Track-HD
Study Protocol

Revised: 2nd September 2013

Version 5.2

Change Log

Date	Description of change(s)	Name
2007-06-20	Initial release	Lifer, S.
2007-06-24	Edits to blood sampling and processing, exclusion criteria, and clause in consent form regarding possible use of video and audio for research purposes.	Tabrizi S.
2007-06-28	Add gait assessment and Dutch and French IQ estimates and relevant references.	Stout J., Queller S.
2007-07-02	Revised consent forms per study sponsor's request.	Tabrizi S.
2008-10-24	Revised assessment battery: • IQ administered at baseline only • Inter-visit graphimetry assessment for follow up visits removed • Details on the number of trials oculomotor task moved to SOP • Details on the number of trials transducer task moved to SOP • Reference to Stopping the Protocol SOP	Tabrizi S.
2008-10-24	Correction to disease staging (Stage 1 = TFC range 11-13, Stage 2 =TFC range 7-10); added Steering Committee members	Tabrizi S.
2008-12-17	Section 5.5.7 amended such that a single ACD tube may be collected at visits other than baseline in cases where it was not previously possible to generate lymphoblastoid cell lines or achieve cryopreservation of lymphocytes	Tabrizi S.

2009-11-10	Revised assessment battery:
	• Cognitive battery
	  ○ Speeded tapping tasks removed and merged with tapping assessment in quantitative motor battery
	  ○ Map search, mental rotation and colour/shape tasks replace previous experimental assessments
	• Quantitative motor
	  ○ Grip force matching task added
	  ○ Tapping tasks modified
	  ○ Posturography modified
	  ○ Gait removed
	• Graphimetry removed
	• Oculomotor
	  ○ Test of visual acuity added
	• Neuropsychiatric
	  ○ Additional apathy items added to PBA
	  ○ Baltimore apathy/irritability scale added
	• Sleep questionnaire added
		Tabrizi S.

2010-02-05	Protocol amended to reflect one year extension to study to 2011 (year 4) and recruitment of additional premanifest patients to replace withdrawn subjects and maintain numbers in this subject group	Tabrizi S.
2010-12-21	New consent and information forms added for fourth study visit and also including clarification of storage and access of pseudonymised data. New assessments have been added as follows: HD-specific Quality of Life questionnaire Physical activity questionnaire	Tabrizi S.
2011-10-25	Protocol amended to reflect three year extension to 2014 (V5, V6 & V7) and recruitment of new participants to replace withdrawals. The informed	Tabrizi S.

	consent has been updated to provide greater transparency and better comply with NRES recommendations (revised March 2011). Patient and control informed consents are now combined in a single study document suitable for all participants. New procedures have been added to the protocol: • Functional Magnetic resonance imaging (fMRI) • Magnetic resonance spectroscopy (MRS) • Diffusion weighted imaging (DWI) • Transcranial Magnetic Stimulation (TMS) • Buccal cheek swab • Cancellation task • Circle tracing with counting backwards • Bimanual tapping • Foot tapping (Pedomotography) • Pronate/supinate alternation (Dysdiadochomotography) And the following assessments have been removed or amended: • UPSIT (removed) • Trails A and B (removed) • Colour shape (removed) • Emotion Recognition (revised) • Speeded tapping cognitive (removed) • Mindstreams visuospatial imagery (removed) • Tongue force (revised) • Posturography (revised) • Problem behaviours assessment (removed) • SF36 (removed) • Quality of Life Index (removed) • PSQI (removed) • ISHD (removed) • Oculomotor (revised)	
2012-04-23	The following changes were made to the TRACK-HD assessments: • Quality of Life Index reinstated • Emotion recognition task removed • Order of MRI scans modified	Tabrizi S.
2013-09	The following changes made to the TRACK-HD assessments: New assessments added to the protocol: • Amide CEST, MTR & NODDI imaging technique • Virtual Maze cognitive task • Quantitative motor Go-No-Go task • TMS-EEG and VEPS added to TMS assessment The following assessments have been amended: • Duration of fMRI • Visual Array comparison task (SPOT7 condition discontinued) • Cancellation task (figure condition discontinued) • Additional 1x 6ml ACD tube to be collected for DNA extraction. • Addition of 2x 2.5ml PAXgene RNA tubes for the isolation of RNA for microarray/biomarker analysis. • Manumotography (light weight discontinued) Assessments discontinued: • Quality of Life Index • HDQoL questionnaire • Physical Activity Questionnaire	Tabrizi S.

	• Oculomotor assessment • Circle tracing cognitive test • Glossometry • Speeded tapping with cognitive load • Short Latency afferent inhibition	

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2. Abbreviations

Abbreviation	Definition
ACD	Acid citrate dextrose
ADM	Abductor digiti minimi
AMT	Active motor threshold
ANART	American national adult reading test
APB	Abductor pollicis brevis
BAIS	Baltimore apathy and irritability scale
BBSI	Brain-boundary shift integral
BRAINS	Brain Research: Analysis of Images, Networks, and Systems
BDI	Becks Depression Inventory
C	Control
CAG	Cytosine-adenine-guanine
CRO	Commercial research organisation
CEST	Amide Chemical Exchange Saturation Transfer
CRF	Case Record/Report Form
CSP	Cortical silent periods
CTMS	Clinical Trial Management System
DNA	Deoxyribonucleic acid
DV	Dependent variables
DWI	Diffusion Weighted Imaging
eCRF	Electronic CRF
EDTA	Ethylenediamine-tetraacetic acid
EEG	Electroencephalography
EHDN	European-HD Network
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyogram
ES	Effect size
FrSBe	Frontal Systems Behaviour Inventory
FDI	First dorsal interossoeus
FHQ	Family History Questionnaire
HADS	Hospital anxiety and depression scale
HD	Huntington's Disease
HD-QoL	HD-specific quality of life
HSG	Huntington Study Group
HVLT	Hopkins verbal learning test
IA	Image analyst
ION	Institute of Neurology
IRB	Institutional review board
LB	Lymphoblastoid
LONI	Laboratory of Neuro Imaging
MEP	Motor evoked potentials
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MTR	Magnetisation Transfer Ratio
NART	National adult reading test
NHNN	National Hospital for Neurology and Neurosurgery
NMR	Nuclear magnetic resonance department of ION
NODDI	Neurite Orientation Dispersion and Density Imaging
OCD	Obsessive-compulsive disorder
P	Psychologist
PAQ	Physical activity questionnaire
PI	Principal investigator
PM	Premanifest
QA	Quality assurance

