulnerability and more rapid clinical progression (Kennedy et al. 2003). We will use a new, state-of-the-art multiplex single-molecule-counting Quanterix immunoassay platform supported by a Wellcome Trust Multiuser

Equipment Grant awarded June 2015; and by our collaborators at Evotec (Wild et al. 2015; Weiss et al. 2012; Weiss et al. 2009; Weiss et al. 2008). A high quality fasted and non-fasted blood sample collection matching the CSF collections will also be generated, which will also be used to compare and evaluate biomarkers and pathways of relevance to HD research and development.

#### **Cognitive-emotional function**

Early deficits in cognition, including social cognition, motivation and emotional processing, will be determined using frontal executive and memory tests from the CANTAB battery (<http://www.camcog.com>). For social cognition and motivational/emotional functioning we will use the newly-developed EMOTICOM battery which has been standardized on 200 volunteers. EMOTICOM tests will include Progressive ratio, Moral Judgement (measuring guilt, shame, anger), and Emotional Intensity Face Morphing Task (including recognition of disgust). We will also use the National Adult Reading Test (Nelson 1982) to estimate premorbid Intelligence Quotient (IQ) and Reinforcement Learning to measure choice behaviour and learning in the context of gains and losses.

#### **Diagnosis and main criteria for inclusion:**

Healthy controls as well as young adult HD gene expansion carriers will be enrolled.

#### **Inclusion Criteria:**

1. All eligible participants:
  - a. Are 18-40 years of age, inclusive; and
  - b. Are capable of providing informed consent and
  - c. Are capable of complying with study procedures
2. For the **Healthy Control** group, participants eligible are persons who meet the following criteria:
  - a. Have no known family history of HD; or
  - b. Have known family history of HD but have been tested for the huntingtin gene CAG expansion and are not at genetic risk for HD (CAG < 36\*).
3. For the **Young Adult Premanifest HD** group, participants eligible are persons who meet the following criteria:
  - a. Do not have clinical diagnostic motor features of HD, defined as Unified Huntington's Disease Rating Scale (UHDRS) Diagnostic Confidence Score < 4; and
  - b. Have CAG expansion  $\geq 40^*$ ; and
  - c. A disease burden score (DBS)  $\leq 240^{**}$  (Penney et al. 1997)

\*Genetic test results must be recorded in a documented report from an accredited genetics laboratory in the medical notes.

\*\*See Appendix B, page 42

#### **Exclusion Criteria:**

4. For all groups, participants are ineligible if they meet any of the following exclusion criteria:

- a. Current use of investigational drugs or participation in a clinical drug trial within 30 days prior to study visit; or
- b. Current intoxication, drug or alcohol abuse or dependence; or
- c. If using any antidepressant, psychoactive, psychotropic or other medications or nutraceuticals used to treat HD, the use of inappropriate (e.g., non-therapeutically high) or unstable dose within 30 days prior to study visit; or
- d. Significant medical, neurological or psychiatric co-morbidity likely, in the judgment of the Principal Investigator, to impair participant's ability to complete essential study procedures; or
- e. Predictable non-compliance as assessed by the Principal Investigator; or
- f. Inability or unwillingness to undertake any of the essential study procedures; or
- g. Needle phobia; or
- h. Contraindication to MRI, including, but not limited to, MR-incompatible pacemakers, recent metallic implants, foreign body in the eye or other indications, as assessed by a standard pre-MRI questionnaire; or
- i. Pregnant (as confirmed by urine pregnancy test); or
- j. Claustrophobia, or any other condition that would make the subject incapable of undergoing an MRI.

For the optional CSF collection only

- k. Needle phobia, frequent headache, significant lower spinal deformity or major surgery; or
- l. Antiplatelet or anticoagulant therapy within the 14 days prior to sampling visit, including but not limited to: aspirin, clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban; or
- m. Clotting or bruising disorder; or
- n. Screening blood test results outside the clinical laboratory's normal range for the following: white cell count, neutrophil count, lymphocyte count, haemoglobin (Hb), platelets, prothrombin time (PT) or activated partial thromboplastin time (APTT); or
- o. Screening blood test results for C-reactive protein (CRP) > 2× upper limit of normal; or
- o. Exclusion during history or physical examination, final decision to be made by the Principal Investigator; including but not limited to:
  - i any reason to suspect abnormal bleeding tendency, e.g. easy bruising, petechial rash; or
  - ii any reason to suspect new focal neurological lesion, e.g. new headache, optic disc swelling, asymmetric focal long tract signs; or
  - iii any other reason that, in the clinical judgment of the operator or the Principal Investigator, it is felt that lumbar puncture is unsafe.

**Sample Size:**

With a type 1 error rate of 5%, a sample of 60 participants/group will provide 80% power to detect a mean difference versus controls of 0.53 adjusted within group standard deviations (effect size), allowing for 5 covariates. Similarly, after allowing for 5 covariates, the sample of 60 CAG-expanded participants allows the same statistical

power for detecting a partial Pearson correlation of 0.36 among outcome measures and between these measures and the CAP score or other potential predictors of HD risk.



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## List of Abbreviations and Definitions of Terms

<b>Abbreviation</b>	<b>Definition</b>
AE	Adverse Event
APTT	Activated partial thromboplastin time
CAG	Cytosine-arginine-glutamine codon whose count in the HTT gene determines the genetic diagnosis of HD
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DBS	Disease Burden Score
eCRF	electronic Case Report Form
GCP	Good Clinical Practice
Hb	Haemoglobin
HD	Huntington's disease
HTT	huntingtin protein
ICH Guidelines	International Conference on Harmonisation Guidance for Industry
KMO	kynurenine mono-oxygenase
KP	kynurenine pathway
MPM	Multi parametric Mapping
MRI	Magnetic Resonance Imaging
NODDI	Neurite orientation and Dispersion and Density Imaging
PT	Prothrombin time
rsfMRI	Resting state functional Magnetic Resonance Imaging
REC	Research Ethics Committee
SAE	Serious Adverse Event
TFC	Total Functional Capacity
TMS	Total Motor Score
UHDRS	Unified Huntington's Disease Rating Scale
VBM	Voxel-based morphometry

## 1. Introduction

### 1.1 Background

Huntington's disease (HD) is an autosomal dominantly inherited, progressive neurodegenerative disorder characterized clinically by a movement disorder (typically chorea), neuropsychiatric disturbances, and cognitive impairment. The clinical features of HD usually emerge in adulthood (mean age of 37 years), after which illness progresses steadily over a period of 15-25 years. Genetic testing (preceded by genetic counselling according to internationally accepted guidelines) allows one to determine whether a clinically normal person harbours the HD mutation and thus predict that a person will go on to develop HD before he or she shows clinical symptoms and signs. HD has a prevalence of approximately 12 per 100,000 in the UK (Evans et al. 2013). HD affects at least 40,000 people living in Europe. In addition, an estimated 80,000 individuals carry the HD mutation but remain as yet unaffected. HD is caused by an expansion of a cytosine-adenine-guanine (CAG) trinucleotide repeat stretch in exon 1 of the HD gene on chromosome 4. Individuals who have 36 CAG repeats or more may develop the clinical symptoms and signs of HD including motor, cognitive and neuropsychiatric abnormalities that cause a progressive loss of functional capacity and shorten life. The course of HD is relentless; to date, there is no treatment which has been shown to alter the progression of the disease (Bates, Harper & Jones 2002).

Since the gene mutation responsible for HD was identified in 1993, considerable progress has been made in understanding the pathogenesis of this disorder and in identifying targets for potential therapies modifying the natural course of the disease (Handley et al., 2006). Currently the only approved treatment for HD is tetrabenazine, but several clinical trials exploring novel therapeutic approaches to treating this disease, including clearance of mutant HTT and antisense oligonucleotide (ASO) or 'gene silencing' compounds, are already in progress. Our previous work in the TRACK-HD study has used structural and functional MRI with behavioural measures to characterise the HD phenotype across a wide spectrum of disease stages, from many years before symptom onset (premanifest) through to established disease (Tabrizi et al. 2009; 2011; 2012; 2013; Klöppel et al. 2015). Premanifest HD (PreHD) cohorts up to 15 years before predicted onset exhibit structural brain differences (Tabrizi et al. 2009; Paulsen et al. 2008), elevated atrophy rates (Tabrizi et al. 2011; 2012; 2013; Aylward et al. 2000; 2011) and subtle functional impairment (Tabrizi et al. 2009; Paulsen et al. 2008). Recently we demonstrated specific brain network alterations in HD with loss of cortico-striatal connectivity early in disease development

(Novak et al. 2015) plus selective vulnerability of highly connected brain ‘hub’ regions (McColgan et al. 2015). Additionally, posterior structural brain hubs show functional downregulation, suggesting loss of function relates to the underlying structural network.

## 1.2 Rationale for Current Study

Pharmacological disease modification aimed at the primary pathogenetic mechanism of the disease is likely to be most effective when administered as early as possible in the cascade of processes underlying the clinical onset of HD. Although we (and others) have provided evidence for structural abnormalities at least 15 years prior to symptom onset (Tabrizi et al. 2009; Paulsen et al. 2008) it is not known whether these are present from the earliest stages of adulthood as a result of abnormal development, or whether they result from degeneration.

Here, by studying a cohort of young adult gene carriers and using approaches we have pioneered and validated as part of TRACK-HD in older cohorts of gene carriers, we aim to establish the earliest time at which disease-related brain abnormalities or behavioural dysfunction are apparent and thereby determine an appropriate time window for therapeutic intervention. Detailed characterisation of such a young gene-positive cohort is currently lacking and this represents the earliest time at which we can gain disease insights *in vivo*, since predictive genetic testing is only permitted in those over 18 years of age in the UK.

A cross-sectional comparison with one visit per participant will be used, as our previous studies show signal-to-noise ratios of current HD-related measures are inadequate to clearly detect, over 3 years or less, the slow rate of longitudinal change in participants far from HD onset (Tabrizi et al. 2011; 2012 & 2013). In contrast, one-time comparison of gene-expanded participants and comparable controls shows the sensitivity of these measures to cumulative disease development (Tabrizi et al. 2009; Paulsen et al. 2008; Weiss et al. 2012) that we will now employ to assess the earliest time point at which neurodegeneration can be detected.

Using non-invasive MRI measures of brain atrophy combined with structural, functional and effective connectivity we aim to detect and characterise any early disease-related effects. The addition of the recently developed novel imaging method NODDI will provide complementary assessments of aspects of pathology that are currently unreported in this young HD cohort.

In parallel we will characterise any functional deficits using targeted cognitive testing. In addition to the HD core assessments, a battery of computerised neuropsychological tests, CANTAB, has been used previously in studies of pre-HD and manifest HD (Lawrence et al.

1996 & 1998). CANTAB planning test scores are neurobiologically-based, validated with good reliability (Insel et al. 2013) and have a significant relationship to the Functional Assessment subsection of the Unified Huntington's Disease Rating Scale (UHDRS) (Ho et al. 2003; Huntington's Study Group 1996). Whilst CANTAB tests 'cold', or non-emotional, cognition, EMOTICOM (developed by Sahakian, Robbins and colleagues) tests 'hot', or social and emotional cognition. EMOTICOM also contains motivation and impulsivity tests, relevant to the behavioural problems of HD, plus the objective measures of aggression and disgust (Calder et al. 2010).

Finally, suitable biomarkers are needed in preparation for future trials to evaluate: (1) how well these novel therapeutics reach their intended target and have a biological effect (pharmacodynamic markers); (2) the effectiveness of these novel therapeutics at improving clinical signs and symptoms (efficacy biomarkers); and (3) the state of disease participants are in throughout the trial (disease progression biomarkers). Cerebrospinal fluid (CSF) is an ideal fluid compartment for assessing HD biomarkers, particularly pharmacodynamics markers, due to its proximity to the brain.

Using our novel mHTT assay in collaboration with CHDI Foundation Inc. and Evotec we will determine the earliest stage at which mHTT is detectable in CSF, and how its level predicts progression by clinical and neuroimaging criteria. The results of these studies will also contribute to establishing the best practices for measuring HTT in CSF from patients before and after HTT lowering therapies. Several CSF biomarker discovery programs have already resulted in the generation of many substances potentially differentially expressed in HD (Ross et al. 2014; Wild & Tabrizi 2008). While promising, these results need to be replicated in a young adult HD cohort to see if there is a relationship to the other variables studied.

A high quality blood sample collection will be generated, which will also be used to evaluate biomarkers and pathways of relevance to HD research and development. If a participant undertakes optional CSF and blood collection, a fasted blood collection will also be generated, exactly matching the conditions of the CSF collection.

#### Neuropsychiatric assessments:

Very little is currently known about early neuropsychiatric signs and symptoms in young adult HD gene carriers. For example, how early do specific neuropsychiatric changes begin and what role do neuropsychiatric measures have in potential clinical trials of young adult premanifest participants? What is the unique predictive contribution of neuropsychiatric signs and symptoms above and beyond that provided by cognitive and motor domains?

An important consideration in designing the neuropsychiatric assessment for this study is the recognition that depression and apathy can influence performance on cognitive and motor measures. Therefore, depression symptom severity and other

neuropsychiatric variables are needed as covariates for analysis of cognitive and motor data. Neuropsychiatric measures may also be important outcome variables for tracking disease progression or effects of therapeutic interventions, although they may have limited sensitivity because they can be influenced both by natural fluctuations as well as disease progression, and thus they may lack sufficient precision.

## **2. Study Objectives**

### **2.1 Primary Objective**

To determine whether young adult gene carriers with a disease burden score (DBS)  $\leq 240$  (Penney et al. 1997) exhibit differences from controls in:

- 1) Regional brain volumes;
- 2) Structural brain connectivity;
- 3) Functional/effective brain connectivity;
- 4) Grey matter and white matter microstructure;
- 5) Myelination;
- 6) Mutant huntingtin levels in the CSF;
- 7) Cognition,

in order to determine which measures (or combination of measures) demonstrate the earliest differences.

### **2.2 Secondary Objective**

Highlight the earliest time points to target intervention, prior to widespread neuronal damage and years before disease onset.

## **3. Study Design**

### **3.1 Study Population**

Two participant cohorts will be included in the study:

1. Healthy controls, n= 60
2. Young Adult Premanifest HD, n=60

#### **3.1.1 Inclusion Criteria**

Healthy controls as well as young adult HD gene expansion carriers will be enrolled.

**All** eligible participants:

- a. Are 18-40 years of age, inclusive; and
- b. Are capable of providing informed consent and
- c. Are capable of complying with study procedures and

For the **Healthy Control** group, participants eligible are persons who meet the following criteria:

- d. Have no known family history of HD (gene negative); or
- e. Have known family history of HD but have been tested for the huntingtin gene CAG expansion and are not at genetic risk for HD (CAG < 36\*) (family control or community control)

For the **Young Adult Premanifest HD** group, participants eligible are persons who meet the following criteria:

- f. Do not have clinical diagnostic motor features of HD, defined as Unified Huntington's Disease Rating Scale (UHDRS) Diagnostic Confidence Score < 4; and
- g. Have CAG expansion  $\geq 40^*$ ; and
- h. A disease burden score (DBS)  $\leq 240^{**}$  (Penney et al. 1997)

\*Genetic test results must be recorded in a documented report from an accredited genetics laboratory in the medical notes.

\*\*See Appendix B, page 42

The rationale for this DBS cut-off is that this boundary corresponds approximately to >18 years to estimated disease onset by the Langbehn formula (Langbehn et al. 2004). DBS cut-offs were used successfully to recruit to the Track-HD study (Tabrizi et al. 2009).

### 3.1.2 Exclusion Criteria:

For all groups, participants are ineligible if they meet any of the following exclusion criteria:

- a. Current use of investigational drugs or participation in a clinical drug trial within 30 days prior to study visit; or
- b. Current intoxication, drug or alcohol abuse or dependence; or
- c. If using any antidepressant, psychoactive, psychotropic or other medications or nutraceuticals used to treat HD, the use of inappropriate (e.g., non-therapeutically high) or unstable dose within 30 days prior to study visit; or



- d. Significant medical, neurological or psychiatric co-morbidity likely, in the judgment of the Principal Investigator, to impair participant's ability to complete essential study procedures; or
- e. Predictable non-compliance as assessed by the Principal Investigator; or
- f. Inability or unwillingness to undertake any of the essential study procedures; or
- g. Needle phobia: or
- h. Contraindication to MRI, including, but not limited to, MR-incompatible pacemakers, recent metallic implants, foreign body in the eye or other indications, as assessed by a standard pre-MRI questionnaire; or
- i. Pregnant (as confirmed by urine pregnancy test); or
- j. Claustrophobia, or any other condition that would make the subject incapable of undergoing an MRI.