Abbreviation	Definition
QC	Quality control
QoL	Quality of life
QOLI	Quality Of Life Index
R&D	Research and development
RNA	Ribonucleic acid
RMT	Resting motor threshold
RT	Reaction time
SAI	Sensory-afferent inhibition
SD	Standard deviation
SDMT	Symbol digit modality test
SEP	Somatosensory evoked potential
SOP	Standard operating procedure
SRB	Scientific review board
TE	Echo time
TFC	Total functional capacity
TIV	Total intracranial volume
TMS	Transcranial Magnetic Stimulation
TMT	Trail-making test
UCL	University College London
UHDRS	Unified Huntington's Disease Rating Scale
VBM	Voxel-based morphometry
VEP	Visual Evoked Potentials
WASI	Wechsler abbreviated scale of intelligence

3. Executive Summary

Track-HD is a multi-centre, multi-national, prospective, observational biomarker study of individuals who have inherited the Huntington's disease (HD) genetic mutation, together with a control group of volunteers not carrying the HD mutation. The original goal of the project - to contribute essential methodology to form the basis for neuroprotective trials in HD – has already yielded a range of quantitative outcome measures suitable for use in potential clinical trials in the early stages of the disease. The aim of visits 5 to 7 study extension is to provide further essential methodological and biological advances in the premanifest HD population. At visit 7 we plan to develop a multi-parametric set of novel and innovative MRI methods to complement the existing Track-HD imaging protocol; to specifically investigate cross sectional alterations in mutant HTT and direct loss of neurons in the brain.

Track-HD complements existing observational studies (e.g., Predict-HD, PHAROS, Registry, COHORT), sharing some features, such as the prospective longitudinal design, but also having areas of unique emphasis, including implementation of multi-site 3T MRI acquisition, and novel quantitative motor, cognitive, oculomotor, neuropsychiatric, and wet biomarker components. Another unique feature of Track HD is the use of only a small number of sites to allow greater flexibility for implementing relatively complex and expensive procedures and the possibility of greater flexibility for modifying study procedures as promising, new methods become available. The protocol describes a study plan, including the study design, participant characteristics, measures, data management and analysis plans, study administration and coordination, and plans for dissemination. Careful attention has been given to the rationale for the measurement approaches.

3.1. Overall study design

The generic Track-HD assessment plan is summarised in *new participants only

	Visit 1 (baseline)	Visit 2 (12 months)	Visit 3 (24 months)	Visit 4 (36 months)	Visit 5 (48 months)	Visit 6 (60 months)	Visit 7 (72 months)
Participants	90	90	90	90	60	60	60
Controls	30	30	30	30	30	30	30
Premanifest HD	30	30	30	30	30	30	30
Early HD	30	30	30	30	-	-	-
Pseudonymise	✓			✓*	✓*		
Check criteria	✓			✓*	✓*		
Demographics	✓			✓*	✓*		
Biosamples	✓	✓	✓	✓	✓	✓	✓
Clinical/motor	✓	✓	✓	✓	✓	✓	✓
Neuropsychiatric	✓	✓	✓	✓	✓	✓	✓
Cognitive	✓	✓	✓	✓	✓	✓	✓
Quantitative motor	✓	✓	✓	✓	✓	✓	✓
MRI	✓	✓	✓	✓	✓	✓	✓
DNA/LB sample	✓			✓*	✓*	✓	✓
Oculomotor	✓	✓	✓	✓	✓	✓	
TMS					✓	✓	✓

*** new participants only**

Table 1 Overall assessment plan

3.2. Participant overview

At the start of the study each centre recruited 90 participants. The target cohort at each centre was 30 control participants, 30 premanifest (PM) HD expansion carriers and 30 participants with early disease (stage 1 or 2). Additional PM participants were recruited after 24-months to replace withdrawals. The study was extended for a further 36 months (Visits 5, 6 & 7) in PM and control participants only. All existing PM and controls participants who met the current inclusion criteria were invited to participate. Additional PM and control

participants were recruited to replace withdrawals and maintain the original target of 30 per group. Early stage participants, previously in TRACK-HD, have been invited to participate in other observational studies or suitable clinical trials.

4. TRACK-HD overview

4.1. Study title

TRACK-HD

4.2. Type of study

Multi-centre, multinational prospective observational biomarker study of premanifest carriers of the HD genetic mutation along with non-carriers as controls with no experimental treatment.