**For the optional CSF collection only:**

- k. Needle phobia, frequent headache, significant lower spinal deformity or major surgery; or
- l. Antiplatelet or anticoagulant therapy within the 14 days prior to sampling visit, including but not limited to: aspirin, clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban; or
- m. Clotting or bruising disorder; or
- n. Screening blood test results outside the clinical laboratory's normal range for the following: white cell count, neutrophil count, lymphocyte count, haemoglobin (Hb), platelets, prothrombin time (PT) or activated partial thromboplastin time (APTT); or
- o. Screening blood test results for C-reactive protein (CRP) >2× upper limit of normal; or
- p. Exclusion during history or physical examination, final decision to be made by the Principal Investigator; including but not limited to:
  - i any reason to suspect abnormal bleeding tendency, e.g. easy bruising, petechial rash; or
  - ii any reason to suspect new focal neurological lesion, e.g. new headache, optic disc swelling, asymmetric focal long tract signs; or
  - iii any other reason that, in the clinical judgment of the operator or the Principal Investigator, it is felt that lumbar puncture is unsafe.

**Participant Categories**

### 3.1.2 Participant Categories

All gene carriers will be defined as;

Pre-manifest: Gene carriers without clinical features regarded as diagnostic of HD

Healthy control participants will be further divided into one of three sub-categories during data acquisition;

Genotype negative: This group includes a first or second degree relative i.e., related by blood to a carrier, who has undergone predictive genetic testing for HD and is known not to carry the HD expansion mutation.

Family Control: Family member or individual not related by blood to carriers (e.g., spouses, partners, caregivers).

Community Control: Individuals unrelated to HD carriers who did not grow up in a family affected by HD.

## 3.2 Recruitment

The healthy controls and pre-manifest gene carriers will be recruited over a period of three years through Enroll-HD, the UK HD Network, HD Association and Youth Organisation and a number of NHS PIC sites (HD outpatient clinics) in line with the eligibility criteria listed in section 3.1 above.

Potential study participants will be referred to NHNN London where the research team will provide them with the participant information sheet. The potential participants will be given at least a week to read the information, discuss it with family and friends, and ask the research team any questions. Should the individual be interested in participating and appear to be suitable for the study, the research team will contact them again to arrange a study visit. At the study visit the participant will attend the NHNN in London where the Principal Investigator or appointed delegate(s) will go through the study information sheet with the participant again before obtaining the participant's informed consent, after which the following assessments will be carried out:

## 3.3 Study Visit and Assessments

Participants will attend a single study visit where they will undergo:

- Cognitive tasks (CANTAB and EMOTICOM batteries)
- 3T Volumetric MRI
- rsfMRI

- NODDI
- MPM
- Huntington’s disease core research assessments.
- Blood sample collection for research genotyping and biomarkers
- Neuropsychiatric self-report questionnaires

**OPTIONAL CSF COLLECTION**

- Clinical review and screening blood sample for CSF collection (Day 1)
- CSF and complimentary fasted blood sample collection (Day 2)

Participants meeting the eligibility requirements for the CSF collection, and who have given their consent to participate in this optional part of the study, will go on to have the lumbar puncture, CSF collection and venous blood draw the morning after the main study visit and stay overnight in a local hotel. The study visit is summarised in table 1 below:

Table 1: study visit timetable

**Day 1**

Estimated Time	Assessment Type	Details	Type of Test	Rating Type	Duration
09:00	Informed Consent	Consent to HD-YAS taken by researcher	-	-	30-45 min
9:45	Clinical review and blood collection	10 ml research genotyping (gene carriers only) ≤40 ml biomarker blood collection Optional 15 ml CSF screening blood sample (safety)	Lab		30 min
10:15	Cognitive	CANTAB EMOTICOM Other Learning from Gains and Loses - optional	Computerised tablet	Objective, computerised	110 min
13:00	LUNCH				
14:00	Imaging	Positioning 3T Volumetric MRI rsfMRI NODDI MPM Learning from Gains and Loses - optional	MRI	-	90 -120 mins
	BREAK				

16:15	HD core research assessments	Demographics, Lifestyle HD history, PMH and conmeds UHDRS (TMS, TFC, IS)	Psychologist/clinician led interview/questionnaires	Clinician/psychologist rated	60 - 75 mins
		PBA-s (& C-SSRS if relevant)			
		SDMT Verbal fluency test (category) Stroop (colour naming) Stroop (word reading)			
17:30	END OF STUDY VISIT				
Day 1 - 7	Neuropsychiatric self-report assessments	To be completed at home within a week of study visit, or following main study visit/overnight if staying for CSF collection	Online/paper questionnaires	Participant self-administered	40 mins

**Day 2 OPTIONAL - Participant arrives fasted for CSF and blood collection**

Overnight hotel stay near study site following Day 1 assessments. Fasting from midnight for CSF and blood collection (water only)					
8:00 - 10:30	CSF collection Blood collection**	Lumbar Puncture and venous blood collection	Biomarkers	Clinician	45 min procedure, plus resting time
1-3 days after CSF collection		Telephone follow-up to check for adverse events following the optional CSF collection. Follow-up details to be entered on to paper CRF by researcher.			

\*Venous blood sample will be obtained immediately after the CSF collection is complete

**3.3.1 Clinical review**

A standard neurological examination will be performed only for CSF volunteers as below, as well as a brief general physical examination. Evidence of possible bleeding tendency such as bruises or petechial rash should be noted if the participant has consented to participate in the optional CSF collection.

- Cranial nerves
- visual acuity
- visual fields to confrontation
- fundoscopy (including appearance of discs and presence / absence of venous pulsations)
- smooth pursuit and saccadic eye movements
- facial sensation
- jaw power
- facial symmetry and power
- bedside auditory acuity
- palatal elevation
- pharyngeal sensation
- cough
- Sternocleidomastoid muscle and trapezius power
- Upper and lower limbs
- Tone
- Proximal and distal power
- Reflexes (-, +/-, +, ++, +++)
- Pinprick sensation
- Plantar responses
- Coordination

**ESTIMATED TOTAL TIME:** 25 minutes

### **3.3.2 Venous Blood Collection Procedure**

Venous blood is drawn in the morning of the study visit. Participants are not required to fast. Collection time is recorded. The following samples are acquired:

- Up to 4 × 10 ml blood in lithium heparin tubes. Gently invert each tube 10 times immediately after collection, and place on ice.
- If venepuncture with vacuum tubes proves challenging, a needle and syringe may be used and the blood transferred immediately into the vacuum tubes, observing safety precautions.

### 3.3.2.1 Plasma Sample Processing

1. Spin lithium heparin tubes at 1300×g for 10 min at 4°C immediately on arrival.
2. Discard any tubes whose plasma is pink due to hemolysis.
3. Combine the supernatant in one tube labelled “plasma” and mix by inverting 10 times. Store on crushed ice.
4. Divide lithium heparin plasma into 300 µl aliquots using supplied pipette tips and cryovials labelled ‘plasma’.
5. Freeze samples on dry ice and store at -80°C.
6. Record time of freezing.

### 3.3.3 Cognitive Tasks

#### **Overview:**

#### ***CANTAB***

- ID/ED Attentional Set Shifting (7 minutes)
- One Touch Stockings (10 minutes)
- Spatial Working Memory (10 minutes)
- Paired Associates Learning (10 minutes)
- Rapid Visual Information Processing (10 minutes)
- Stop Signal Task (SST) (20 minutes)

#### ***EMOTICOM***

- Emotional Intensity Face Morphing (5 minutes)
- Moral Judgement (10 minutes)
- Progressive Ratio (20 minutes)

#### ***Other***

- Reinforcement learning (5 minutes)
- National Adult Reading Test (3 minutes)
- Cats and Dogs Visual Processing task (15 mins)

**ESTIMATED TOTAL TIME:** 125 minutes

**Description:****CANTAB battery**

The CANTAB computerised neuropsychological tests (<http://www.camcog.com>) have been used previously in studies of premanifest and manifest HD and are neurobiologically-based and validated, with good reliability.

The following CANTAB tests are proposed for inclusion in the battery:

- Intra-dimensional and extra-dimensional (ID/ED) Attentional Set Shifting

Measure of cognitive flexibility that involves rule acquisition and reversal learning in response to simple and compound visual stimuli. Participants are required to adapt their responding to changes in outcome contingency and shift their attentional focus within the same (ID) or different (ED) stimulus dimensions. Previously shown to be sensitive to deficits in both early (Lawrence et al. 1996) and premanifest (Lawrence et al. 1998) HD.

- One Touch Stockings

A test of visuospatial planning and working memory based on a modified version of the Tower of London. Participants are shown example configurations of three coloured balls and must determine the minimum moves required to match their display to the example without actually moving the balls. Previously shown to be sensitive to disease progression and to correlate with functional assessment scores in early HD (Ho et al. 2003).

- Spatial Working Memory

Examines memory for spatial locations already visited in an array of visual stimuli, assessing an individual's ability to retain and manipulate visuospatial information and to apply an effective search strategy. These aspects of visuospatial working memory have been found to be impaired in premanifest HD (You et al. 2014; Tabrizi et al. 2009).

- Paired Associates Learning

A sensitive measure of episodic memory and new learning. Patterns are briefly displayed at various locations on the screen, before they are hidden and the participant must recall their location. The number of patterns to be recalled increases from 2, 3, 6, to 8. Elevated error rates on this task are apparent in manifest HD (Lawrence et al. 2000) and predictive of time to disease onset in premanifest HD (Begeti et al. 2016).

- Rapid Visual Information Processing

Measures sustained attention by presenting a rapid stream of digits and requiring participants to detect target sequences. Accuracy measures indicate sustained attention ability and reaction times provide a measure of visuomotor processing speed.

- Stop Signal Task

Measures response inhibition (impulse control). Participants respond to an arrow stimulus, however if an audio tone is present the subject must inhibit that response. Performance indicates how quickly and efficiently participants can inhibit a prepotent motor response.

### **EMOTICOM battery**

EMOTICOM is a newly developed battery to assess social cognition and motivational/emotional functioning which has been standardised on 300 volunteers (Bland et al. 2016).

The following EMOTICOM tests are proposed for inclusion in the battery:

- Emotional Intensity Face Morphing

Assesses emotion recognition by determining the point of emotional intensity at which participants can recognise a facial emotion. Participants view faces that either increase or decrease in emotional intensity and are asked to respond at points when they can detect (or no longer detect) a given emotion. It is anticipated this task will be sensitive to emotion recognition deficits documented in early and premanifest HD (Henley et al. 2012).

- Moral Judgement

Examines emotional responses to social situations. Cartoon figures depict a range of moral scenarios involving accidental or intentional harm. Participants are required to imagine how they would feel as either the victim or actor and to rate their feelings across a range of emotions including guilt, shame, anger and feeling “bad”. This task will provide a detailed measure of emotional responsiveness and social processing.

- Progressive Ratio

Determines a motivational ‘breakpoint’ at which participants will stop responding in order to receive a reward that becomes progressively smaller and requires greater effort as the task progresses. Participants make their responses to visual stimuli on the screen and are told they can give up at any point. The breakpoint measure has been widely used to gauge motivation and willingness to exert effort in human and animal studies.

### ***Other***

- Reinforcement learning

The task measures learning and choice behaviour in the context of both gains and losses.



Participants are shown two sets of symbols and need to learn via trial-and-error which symbol in each set is correct. Shown to be sensitive to pharmacological manipulation with a D2 receptor antagonist (Eisenegger et al. 2014), this task is anticipated to be sensitive to early indirect pathway dysfunction in premanifest HD.

- National Adult Reading Test: Reading test used as an estimate of premorbid Intelligence Quotient (IQ)
- Cats and Dogs visual processing task: asks participants to determine whether a black and white picture is either a cat or a dog. The pictures are shown with increasing degrees of skew increasing the difficulty to determine with the picture is a cat or a dog. Participants are briefly shown the skewed image and then make their choice by pressing the left and right button on the keyboard. The participant is shown a series of these pictures are a variety of difficulty levels. This task has been shown to be sensitive to visual processing deficits in other neurodegenerative conditions such as Parkinson's disease (PD). (Weil *et al.* 2017)

### **Learning From Gains and Losses in HD-YAS – Sub-study**

Learning from Gains and Losses forms a cognitive sub-study in HD-YAS comprising a social learning task and extended reinforcement learning task mapped to fMRI outcomes. Only participants who do not take antipsychotic medications will be invited to participate. The participant will be given the Learning from Gains and Losses Participant Information Sheet to read in advance, and if they choose to participate they will be asked to sign the consent form at the study visit.

#### **3.3.4 Imaging assessment**

All participants will undergo structural (T1, NODDI and MPM) and functional (resting state) MRI on a 3T scanner. Protocol details will be provided in the Standard Operating Procedure (SOP) documents. The structural T1 modality was chosen because it can provide images suitable for the most widely used and discriminating analysis techniques (e.g. Aylward et al., 2004; Henley et al., 2006; Rosas et al., 2005; Kassubek et al., 2004,). NODDI, MPM and rsfMRI are exploratory techniques which have been added to provide complementary assessments of hallmarks of HD. NODDI has the ability to highlight subtle changes in tissue microstructure (eg. Jones et al., 2006; Zhou et al, 2003; Filippi et al., 1998; van den Bogaard et al.,2012; Zhang et al, 2012) and MPM will provide a measure of myelination within the white matter. These measures, together with the rsfMRI will be used to identify any alterations in network connectivity.

**ESTIMATED TOTAL TIME: 90 minutes**

A 3T MRI scanner has been chosen for the HD-YAS study, for the following reasons:

- All the planned image analysis techniques can be applied to 3T scans;
- There is better grey-white definition for the same scan duration.
- 3T scanners are using cutting edge technology and as new imaging techniques become available, the 3T MRI collection will be invaluable for further analyses in the future, in addition to the morphometric studies planned for the HD-YAS study.
- 3T imaging represents technology that will become dominant, which means that the HD-YAS study is undertaking an imaging protocol which will be at the forefront of research and trials.
- All main centres with MRI facilities will be changing to 3T scanners, and this is currently occurring at a rapid pace.

**Local Radiologist Read**

A radiological read will be performed for any incidental pathology, this will ideally be done within five working days of image collection. If abnormalities are seen UCL will liaise with the recruiting site and ensure the participant's GP and/or local neurologist are informed if relevant.

The following analysis will be performed by the dedicated image analysis team at UCL:

- 1) Whole brain, ventricular and caudate volumes. Assessment of neurite density using Neurite Orientation and Dispersion Diffusion Imaging (NODDI) to detect subtle changes in microstructure which are complementary to macrostructural information provided by volumetric imaging.
- 2) Grey- and white-matter density using voxel-based morphometry (VBM) (Ashburner & Friston, 2000)
- 3) Automated segmentation of regions of interest using the Freesurfer software which integrates reliable and validated image analysis tools for large neuroimaging studies.
- 4) Resting state fMRI region analyses and principal component analyses. Seed connectivity will be used to identify brain regions which are simultaneously activated at rest. Dynamic causal modelling will be used to explore causal interactions between the brain and these regions.

- 5) Brain cellular microstructure and structural connectivity will be examined using volumetric MRI combined with neurite orientation dispersion and density imaging (NODDI). Computational approaches will be applied to visualize neural tracts and to determine the relative strength of anatomical connections between brain regions. Graph theory will be used to detect changes in the organization of brain networks such as integration and efficiency. Statistical parametric mapping will be used to identify any differences in myelination within gene carriers.

### **3.3.5 HD Core Research Assessments**

The following assessments will be completed as part of the core HD core research assessments at the study visit. These validated assessments are commonly used in HD research.

#### **Unified Huntington's Disease Rating Scale (UHDRS)**

The Unified Huntington's Disease Rating Scale (UHDRS), developed by the Huntington Study Group to provide a uniform assessment of the clinical features and course of HD has undergone reliability and validity testing that support its use in longitudinal studies (Huntington Study Group 1996). The scale assesses four domains associated with HD: motor function, cognitive function, behavioural abnormalities and functional capacity.

#### **UHDRS Total Functional Capacity Scale (TFC)**

The TFC represents the Investigator's assessment of the participant's capacity to perform a wide range of activities of daily living including working, chores, managing finances, eating, dressing and bathing. It is based on a brief interview with the participant and the study partner (if relevant). Scores range from 0 to 13, and higher scores represent better functioning.

#### ***UHDRS Independence Scale***

The patient's independence scale is the Investigator's assessment of the participant's degree of independence. The scale consists of 19 discrete levels ranging from 10 to 100 (by 5) where no special care needed corresponds to a scale of 100 and tube fed and total bed care corresponds to a scale of 10.