4.3. Study Centres

Data collection for Track-HD began in early January 2008. Data collection for the study extension began in March 2012. Sample size calculations have shown that the existing 4 study sites will be sufficient to power the study extension and the introduction of new imaging techniques at V7:

Institute of Neurology, UCL, London
Sarah Tabrizi, MD, PhD (ST); PI of Track-HD

University of British Columbia, Vancouver, Canada
Blair Leavitt, MD (BL)

Université Pierre and Marie Curie, Paris, France
Alexandra Dürr, MD PhD (AD)

Universiteit Leiden, Netherlands
Raymund Roos, MD (RR)

Other sites in Europe and North America may be necessary depending on future power calculations and ongoing data analysis.

4.4. Steering Committee

The Track-HD steering committee will be ad hoc and dynamic, but will contain key members representing each area of study as follows:

Beth Borowsky, PhD (Science Director)
CHDI Foundation

Alexandra Dürr, MD PhD (PI, Clinical assessment site)
University Pierre & Marie Currie

Chris Frost, MA (Statistical analysis)
London School of Hygiene and Tropical Medicine

G. Bernhard Landwehrmeyer, MD (Database repository, data monitoring)
Ulm University

Douglas R. Langbehn, MD, PhD (Statistical analysis)
University of Iowa

Blair Leavitt, MD (PI, Clinical assessment site)
University of British Columbia

Raymund Roos, MD (PI, Clinical assessment site)
Leiden University

Julie Stout, PhD (Data QA/QC & analysis – cognitive, functional, & QoL assessments)
Monash University

Sarah Tabrizi, MD, PhD (Global Principal Investigator, PI, Clinical assessment site, Steering Committee Chair)
Institute of Neurology, University College London

4.5. Other key investigators and expert advisors in TRACK-HD

Additional consultants and investigators will be utilized during the course of the Track-HD study.

4.6. Funding

Track-HD is funded by CHDI Foundation Inc, New York, NY USA, known formerly as the CHDI/High Q Foundation Inc. This is a philanthropic not-for-profit charitable organisation aimed at finding treatments for Huntington's disease.

4.7. Study period

Track-HD is a prospective study for which each participant was originally enrolled for 24 months. The study was extended for a further 12 months in the original participants, and new PM participants were recruited in the third year of the study to replace withdrawals. The study has been extended for a further 36 months in controls and PM participants only. The study duration per centre is therefore 72 months from January 2008 to the projected end date of December 2014.

4.8. Study objectives

The primary aim of the study - to provide essential methodological advances needed for optimizing neuroprotective clinical trials in HD – has already yielded a range of quantitative outcome measures suitable for use in potential clinical trials in the early stages of the disease. Track-HD will now focus on individual and combined clinical and biological outcome measures for tracking progression in the premanifest stages of the disease, in which despite significant progressive regional and whole-brain atrophy and clear cross-sectional deficits compared with controls, there has so far been limited detectable cognitive or motor decline (Tabrizi et al. 2011).

4.9. Study population

At the start of TRACK-HD, clinical trials in HD were uncommon and lacked the appropriate outcome measures. However, a number of candidate therapeutics with potential disease-modifying effects are currently being tested in early stage disease and it is therefore appropriate that eligible individuals in TRACK-HD be offered the opportunity to participate in these trials, rather than continue observation in our study. For this reason, early stage participants were not included at TRACK-HD visits 5, 6 and 7. Any early-HD participant who has developed advanced symptoms of HD and is not eligible for clinical trials or further participation in TRACK-HD, will be offered participation in other observational studies such as Registry. Although some of the original PM participants may also have advanced to early stage disease, this cannot be disclosed as part of a research study and therefore these individuals cannot be offered participation in a clinical study for early stage disease, unless clinical onset has been confirmed by their doctor. In these cases, the PM participant may also be invited to take part in a clinical trial. However, if they are unaware of their current disease status, they will be allowed to continue in TRACK-HD.

Each centre initially recruited 90 participants drawn from the population of its HD clinical service, including 30 control participants, 30 premanifest HD expansion carriers (PM) and 30 participants with early disease (stage 1 or 2). Additionally, new premanifest participants were recruited during the third year of the study to replace withdrawn participants. A further 33 controls and 30 PM participants were recruited across sites at visit 5 to replace withdrawals and maintain the statistical power of the study.

In order to increase the yield of disease-related changes in the premanifest cohort, a burden of pathology selection criterion is used. Burden of pathology severity is given by (CAG-35.5) × age. A threshold of >250 CAG-years will be set, which approximates to 15 years to estimated onset (calculations based on Predict-HD dataset by Doug Langbehn).

Control participants are normal repeat length siblings not carrying the expansion mutation, non-family persons known not to carry the expansion mutation and partners or spouses of research participants. Such individuals share genetic, environmental, social and dietary exposure as well as some psychological burden of living with HD. The decision to use partner/spouse controls and normal repeat length persons rather than untested at-risk individuals was reached after a careful consultation process and is based on the following rationale:

- Untested at-risk individuals may individually have certain characteristics (such as motivation) that make them good controls for certain neuropsychiatric tasks.
- However, using at-risk individuals effectively reduces by 50% the number of "true" gene-negative controls.
- At-risk participants who tested positive would tend to be so far from onset that analyses using their data would be poorly powered.

4.10. Study design

Given the greater challenge in the design of outcome measures for trials in a premanifest population (Tabrizi et al 2011), we aim to analyse data longitudinally. At each visit, participants will undergo clinical, motor, cognitive, neuropsychiatric, structural and functional MRI, TMS, MRS, and oculomotor assessment (the latter to visit 6 only), as well as donating blood samples and buccal cheek swab (Table 1). Each visit will last approximately 7 hours.