#### ***UHDRS Total Motor Scale (TMS)***

The TMS is the sum of the individual motor ratings obtained during administration of the motor assessment portion of the UHDRS. Scores range from 0 to 124, and higher scores represent more severe impairment.

#### **Problems Behaviour Assessment for Huntington's Disease – Short Form (PBA-s)**

The PBA-s assesses common behavioural and psychiatric manifestations of HD, including affect, irritability, loss of motivation, perseverative phenomena and psychotic symptoms. The test administrator interviews the participant (and companion if relevant) and rates the participant's behaviour over the prior four weeks according to the guidelines for the test. The C-SSRS will also be activated and completed if triggered by responses to items in the PBA-s which relate to self-harm and suicidal ideation. If the responses to the C-SSRS indicate the subject is at risk, in line with local standard operating procedures, the clinical care team will be informed and the relevant referrals made.

### **Verbal Fluency (Category)**

Verbal fluency is a commonly used neuropsychological test which examines the ability to spontaneously produce words orally within a fixed time span. For category fluency, words must be produced according to semantic constraints. The measure of performance used will be the number of correctly generated words within 60 seconds.

### **Stroop (Colour & Word)**

The Stroop Colour and Word Reading tests are commonly used neuropsychological tests. They involve naming colors (e.g., red, green, blue) and reading the words for colors in black ink.

### **Symbol Digit Modality Test (SDMT)**

SDMT involves a simple substitution task. Using a reference key, the examinee has 90 seconds to pair specific numbers with given geometric figures. The score is the number of correct responses achieved in 90 seconds.

**ESTIMATED TOTAL TIME:** 60 minutes

### **3.3.6 Neuropsychiatric self-report assessments**

All questionnaires are self-report and designed to be completed by participants independently in a take-home pack or online, depending on preference/availability.

- Frontal Systems Behaviours Inventory (FrSBe) 7 mins
- Toronto Alexithymia Scale (TAS-20) - 20 items; 4 minutes
- Toronto Empathy Questionnaire (TEQ) - 16 items; 3 minutes
- Zung self-rating depression scale (SDS) - 20 items; 3 minutes
- State/Trait Anxiety (STAI) - 40 items; 5 minutes

- Barratt Impulsivity scale (BIS-11) - 30 items; 4 minutes
- Obsessive-Compulsive Inventory (OCI-R) - 18 items; 3 minutes
- Apathy-Motivation Index - 18 items; 3 minutes
- Pittsburgh Sleep Quality Index (PSQI) -19 items; 3 minutes
- MOS 36-Item Short-Form Health Survey (SF-36) - 36 items; 4 minutes

**ESTIMATED TOTAL TIME:** 40 minutes

Description:

- FrSBe

The goal of the FrSBe is a 46-item behaviour rating scale that is intended to measure behaviour associated with damage to the frontal systems of the brain. Separate rating forms are available for the participant (Self-rating) and the companion (Family Rating). Each FrSBe form yields a Total score and scores for subscales measuring Apathy (14 items), Disinhibition (15 items), and Executive Dysfunction (17 items). Each item is rated on a 5-point Likert scale.

The FrSBe has been used in the Predict HD study and preliminary analyses demonstrate some sensitivity in premanifest HD. Hamilton et al. (2003) showed that this rating scale was sensitive to changes occurring between the premanifest period and early HD.

- TAS-20

Widely used and validated self-report instrument to measure the construct of alexithymia, i.e., difficulties in identifying and describing emotions, reduced emotional engagement and tendencies towards externally focused attention. Items are scored on a five-point scale from 'strongly disagree' to 'strongly agree' (Bagby et al. 1994).

- TEQ

Validated brief self-report measure that covers multiple facets of empathy, including its cognitive and affective components. Items are scored on a five-point scale ranging from 'never' to 'often' (Spreng et al. 2009).

- SDS

Self-rated scale that is a well validated screening tool for depression. Covers affective, psychological and somatic symptoms associated with depression, items are scored on a four-point scale (Zung et al. 1965).

- STAI

Well validated and commonly used self-report measure of anxiety, providing assessment of both state and trait levels of anxiety. Items are scored on a four-point scale that reflects frequency of anxious thoughts/behaviours (Spielberger et al. 1983).

- BIS-11

Self-report questionnaire designed to assess the personality/behavioural construct of impulsiveness. Measures three factors, including attentional, motor and non-planning impulsivity, items are scored on a four-point scale relating to frequency of behaviours (Patton et al. 1995).

- OCI-R

Brief self-report instrument to determine severity of obsessive and compulsive behaviours. Items are scored on a five-point scale identifying how often an individual is distressed by behaviours relating to washing, checking, ordering, obsessing, hoarding, neutralising (Foa et al. 2002).

- AMI

The Apathy-Motivation Index (AMI) is an 18 item scale that assesses motivation, which was developed for use in healthy participants. Based on the Lille Apathy Rating Scale (LARS) (Sockeel et al. 2006), the AMI had been modified to be sensitive to variations in motivation in healthy people. The scale covers behavioral, social and emotional domains of apathy, and can identify profiles of apathy that are associated with depression, anhedonia and fatigue.

- PSQI

Self-report questionnaire assessing several sub-categories including, subjective quality of sleep, sleep onset latency, sleep duration, sleep efficiency, presence of sleep disturbances, use of hypnotic-sedative medication and presence of daytime sleepiness (Buysse et al. 1989).

- SF-36

Self-report scale assessing eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical/emotional problems; 3) limitations in usual role because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions (Ware & Sherbourne 1992).

### **3.3.7 Optional CSF and associated venous blood sample Collection**

Lumbar puncture is to be performed between 8:00 and 10:30 am local time. All participants will be asked to fast from midnight the night before their appointment, but are permitted to drink water freely. Compliance with instructions to fast will be recorded.

The participant's continued consent to participate will be confirmed and recorded in the medical notes prior to CSF and blood collection, and the results of the routine laboratory examination are to be reviewed and recorded together with measurement of vital signs. Venous blood sampling is performed immediately after CSF collection is complete. (See Section 3.3.8 for complete instructions).

### **3.3.8 Lumbar CSF Collection Procedure**

1. Ensure that all equipment is on hand and that ice is available for CSF collection and transportation of samples to the lab.
2. Ensure availability and settings of centrifuges for appropriate temperatures and timely processing of CSF and blood samples.
3. Pre-cool CSF collection tubes on ice.
4. Prepare a sterile field containing all equipment needed, label tubes.
5. Place participant into lateral decubitus position with pillow between knees.
6. Identify L4/5 or L3/4 space using surface markings.
7. Disinfect skin using pre-filled antiseptic sponge.
8. Inject up to 5ml of 2% lidocaine for local anaesthesia. Use the 25g needle and inject lidocaine to raise a skin wheal. Then inject lidocaine more deeply.
9. Obtain CSF using a 22G spinal needle. If the participant is thin, do not insert the deep infiltration needle all the way. Use only about 2/3 of its length (to prevent entering the subarachnoid space with anything other than the pencil-point spinal needle).
10. If CSF cannot be obtained, up to three needles may be used.
11. An adjacent space may be used (with further lidocaine, max. total 10 ml, if needed).
12. If necessary, CSF space may be located by sitting participant up, but once CSF is seen, it is recommended to have participant lie back in lateral decubitus position for 30 seconds before collection begins. Document positions of participant during puncture and collection in the eCRF.

13. Document the space used for lumbar puncture, the number of attempts and volume of lidocaine used in the eCRF.
14. Omit pressure measurement for all participants (because polypropylene spinal manometers are not available).
15. CSF is collected in 50ml tubes placed on ice in the Styrofoam cup.
16. Collect the first 1 ml of CSF into the supplied tube labelled 'CSF'. If the first 1 ml (approx. 15 drops) is not macroscopically bloody, continue sampling CSF in the same tube up to 20 ml, keeping the tube in the ice cup. If the first 1 ml is macroscopically bloody, stop collecting CSF by reinserting the stylet partially, discard the tube, and collect a second 1 ml in a new pre-cooled 'CSF' tube, and examine it visually for blood contamination. If it is free of blood, continue collecting CSF up to 19 ml. If the second separately collected ml of CSF is also macroscopically bloody, discard the tube, and continue to collect 18 ml of CSF in a third pre-cooled 'CSF' tube. Stop collecting CSF when sampling time exceeds 20 minutes. Document these details in the eCRF.
17. Place cap on tube and leave on crushed ice until further processing.
18. Reinsert the stylet before withdrawing the needle.
19. Cover the puncture site with sterile dressing.
20. Record time of CSF collection.
21. Participants can mobilise or remain lying for an hour at their discretion.
22. Transport samples immediately to biomarker laboratory for processing.