4.11. Quality control and quality assurance

Stringent local and central QC/QA measures will be in place. All personnel are trained and assessed for inter-rater reliability before beginning participant assessments and on an ongoing basis with annual retests. Imaging QC will ultimately be centralised under the control of an imaging CRO contracted by CHDI for the purpose of site specification and QC/QA. All measures will be automated or computer-administered to the maximum possible extent and careful oversight of UHDRS administration will be provided by each clinical site PI.

4.12. Data storage and security

Phenotypic and imaging data will be pseudonymised and securely stored by CTMS, Ulm and LONI, Los Angeles, respectively. Pseudonymised biosamples will be stored by the biorepository at Biorep, Milan. Data may also be stored on behalf of CHDI Foundation, Inc at other central repositories. All agencies responsible for data storage will observe the highest precautions to ensure data integrity and security.

4.13. Data flow

Data and biosamples will be stored, checked and monitored centrally by appointed data repositories and monitors. The pathways for data collection, storage, checking and analysis are outlined below (**Figure 1**).

After minimal essential **local QA** (e.g. entering Participant ID into the oculomotor data), all data will be transmitted to a central server.

Central QA will be conducted by nominated agencies (e.g. Imaging CRO for imaging data). "Clean" data will then be stored in the distributed central data repository (i.e. LONI for imaging at UCLA in Los Angeles, CTMS for clinical data at EHDN in Ulm, CRB for biological samples at Biorep in Milan) and distributed to study centres for en masse **modality-specific processing** – e.g. caudate segmentation, cortical thickness measurements, CAG sizing, etc. See Section 5.15.

The **key question** of the study — what combination of measures best captures disease progression in premanifest HD and to provide further essential methodological and biological advances in the premanifest HD population. — should be centrally managed by the Steering committee with our biostatisticians. Clinical Neurology fellows and Psychologists at the 4 study sites may be involved in data analysis in the interim period between assessments.

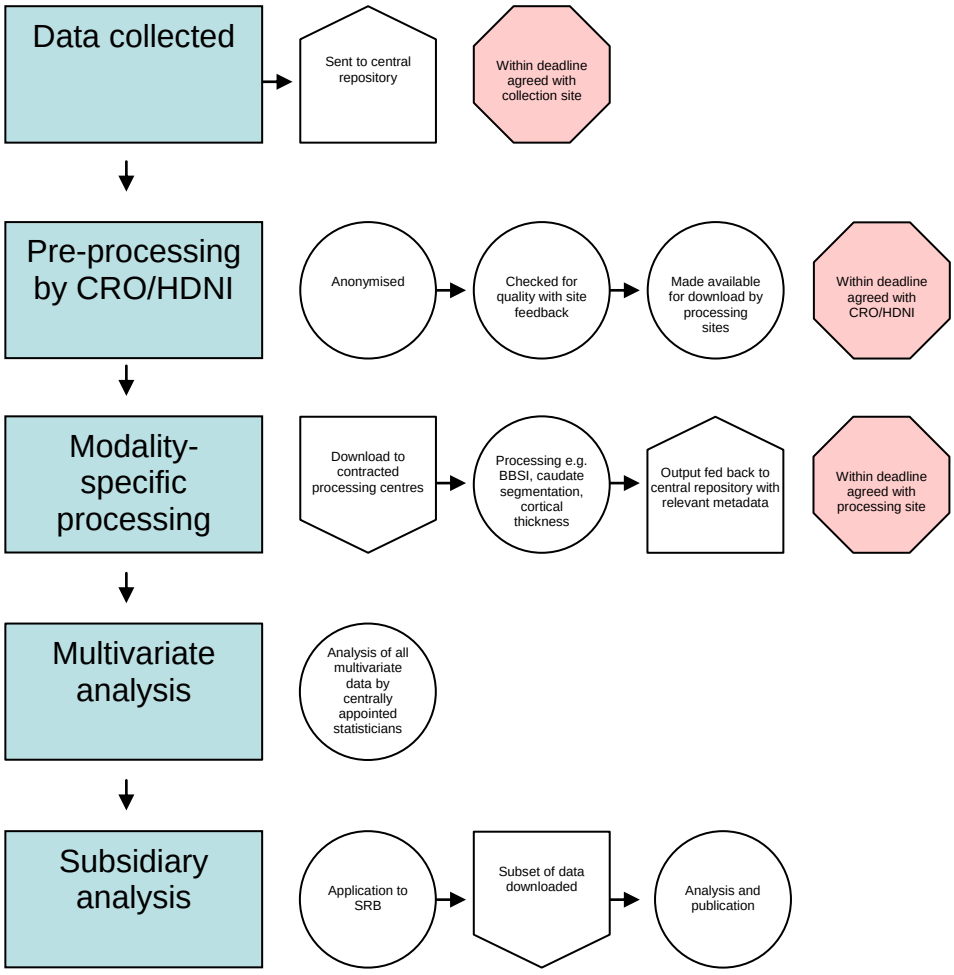


Figure 1 Data flow schematic

4.14. Organisation

The Principal Investigator is Professor Sarah Tabrizi, London. She will head a Central Coordination Team consisting of a full-time Clinical Trial Manager, Study coordinator and Study Administrator. The Central Coordination Team will be responsible for finalising the study protocol and liaising with sites and other agencies (data repositories, data monitors, expert advisors) to ensure that the new tasks added at Visit 7 are ready to begin at all sites by March 2014. The Team will be guided by CHDI Foundation Inc, and the Steering committee.

4.15. Study management

Besides the Imaging CRO, Track-HD will not involve a CRO. Instead, the roles that might be occupied by a CRO will be devolved to other organisations already involved in the study, as detailed below (Table 2).

Role	Responsibility
Project management and planning	• Study coordination team • Steering committee • CHDI Foundation Inc.
Regulatory documents (IRB)	• Study coordination team
Conducting investigator meetings	• Study coordination team
Conducting study expert meetings	• Study coordination team
Training of personnel (incl. cognitive, motor, imaging and TMS)	• Study coordination team • Key investigators/EHDN
Site identification and selection	• CHDI Foundation Inc. • Study coordination team
Initiation visits	• Study coordination team
Monitoring visits	• Study monitoring team at EHDN, Ulm • Direct on-line data monitoring
Management of laboratory samples	• Biorep
Quality assurance	• Imaging CRO • EHDN • Biorep • Cognitive (Julie Stout and colleagues)

Table 2 Study management roles and responsibilities

CHDI will contract an imaging CRO to establish and coordinate aspects of the imaging infrastructure, namely:

- Study set-up
- Site qualification
- Protocol definition
- Training
- Data anonymisation and transfer
- Clinical read of baseline scans
- Assessment of scan quality and consistency
- Site communication and troubleshooting

5. Detailed study background

5.1. 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 5-10 per 100,000 in the general population of the Western hemisphere. 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). Systematic screening efforts to identify compounds with disease-modifying properties are under way, and some compounds have been reported to result in beneficial effects when applied in model systems of HD (Ona et al., 1999; Hockly et al., 2003) thus providing a rationale for identifying well-tolerated and clinically effective novel treatments for HD. However, currently the predictive value of these promising results obtained in model systems for HD patients is unknown. Despite these advances, a more seamless integration of basic, translational and clinical HD research is required to plan and conduct future clinical studies, e.g. by identifying and validating biological markers that track the course of HD ('state biomarkers'), and by identifying factors that influence the onset and progression of illness.

5.2. Rationale

Candidate therapeutics with potential disease-modifying effects in HD are currently being tested (Ross and Tabrizi, 2011). Selective reduction of mutant HTT expression is one approach already being explored in preclinical studies, and compounds suggested to promote clearance of mutant HTT are being studied in phase Ib and phase IIa trials. However, there have so far been no successful disease-modifying phase III clinical trials in HD; such trials ultimately rely on the development of objective and quantitative outcome measures, particularly when considering trials before overt symptom onset. Therefore, it is highly desirable to validate measures that allow efficacy-testing over relatively short intervals using practical sample sizes in a cohort which may best respond to therapeutic interventions, namely those in the earliest stages of the disease.

Track-HD is an international, multi-centre study which is designed to determine the individual and joint utility of a selected set of clinical and biological outcome measures. Track-HD integrates prospectively- and systematically-collected clinical research data (e.g. phenotypic clinical features, family history, demographic characteristics) with biological specimens. We will measure clinical and biological markers, including neuropsychiatric, cognitive, quantitative motor, oculomotor, imaging markers, and laboratory biomarkers.

Several imaging, cognitive, and motor measures have been proposed as suitable candidates for tracking disease progression, but so far most studies have relied on cross-sectional data to infer usefulness (for review see Weir et al 2011). Such inferences risk overestimating the sensitivity of measures because cross-sectional group differences might represent the accumulation of up to 25 years of neurodegeneration. Longitudinal studies over durations relevant to clinical-trial timelines are therefore essential, but few studies have described change within premanifest and early stage HD, particularly over short periods. In TRACK-HD we have previously reported significant progressive white-matter, regional, and whole-brain atrophy over 12 months across the spectrum of

disease from premanifest to early stage HD (Tabrizi et al 2011); we have also observed accompanying deterioration in clinical, motor and cognitive measures in early HD.

In the premanifest cohort, significant global, regional and grey/white matter structural changes have been detected, e.g. using boundary shift integral (BSI) analysis and Voxel Based Morphometric (VBM) analyses of 3T MRI data (Tabrizi et al. 2011). However, at 24 months there were no reliable functional markers of disease progression in any modality. It was not until 36-months, that we were able to demonstrate detectable worsening on a number of key clinical, quantitative-motor and cognitive tasks that accompanied this widespread macro- and micro-structural loss. However, even at 36 months these changes are only evident in individuals who are fewer than 10 years to predicted symptom onset, and in those further from predicted onset there is still little evidence of significant functional decline (Tabrizi et al. 2013). We must therefore conclude that in order to measure longitudinal change in a premanifest population the sensitivity range of measurement within each assessment needs to be recalibrated. It is a basic psychometric principle that sensitivity of measurement in one functional range, e.g. individuals with early HD, comes at the expense of loss of sensitivity in other potentially measurable parts of the disease, e.g., healthy, premanifest individuals with clean motor scores. Furthermore, disease progression will continue with the TRACK-HD premanifest cohort and the natural markers of progression that we are already able to detect, e.g. increasing motor and total functional capacity scores, are expected to increase resulting in a perimanifest disease group who may show detectable functional decline within the 36-months of the study extension.

Functional MRI measures blood flow within the brain and has already demonstrated dysfunction in the premanifest stages of HD (Saft et al. 2008, Kloppel et al. 2009) In addition to task-dependent fMRI, resting state fMRI has recently emerged as a technique which may be sensitive to functional reorganization in degenerative conditions such as Alzheimer's disease (Wu et al. 2011), and it is yet to be established whether this method would be sensitive to functional changes in HD and whether longitudinal change can be detected. Resting state fMRI was therefore included alongside the existing structural measures, which will enable us to understand the relationship between the two and is a significant advantage over stand-alone fMRI studies. It will also be possible to compare both structural and functional changes in those that show the greatest disease progression versus those that show the least change.