### **3.3.8.1 CSF Participant Discharge**

Participants are observed for potential complications for at least an hour and discharged once appropriate. Any AEs are recorded.

Participant is discharged by nurses with instructions for over-the-counter pain medication and hydration in the event of headache.

### **3.3.8.2 CSF Follow-up Telephone Call**

Participants will be contacted 24 to 72 hours following the Sampling Visit to collect any AE and/or concomitant medication data.

### **3.3.8.3 CSF Sample Processing**

1. All CSF processing should be done on ice, beginning within 30 minutes of completion of collection.



2. Agitate the entire CSF sample for 10 seconds to homogenise CSF.
3. Use 200 µl of the CSF to determine white blood cell count and erythrocyte count per µl according to local GLP-approved laboratory practice. This should be done in triplicate within 60 minutes of collection and all values recorded in the eCRF.
4. Centrifuge the 50 ml tube containing residual CSF at 400 × g for 10 min at 4°C to remove cells while preserving cell integrity for potential future use.
5. Pipette supernatant into a single tube labelled “CSF supernatant” and agitate for 10 seconds to homogenise CSF
6. Aliquot the CSF into 300 µl aliquots, using supplied pipette tips and cryovials labelled “CSF”.
7. Gently resuspend pellet in 300µL of supplied preservative solution and transfer to the cryovial labelled “Cells from CSF”.
8. Freeze CSF aliquots and resuspended cells on dry ice and store at -80°C.
9. Record time of freezing

### **3.3.9 Venous Blood Collection Procedure**

Venous blood is drawn immediately after CSF collection is complete, recording the time. The following samples are acquired:

- 1 × 8.5 ml serum tube.
- 4 × 10 ml blood in lithium heparin tubes. Gently invert each tube 10 times immediately after collection, and place on ice.
- If venepuncture with vacuum tubes proves challenging, a needle and syringe may be used and the blood transferred immediately into the vacuum tubes, observing safety precautions.

#### **3.3.9.1 Serum Sample Processing**

1. Spin serum tube at 2000×g at room temperature for 10 min immediately upon arrival in the biomarker laboratory
2. Transfer 1500 µl of the supernatant into each of 2 separate 2 ml cryovials labeled “serum”, freeze on dry ice and store in -80°C.
3. Record time of freezing

#### **3.3.8.2 Plasma Sample Processing**

1. Spin lithium heparin tubes at 1300×g for 10 min at 4°C immediately on arrival.
2. Discard any tubes whose plasma is pink due to hemolysis.

3. Combine the supernatant in one tube labelled “plasma” and mix by inverting 10 times. Store on crushed ice.
4. Divide lithium heparin plasma into 300 µl aliquots using supplied pipette tips and cryovials labeled ‘plasma’.
5. Freeze samples on dry ice and store at -80°C.
6. Record time of freezing.

### **3.3.9 Sample storage**

- Store samples in a -80°C freezer.
- Log samples on papercrf and secure HD-YAS database.

### **3.3.10 Sample Quality Control**

The following quality control measures will be carried out to identify and flag samples subject to potential confounders:

- Microscopic erythrocyte count in CSF is performed locally in triplicate and recorded on the secure HD-YAS database. Cut-off for flagging: > 1000 cells/µl.
- Microscopic leukocyte count in CSF is performed locally in triplicate and recorded on the secure HD-YAS database. Cut-off for flagging: ≥ 5 cells/µl.

### **3.3.11 Analysis of CSF and plasma samples**

CSF and plasma samples will be analysed locally at UCL Institute of Neurology or by collaborators authorised by the Principal Investigator. This may include collaborators outside the EU from academic or commercial entities for the purpose of research (1) to better understand HD or other diseases being studied, (2) that furthers the development of treatments for HD or other diseases or (3) that furthers biomedical research. Any shared samples will be coded and linked-anonymised.

Analyses of huntingtin protein and the kynurenine pathway are specifically planned. Specifically, the levels of the following KP metabolites will be measured in CSF: kynurenine, kynurenic acid, 3-OH-kynurenine, quinolinic acid and anthranilic acid. In addition, the plasma levels of tryptophan will be determined in the fasted plasma collection, which will allow for an additional control for lack of compliance with the stipulation of an overnight fast.

Additional measurements, including but not limited to other KP metabolites or precursors, the levels of soluble HTT, and other putative biomarkers may also be measured at appropriate laboratories.

The primary outcome measurements are of unknown clinical significance. The detailed analysis may include measurements of potential clinical significance in relation to conditions other than HD, such as oligoclonal bands. However, patients with other neurological diagnoses or unexpected examination findings will be excluded. Therefore any abnormal results, obtained on a linked-anonymised basis, will remain of indeterminate clinical significance and will not be fed back to the participant.

A portion of each participant's CSF and fasted blood samples will be shared alongside phenotypic data with CHDI Foundation Inc. a collaborator of the Principal Investigator and partial funder of HD-YAS to investigate the huntingtin protein, the kynurenine pathway and other biomarkers and pathways of relevance to HD.

### **3.3.12 CSF and blood draw Safety**

The procedures for performing lumbar punctures and venous blood draws have been designed to maximize participant safety.

Study-related risks are explained in the informed consent document. In particular, the following risks may be associated with lumbar puncture: pain; headache (approximately 5%), infection, bleeding and nerve root damage. Most headaches resolve spontaneously but occasionally a headache may be persistent; in rare cases this may necessitate treatment, which may include a second procedure (a blood patch), carried out in a clinical setting.

See Appendix A, page 40 –Principal Investigator Obligations for additional information.

## **3.4 Criteria for Termination of the Study**

If the study is prematurely terminated or suspended for any reason, the Principal Investigator/institution will promptly inform the study participants and should assure appropriate follow-up for them. The Principal Investigator will also inform the appropriate Research Ethics Committee and Trust R&D Office.

## 4. Adverse Event Reporting and Documentation

### 4.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence during a clinical investigation and that does not necessarily have a causal relationship with study treatments or procedures. An AE is therefore any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of study procedures.

The Principal Investigator or appointed delegate(s) will probe, via discussion with the participant, for the occurrence of AEs during each participant visit, after the screening visit, and record the information in the site's source documents. AEs will be recorded on paper CRFs and in the HD-YAS secure database. AEs will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study procedures if applicable, or if unrelated, the cause.

#### 4.1.1 AE Severity Grading

The severity of an AE will be graded on a 5-point scale (Common Terminology Criteria for Adverse Events v3.0 (CTCAE);

[http://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/ctc.htm](http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)) defined as follows:

Grade 1	Mild AE
Grade 2	Moderate AE
Grade 3	Severe AE
Grade 4	Life-threatening or disabling AE
Grade 5	Death related to AE

#### 4.1.2 AE Relationship to study procedures

The relationship of an AE to the study procedures will be evaluated according to the following guidelines:

Probable: This category applies to AEs which are considered with a high degree of certainty to be related to the study procedure. An AE may be considered probably related to the study procedure if:

1. It follows a reasonable temporal sequence from administration of the study procedure;
2. It cannot be reasonably explained by the known characteristics of the participant's clinical state, or by environmental or toxic factors;

3. It follows a known pattern of response to the study procedure;

Possible: This category applies to those AEs in which the connection with the study procedure appears unlikely but cannot be ruled out with certainty. An AE may be considered as possibly related if it has at least two of the following:

1. It follows a reasonable temporal sequence from the study procedure
2. It may readily have been produced by the participant's clinical state, or by environmental or toxic factors;
3. It follows a known response pattern to the study procedure.

Unrelated: This category applies to those AEs which are judged to be clearly and incontrovertibly due to extraneous causes (disease, environment, etc.) and do not meet the criteria for study procedure relationship listed under possible or probable.

## 4.2 Serious Adverse Events

A Serious Adverse Event (SAE) is defined as any AE that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed.

An AE is considered to be life-threatening if, in the view of the Principal Investigator, the participant was at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more serious form, might have caused death.

### 4.2.1 Serious Adverse Event Reporting

SAEs (as defined in Section 4.2) must be reported to the study Sponsor immediately and in no case later than within 24 hours of awareness of the event.

All SAEs that occur (whether or not related to study procedures) will be documented. The collection period for all SAEs will begin from the time of informed consent until all the study procedures have been completed, unless further follow-up is requested by the Investigator.