The addition of the recently developed novel imaging methods CEST, MTR and NODDI at V7 will provide complementary assessments of aspects of pathology that are currently unreported in HD. These techniques are exploratory yet, if specificity within HD and reproducibility across time and sites can be established, they could be highly relevant to future drug development. The possibility of developing an MR-based method to measure mutant HTT in the living brain has unprecedented potential for the field.

Since its initial description in 1872, it has been clear that HD has a strong hereditary contribution resulting in the generational transmission of the disease from parent to offspring, regardless of gender (Bates, Harper, & Jones, 2002). Beginning in 1981 and through the collection of clinical and family history information and biological material (DNA) from HD families the gene and the mutation causing HD was identified in 1993 (The Huntington's Disease Collaborative Research Group, 1993). The unstable, expanded CAG repeat within the coding region of the HD gene at 4p 16.3 explains many of the genetic features of the disorder, including the variable age at onset, the tendency for juvenile disease to be inherited from fathers, and the (rare) appearance of new mutations. There is a strong and consistent inverse relationship between the length of the CAG repeat and the age at clinical onset of HD (The Huntington's Disease Collaborative Research Group, 1993; Langbehn et al., 2004; Penney et al., 1997). However, the size of the CAG repeat accounts for only about 60-70% of the variance in age at onset; other, as yet unidentified factors influence age at onset and the cascade of pathogenic events resulting in the HD phenotype. Recent studies suggest that the remaining variation in age at onset of HD is strongly heritable (Wexler et al., 2004). These findings indicate that the onset of HD is substantially influenced by factors other than repeat size, and that other modifier genes may determine the remaining variation in age at onset.

Owing to the limited availability of prospectively collected, longitudinal data of sufficient quality, studies to identify genetic modifiers of the rate of disease progression or the pace and extent of abnormalities seen on neuroimaging have not been performed to date. Identification of genes that modify the pathogenic process in HD offers a direct route to validate targets for development of HD experimental therapeutics. Track-HD will provide a wide range of HD-associated phenotypes by which to identify modifier genes. Initially, the

phenotypes available will be derived from clinical assessments (UHDRS), but the collection of biological samples will also permit the study of additional phenotypes at the levels of RNA, protein, metabolites and cultured cells. The combination of phenotypic and genotypic information will permit analysis of relationships between individual polymorphisms and genes and the effect they have on modifying the phenotypic presentation, rate of progression and response to treatment of HD using genetic linkage and genome-wide association strategies.

The clinical database on HD and the biomaterials to be collected for the Track-HD study will be used for a variety of analyses which may be broadly categorized as either cross-sectional or longitudinal. The sample size was selected to ensure sufficient statistical power for determining the sensitivity of selected assessment tools for monitor the progression of HD and for detecting molecular determinants or markers for clinically relevant phenotypic characteristics or outcomes (e.g. progression of HD and a better definition of the clinical onset of disease). This will, in turn, improve the efficiency of therapeutic trials by providing more and more clearly defined endpoints (e.g. delaying onset of clinical disease).

5.3. Objectives

Track-HD is designed to relate phenotypic characteristics in as many modalities as can be measured (clinical, cognitive, quantitative motor, oculomotor, neuropsychiatric, imaging, laboratory) and genetic factors, in order to relate phenotypic characteristics, genetic factors ('genetic modifiers'), data derived from the study of blood ('wet biomarkers') and imaging data ('dry biomarkers').

In order to measure disease progression in a premanifest population we already introduced suitable measures for detecting compensatory mechanisms at Visit 5, which may explain the absence of longitudinal differences over 24 months between premanifest and control participants despite profound structural changes in the brain. Furthermore, we recalibrated the sensitivity range of the existing TRACK-HD assessments, e.g., cognitive and quantitative motor, to better detect disease progression in a premanifest cohort. Our 36-month data do show detectable differences in premanifest individuals closest to onset, which suggests that compensatory mechanisms may start to fail around this time (Tabrizi et al. 2013), but a recalibrated battery may be more sensitive to these changes over time periods shorter than 36-months which is likely to be more relevant for clinical trials. It may also enable us to detect the very earliest functional changes in those further from predicted onset, when there is arguably the most to rescue.

The primary objective of this study is to determine what combination of measures is the most sensitive for detecting change over the natural course of HD leading up to clinical disease onset, with a view to identifying clinical measures that predict disease progression and which could be used for potential stratification of premanifest individuals into clinical trials; and to provide further essential methodological and biological advances in the premanifest HD population.

5.4. Study design

Track-HD is an ongoing longitudinal natural history study of premanifest and early HD (stage 1 and 2; Shoulson, 1981). Each centre initially recruited 90 participants: 30 control participants, 30 premanifest individuals (PM) and 30 early disease participants and additional PM participants were recruited to replace withdrawals in year 3. The study was extended for a further 36 months in controls and PM participants only. Participants undergo clinical, neuropsychiatric, cognitive, quantitative motor, and structural and functional MRI assessment, as well as donating blood samples and buccal swabs for a further 3 years (visits 5-7), and oculomotor for 2 years (visits 5-6)

Clinical phenotypic data will be assessed and documented based on information obtained from three sources:

- Trained assessors who record their clinical impression using rating scales (i.e. UHDRS motor);
- Participants themselves who report on their subjective experience (i.e. Hospital Anxiety and Depression Scale, Snaith Irritability Scale, and Beck Depression Inventory-II);

- Companions who report on the level of function and neuropsychiatric aspects of the participant.

For a given participant, the same investigator should carry out the assessment throughout the study where possible.

5.5. TRACK-HD study assessments

5.5.1. Demographic information

Participant group (Control/PM); Invariable demographical data (date of birth; sex; Ethnicity; Handedness; Education level; Education years) and Variable demographic data (Height (cm); Weight (kg); Occupation; Employment (full-time; part time; unemployed; retired); Marital status).

5.5.2. Clinical assessment

The steady worsening of the motor, cognitive, and neuropsychiatric capacities of individuals affected by HD results in progressive functional decline. Clinical rating scales aimed at capturing the clinical phenotype and mirroring the progression of the illness have been widely used to establish the rate of functional decline in a variety of HD populations. The Unified Huntington's Disease Rating Scale (UHDRS) was developed by the Huntington Study Group (HSG) in 1993 and revised in 1999 as UHDRS 99 (The Huntington Study Group, 1996; Marder et al., 2000). The UHDRS 99 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 early stages of the illness. The UHDRS 99, of which the motor and functional domains will be employed in Track-HD, has been used in all clinical sites collaborating as HSG in North America, Europe, and Australia. The UHDRS has undergone extensive testing of reliability and internal consistency (The Huntington Study Group, 1996; Marder et al., 2000) and has been shown to have a good inter-rater reliability for the total motor score. The motor section of the UHDRS correlates strongly and significantly with the functional component of the UHDRS. Internal consistency, as measured by Cronbach's alpha, was 0.95 for the motor component and 0.95 for the functional component of the UHDRS (Shoulson & Fahn, 1979). The UHDRS has been used widely in HD clinical trials. (Hersch et al., 2006; Tabrizi et al., 2005).

Motor assessment: The UHDRS motor examination will be administered; this is the gold standard for HD.

Past medical history: Birth trauma or neonatal illness; Birth / neonatal illness details; Childhood illness <12; Illness <12 details; Illness 13-17; Illness 13-17 details; Surgery; Surgery details; Alcohol units per week; Alcohol status (never abused; previous abuse; current abuse); Recreational drug use; Tobacco (Current; ex; never); Cigarettes per day; Years of smoking; Allergies.

Medication: Name; dose; duration for each; Active medical conditions.

Huntington's disease history: Affected parent; Parental onset age; Onset age according to participant; Onset age according to family; Onset age according to rater; First symptom according to participant; First symptom according to family; First symptom according to rater (evaluation of clinical onset will be detailed and based on the EHDN "symptom age at onset" questionnaire (see Track-HD SOP documents); Date of genetic test; Analysing laboratory; Small allele length; Large allele length.

Psychiatric history: Previous depression; Previous anxiety disorder; Previous OCD diagnosis; Previous psychotic illness; Previous suicide attempt; Previous self-harm; Previous suicidal ideation.

5.5.3. Family history questionnaire (FHQ)

A questionnaire will be handed to consenting participants to share their family tree by indicating their siblings, children and relatives up to the second degree and by volunteering the following information on each person within the family tree: gender, year of birth, alive/dead (for those deceased: year of death/age at death and – as best as participants can tell – cause of death), opinion whether in the view of the contributor a member of a family is affected with HD/carries the HD mutation, (for those affected with HD/carrying the HD mutation: age

at time of HD diagnosis/predictive testing, first signs and symptoms and whether the diagnosis of HD was confirmed by physician/genetic testing). These data will be recorded first in a source data file document (see Source Data File Family History in Track-HD SOP documents). From these data a graphical representation of a family tree will be generated using appropriate software (see Track-HD SOP documents). In order to protect the confidentiality of the information contained within the family tree, annotations (affected by HD, mutation carrier, participant in Registry/Cohort) will be visible only on demand. Within this family tree the symbols representing those members of the family who consented to participate in Registry will be annotated with their pseudonyms; family members who did not consent to participate in Registry will be represented with symbols without an annotated pseudonym. By using this procedure, biosamples and clinical data of related participants (which is essential e.g. to identify genetic modifiers by sib pair analysis) can be linked while protecting the privacy of individuals volunteering information through the use of pseudonyms (participants in Registry/Cohort) or anonymous codes (not participating in Registry/Cohort), respectively.

The source data file for the FH component is provided in the Track-HD SOP documents.

5.5.4. Functional and quality of life (QoL) assessments

5.5.4.1. Overview

Time required: up to 30 minutes (5 minutes at home for companion if available)

Summary

The goal of the Track-HD functional/QoL battery (Table 3) is briefly to assess current functional abilities and participants' subjective report of their quality of life and to relate findings to progression of disease and to cognitive, motor/oculomotor, imaging, neuropsychiatric and wet biomarker measures in the Track-HD study.

For participants:

1. a brief interview to assess current functional abilities, which will be administered by a trained rater as part of the study visit (UHDRS TFC)
2. the HD-specific Quality of Life (HD-QOL), PM participants only
3. the Physical Activity Questionnaire (PAQ)
4. Quality of Life Index (QOLI),

For companions, if available (to be completed at home):

1. a companion version of the HD-QoL

List of Tests	Abbrev	Rating Type	Time (min)
UHDRS Total Functional Capacity	TFC	Clinician rated	5
HD-specific Quality of Life* (PM and companions only)	HD-QoL*	Self rating	5* (Home 5 for companions)
Physical Activity* Questionnaire	PAQ*	Self rating	10*
Quality of Life Index*	QOLI*	Self-rating	10*

*** removed at V6**

Table 3 Functional & QoL assessments

Rationale for task selection

Whereas functional changes are well documented in middle to late stage HD (Marder et al., 2000), less known about this aspect of premanifest and early HD. Yet, subtle cognitive, psychiatric and motor changes may lead to altered productivity in the home, community and workplace. For example, a person with above average work performance may have to work more hours to maintain the same level of productivity. Alternatively, a person working at above average skill level may decline into an average level of performance, and therefore not be considered to have a significant impairment. In both of these cases, productivity changes would not be evident from simply looking at occupational status.