In accordance with the standard operating procedures and policies of the REC, the Principal Investigator will report SAEs to the REC.

### **4.3 Post-study Follow-up of Adverse Events**

Any AE, including clinically significant physical examination findings, must be followed until the event resolves, the condition stabilises, the event is otherwise explained, or the participant is lost to follow-up. If resolved, a resolution date should be documented on the secure HD-YAS database and in the source documents. The Principal Investigator is responsible for ensuring that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals as is medically indicated.

## **5. Data Analysis, Power Calculations and Statistical Methodology**

We will test for adjusted mean differences between groups, typically via covariate-adjusted least square comparisons. Covariates will include age, gender, and IQ and, nested within the gene-carrier group, CAG length and its interaction with age.

We will adjust for potential group differences in residual variances, and if substantial violations of test-assumption data distributions are noted we will adjust the analyses by outcome-variable transformation if possible. Failing this, we will use nonparametric bootstrapping for inference and confidence level estimation.

For objectives 2 and 4 (See section 2.1), group differences will be tested using non-parametric permutation testing with inclusion of age, gender, site and IQ as covariates. In secondary analyses, we will assess correlations between outcomes and the CAG-length-Age Product measures (CAP) statistic that has proved a powerful predictor of HD progression in older participants (Ross et al. 2014)

With a type 1 error rate of 5%, a sample of 60 participants/group will provide 80% power to detect a mean difference versus controls of 0.53 adjusted within-group standard deviations (effect size), allowing for 5 covariates. Similarly, after allowing for 5 covariates, the sample of 60 CAG-expanded participants allows the same statistical power for detecting a partial Pearson correlation of 0.36 among outcome measures and between these measures and the CAP score or other potential predictors of HD risk.

In Track-HD, participants less than 40 years old who, based on age and CAG length, were estimated to be more than 11 years from onset, the effect size for caudate volume difference was 0.78 when compared to an age-matched subsample of controls from that

study. Hence, we believe that the detectable effect size of 0.53 is a reasonable conjecture of potential differences for the younger population sampled for this project.

#### *Implications of the Null Hypothesis*

These analyses will test the null hypothesis of no significant difference in phenotypes between HD gene carriers and controls. A lack of group differences would also be an important result, as it would suggest that neuronal structure and function may be essentially normal (within the limits of our measurement precision) until a tipping point occurs in disease progression in preHD.

#### **Expected outcomes**

We will determine whether young adult gene carriers with a disease burden score (DBS)  $\leq 240$  (Penney et al. 1997) from expected symptomatic onset exhibit differences from controls in:

- 1) Regional brain volumes;
- 2) Structural brain connectivity;
- 3) Functional/effective brain connectivity;
- 4) Grey matter and white matter microstructure;
- 5) Myelination
- 6) Mutant huntingtin levels in the CSF;
- 7) Cognition.

This range of complementary measures, assessed using rigorous hypothesis-led testing, will allow us to clearly articulate which measures (or combination of measures) demonstrate the earliest differences. This will potentially provide a new boundary for the earliest time at which therapy could (or should) start.

## **6. Study Management**

### **6.1 Ethics and Regulatory Considerations**

The investigator will conduct the study in compliance with the protocol and in accordance with the ICH for GCP and the appropriate regulatory requirement(s). The study will also be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

Participants must give informed consent prior to undertaking study procedures and these informed consents must be obtained by clinical site staff using approved processes. Signed consent forms will be maintained in a secure designated location.

### **6.1.1 Audits and Inspections**

The study may be subject to inspection and audit by University College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition). Audits and/or inspections may also be carried out by local authorities, or authorities to which information on this trial has been submitted. All documents pertinent to the trial will be made available for such inspection after an adequate announcement.

### **6.1.2 HRA/Ethics Committee Approval**

The Investigator will obtain approvals from the HRA/Research Ethics Committee (REC) The Investigator will require confirmation of capacity and capability to deliver the study before accepting participants into the study. Substantial amendments to the protocol will require written approval / favourable opinion from the HRA/REC prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to participants. Deviation from the protocol required to eliminate an immediate hazard(s) to participants will be fully documented in the CRF and source documentation.

### **6.1.3 Confidentiality**

In order to maintain subject privacy, all case report forms (CRFs), study reports and communications will identify the subject by initials and the assigned Subject number. The investigator will preserve the confidentiality of participants taking part in the study in accordance with the Data Protection Act.

### **6.1.4 Sponsorship and Funding**

University College London will act as the Sponsor for this study.

This study is funded by the Wellcome Trust as part of the Collaborative Award in Science presented to Professor Tabrizi as part of the TREAT-HD project, and the CSF collection is funded by CHDI Foundation Inc., a not-for-profit foundation that only works on HD.

### **6.1.5 Indemnity**

University College London holds insurance against claims from participants for injury caused by their participation in the trial. Participants may be able to claim compensation if they can prove that UCL has been negligent. However, as this trial is being carried out in a hospital, the hospital continues to have a duty of care to the participant of the



trial. University College London does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or otherwise.

Participants may also be able to claim compensation for injury caused by participation in this trial without the need to prove negligence on the part of University College London or another party. Participants who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to the Sponsor's Insurers, via the Sponsor's office.

Hospitals selected to participate in this trial shall provide negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to University College London, upon request.

## **6.2 Informed Consent Procedure**

Consent to enter the study will be sought from each participant only after a full explanation of the study has been given, a participant information sheet offered and time allowed for consideration. Signed participant consent will be obtained. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

The right of the participant to refuse to participate without giving reasons will be respected. After the participant has entered the study the investigator remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

## **6.3 Biological samples (handling, processing and storage)**

Biological material from the blood collection (plasma and DNA), as well as CSF and fasted blood from the optional part of the study, will be collected from participants in accordance with the participant consent form and participant information sheet and shall include all biological materials and any derivatives, portions, progeny or improvements as well as all participant information and documentation supplied in relation to it. These biological samples will be stored at UCL Institute of Neurology for the processing described in sections 3.3.7.3, 3.3.8.1 and 3.3.8.2 of this protocol, and for use in future HD research. This will prepare samples for shipment to collaborators in accordance with the analytical plan agreed with the Principal Investigator. A portion of the coded samples will be shared with

CHDI, and these samples will be stored at a biological storage facility called BioRep, located in Milan, Italy. The PI and his delegated representatives will process, store and dispose of samples in accordance with all applicable legal and regulatory requirements, including the Human Tissue Act 2004 and any amendments thereto.

### **6.3.1 Research Genotyping**

Only participants with a confirmed predictive genetic test result are eligible for HD-YAS. Participants will have undergone extensive genetic counselling prior to predictive testing through their local service and all participants will be aware of their genetic status i.e., whether they will go on to develop HD or not. The exception to this are family and community controls who are not at risk of inheriting the expansion mutation, and therefore, predictive testing is not necessary.

All pre-manifest HD participants who already have a positive predictive genetic test will donate a sample for research genotyping to confirm the HD expansion mutation size. The purpose of this is to standardise the way the test is performed for study analyses. A DNA sample will not be collected from control participants.

Ten ml of peripheral blood will be collected in 2 x 5ml purple topped EDTA tubes. Samples will be transported on the day of collection to the Neurogenetics Laboratory, National Hospital for Neurology and Neurosurgery for DNA extraction and research genotyping. DNA extraction and genotyping will be performed according to standard procedure. The results will be fed back to Professor Tabrizi and the study team, but since these are obtained for research they will not be fed back to the participant.

DNA will be stored at UCL Institute of Neurology under custodianship of the PI. Samples will be donated with the understanding that it is used for HD-related research. In addition to re-sizing of the CAG expansion mutation within the HD gene, DNA may also be used to identify genetic modifiers of HD, in particular genetic modifiers of age of onset, rate of progression and phenotypic characteristics presentations.

## **6.4 Data Collection, Retention and Monitoring**

### **6.4.1 Blinding**

The Principal Investigator and psychologist carrying out the neurological and UHDRS assessments will be blinded to subject status (pre-manifest gene carrier versus healthy control) to prevent introduction of any rating bias; only individuals involved in recruitment or responsible for data monitoring within the HD Research centre will be aware of, or have access to, the subject status.

#### **6.4.2 Data transfer (handling, processing and storage)**

In the study, name, date of birth, medical history, ethnicity and cognitive data will be collected from participants in accordance with the participant consent form, participant information sheet and section 6 of this protocol.

The participant data will be stored securely at UCL Institute of Neurology and collaborators authorised by the Principal Investigator for statistical analysis, and UCL will act as the data controller of such data for the study.

The Principal Investigator and her delegated representatives will process, store and dispose of participant data in accordance with all applicable legal and regulatory requirements, including the Data Protection Act 1998 and any amendments thereto. Data held on paper will be stored at the UCL Institute of Neurology Huntington's Disease Research Centre under secure access control, in a locked filing cabinet controlled by the Investigator.

All transfers of data and/or samples will be covered by materials transfer agreements.

#### **6.4.3 Data Entry and Quality Control**

All HD-YAS assessments will be collected on paper case report forms, scored and filed in the participants medical notes, the scores will be entered onto the HD-YAS secure database. For the CANTAB and EMOTICOM data is recorded electronically direct into a tablet as the participant completes the assessments.

Source Documents: The Principal Investigator will maintain source documents for each participant enrolled in the study. Source documents such as local laboratory ranges and reports, participant charts and doctors' notes will be kept as part of the participants' medical records. For participants who do not have a medical record per se, another method of documentation and record keeping will be employed, along with the obligation to retain source documents, such as laboratory reports, for the period of time specified in the site agreement. Participant files including medical records and signed participant informed consent forms must be available for review in the event the site is selected for monitoring, audits, or inspections.

#### **6.4.4 Monitoring**

The Principal Investigator, on behalf of the Sponsor, is responsible for ensuring the proper conduct of the study with regard to ethics, protocol adherence, site procedures, integrity of the data, and applicable laws and/or regulations.

A senior member of the HD Research centre who is not involved with the study directly will carry out HD-YAS data monitoring and will ensure compliance with the study protocol.

The Principal Investigator will make study data accessible to the designated study monitor, to other authorised representatives of the Sponsor, and to regulatory inspectors.

The Chief Investigator will ensure there are adequate quality and number of monitoring activities conducted by the study team. This will include adherence to the protocol, procedures for consenting and ensure adequate data quality.

The Chief Investigator will inform the sponsor should he/she have concerns which have arisen from monitoring activities, and/or if there are problems with oversight/monitoring procedures.

#### **6.4.5 Intellectual Property Rights**

All background intellectual property rights (including licences) and know-how used in connection with the study shall remain the property of the party introducing the same and the exercise of such rights for purposes of the study shall not infringe any third party's rights.

All intellectual property rights and know-how in the protocol and in the results arising directly from the study, but excluding all improvements thereto or clinical procedures developed or used by each participating PIC site, shall belong to UCL. Each participating PIC site agrees that by giving approval to conduct the study at its respective site, it is also agreeing to effectively assign all such intellectual property rights ("IPR") to UCL and to disclose all such know-how to UCL.

Each participating PIC site agrees to, at the request and expense of UCL, execute all such documents and do all acts necessary to fully vest the IPR in UCL.

Nothing in this section shall be construed so as to prevent or hinder the participating PIC site from using know-how gained during the performance of the study in the furtherance of its normal activities of providing or commissioning clinical services, teaching and research to the extent that such use does not result in the disclosure or misuse of confidential information or the infringement of an intellectual property right of UCL. This does not permit the disclosure of any of the results of the study, all of which remain confidential.

### **6.5 Amendments**

Any amendments to the protocol will be written and approved by the Principal Investigator and submitted to the REC for approval prior to implementing the changes. In some instances, an amendment may require changes to the informed consent form, which also must be submitted to the HRA/REC and Trust R&D department prior to administration to study participants.

## **6.6 Record Keeping**

### **6.6.1 Statutory compliance**

The Principal Investigator agrees to comply with all applicable laws and regulations relating to the privacy of patient health information.

### **6.6.2 Retention of Study Documents**

UCL and each participating site recognise that there is an obligation to archive study-related documents at the end of the study (as such end is defined within this protocol). The Chief Investigator confirms that he/she will archive the study master file at University College London for the period stipulated in the protocol and in line with all relevant legal and statutory requirements. The Principal Investigator at each participating site agrees to archive his/her respective site's study documents for 20 years and in line with all relevant legal and statutory requirements.

## **7. Appendix A –Principal Investigator Obligations**

The study protocol and the final version of the participant informed consent form will be approved by the HRA/REC before enrolment of any participants. The opinion of the HRA/REC will be dated and given in writing.

The Principal Investigator will ensure that the HRA/REC will be promptly informed of all changes in the research activity and of all unanticipated problems including risk to participants. The Principal Investigator will not proceed with changes to the protocol until HRA/REC approval has been obtained.

Written informed consent must be given freely and obtained from every participant prior to clinical study participation. The rights, safety, and well-being of the study participants are the most important considerations and should prevail over interests of science and society.

As described in GCP guidelines, study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective task(s). Study personnel will not include individuals against whom sanctions have been invoked after scientific misconduct or fraud (e.g., loss of medical licensure, debarment). Quality assurance systems and procedures will be implemented to assure the quality of every aspect of the study.

### **HRA/REC Review/Approval/Reports:**

The protocol and informed consent for this study, including advertisements used to recruit participants, must be reviewed and approved by the HRA/REC prior to enrolment of participants in the study. It is the responsibility of the Principal Investigator to ensure that all aspects of the ethical review are conducted in accordance with the current Declaration of Helsinki, ICH, GCP, and/or local laws, whichever provide the greatest level of protection. Amendments to the protocol will be subject to the same requirements as the original protocol.

A progress report with a request for re-evaluation and re-approval will be submitted by the Principal Investigator to the REC at intervals required by the REC.

After completion or termination of the study, the Principal Investigator will submit a final report to the REC. This report should include: deviations from the protocol, the number and types of participants evaluated, and significant AEs, including deaths.

### **Study Documentation:**

The Principal Investigator is required to maintain complete and accurate study documentation in compliance with current Good Clinical Practice standards and all applicable federal, state, and local laws, rules, and regulations related to the conduct of a clinical study. Study documentation includes REC correspondence, protocol and amendments, information regarding monitoring activities, participant exclusion records, eCRFs, and data queries.

**Confidentiality:**

The anonymity of study participants must be maintained. Study participants will be identified by an assigned participant number on eCRFs and other documents submitted to the study monitors. Documents that will be submitted to the study monitors and that identify the participant (e.g., the signed informed consent document) must be maintained in strict confidence by the Principal Investigator, except to the extent necessary to allow auditing by regulatory authorities or the clinical monitor.

**Study Facilities:**

The Principal Investigator must ensure that there is a robust institutional policy on freezer failure that includes checks, alarms, emergency contact details, backup power supplies, CO<sub>2</sub> cylinders and an infrastructure to transfer samples to an off-site facility if necessary.

## 8. Appendix B –Disease Burden Score Table

Age	CAG							
	42	43	44	45	46	47	48	49
18	117	135	153	171	189	207	225	243
19	123.5	142.5	161.5	180.5	199.5	218.5	237.5	256.5
20	130	150	170	190	210	230	250	270
21	136.5	157.5	178.5	199.5	220.5	241.5	262.5	283.5
22	143	165	187	209	231	253	275	297
23	149.5	172.5	195.5	218.5	241.5	264.5	287.5	310.5
24	156	180	204	228	252	276	300	324
25	162.5	187.5	212.5	237.5	262.5	287.5	312.5	337.5
26	169	195	221	247	273	299	325	351
27	175.5	202.5	229.5	256.5	283.5	310.5	337.5	364.5
28	182	210	238	266	294	322	350	378
29	188.5	217.5	246.5	275.5	304.5	333.5	362.5	391.5
30	195	225	255	285	315	345	375	405
31	201.5	232.5	263.5	294.5	325.5	356.5	387.5	418.5
32	208	240	272	304	336	368	400	432
33	214.5	247.5	280.5	313.5	346.5	379.5	412.5	445.5
34	221	255	289	323	357	391	425	459
35	227.5	262.5	297.5	332.5	367.5	402.5	437.5	472.5
36	234	270	306	342	378	414	450	486
37	240.5	277.5	314.5	351.5	388.5	425.5	462.5	499.5
38	247	285	323	361	399	437	475	513
39	253.5	292.5	331.5	370.5	409.5	448.5	487.5	526.5
40	260	300	340	380	420	460	500	540

Penney disease burden scores for age-CAG combinations  
disease burden = age \* (CAG - 35.5)

Scores are for age-CAG combinations indicated in the margins. Highlighted values are maximum scores corresponding to estimated age until onset > 18 years as estimated by Langbehn et al. (2004).



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