Thus, for a study of premanifest HD, it is essential to identify measures sensitive to the subtle functional changes that may occur prior to diagnosis with HD. One goal of the study is to better understand how changes in everyday functioning are related to cognitive, psychiatric, and motor function as well as neuropathology. A second goal is to contribute to efforts aimed at identifying functional and quality of life measures that will be useful in future clinical trials.

In this assessment category, the domains of greatest interest during premanifest HD are:

- Productive activities outside the home
- High order activities of daily living (e.g. finances, homemaking)
- Social functioning and adjustment
- Life satisfaction
- Physical activity

The measures selected for the functional/QoL battery will provide a broad assessment of these five domains.

Scientific questions to be addressed by the Functional/QoL battery

Compared to the cognitive and motor domains, much less is known about how the course of functional decline in the transition from health to illness. For example, when, relative to onset, do specific functional and QoL changes begin, and what is the nature and rate of change across time? Do functional and QoL measures make a unique predictive contribution to measures of disease progression above and beyond that provided by other clinical outcome measures?

5.5.4.2. Information on specific instruments

UHDRS TFC

The goal of the UHDRS TFC is to obtain a clinician's assessment of the participant's capacity to perform in each of five functional domains including occupation, finances, domestic chores, activities of daily living, and care level. The TFC is a clinician rated 14-unit scale (range 0-13) with higher scores indicating higher function.

The UHDRS Total Functional Capacity Scale (TFC) was selected because it is a standard in the field for diagnosed HD. Thus, it will allow comparison of Track-HD findings with those of other studies. One limitation of the TFC is a lack of sensitivity at the upper end of the functional spectrum, likely to occur in premanifest HD. For example, subtle productivity changes in work performance over time, would lead to only a one point change in TFC score. However, disease progression will continue within the TRACK-HD premanifest cohort and natural markers of progression such as decreasing total functional capacity scores are expected to result in a detectable functional decline within the next 36 -months. The other functional measures of the UHDRS, the Functional Checklist and the Independence Scale, have a relatively greater focus on more basic activities of daily living (i.e., toileting, ambulation) compared to the TFC and thus, are less relevant to the target sample of Track-HD. The Functional Checklist and Independence Scale will not be included in Track-HD.

HD-Specific Quality of Life (HD-QoL) (Visits 4 and 5 only)*

Existing quality of life scales such as the QOLI and SF36 have shown high reliability and validity in a variety of clinical populations, but none have been specifically developed for use with individuals living with HD and their companions or family carers. A new HD-specific measure has been developed to help understand the psychosocial aspects of life of both (Ho Clinical Genetics 2009). Results from pilot studies suggest that the impact of disease is greater on patients in terms of communication, but while some aspects of mood, cognition, and social interaction are more commonly affected in HD patients, there are aspects that are similarly relevant for carers. HD-QoL has therefore been selected for inclusion. The new measure was included at Visits 4 and 5 to provide 12-months of longitudinal data for analysis before continuing further assessment.

Physical Activity Questionnaire (Visits 4 and 5 only)*

There is evidence that environmental factors such as leisure activities, occupation, and diet influence the onset and progression of neurodegenerative disorders such as Alzheimer's and Parkinson's disease, either directly or by interacting with genetic risk factors. It is therefore possible that these factors may also be important for HD and it was recently reported in retrospective study of physical activity, that those who led a more passive lifestyle had an earlier age of onset than those who were less passive (Trembath 2010). A physical activity questionnaire has therefore been developed, which is based on a physical activity coding system (Ainsworth 1993).Using this system it is possible to code different types of activity to estimate levels of strenuous, moderate and light physical activity (Godin 1997). This information will be collected retrospectively to distinguish the most and least physically active individuals. This questionnaire was included at 2 time points to capture retrospective data from participants who participated at and prior to Visit 4, as well as new participants recruited at Visit 5.

Quality of Life Index (QOLI) (Visits 1 to 6 only)*

The goal of the QOLI is to obtain self-report of overall life satisfaction, as well as ratings of specific aspects of quality of life including health/daily functioning, social/ economic, psychology/spiritual, and family life. The QOLI, developed for use in clinical populations, is a 32-item self-report questionnaire which is commercially available. Participants are asked to rate each item 3-point rating scale for importance and a 6-point rating scale for satisfaction. Scores on individual items are summed to obtain an overall score and several subscales, with higher total scores indicating better quality of life.

The QOLI was selected because it has high internal consistency, test-retest reliability and validity in a variety of clinical populations (Ferrans & Powers, 1992). The QOLI is also being used in the Predict-HD sub-study on functional ability (led by Carissa Nehl and Jane Paulsen), and therefore, inclusion of this measure in Track-HD will provide a link between the two studies.

The QOLI was introduced at Visit 1, but since there were no significant changes between groups or across time the questionnaire was discontinued at Visit 6 in order to further interrogate the existing data.

5.5.5. Neuropsychiatric assessment

5.5.5.1. Overview

Time required: up to 20 minutes at study visit-participant, (10 minutes at home for companion if available)

Summary

The goal of the Track-HD neuropsychiatric battery (Table 4) is briefly to assess neuropsychiatric symptoms and relate findings to progression of disease and to cognitive, motor/oculomotor, functional/quality of life, imaging, and wet biomarker measures in the Track-HD study. To achieve this goal, the neuropsychiatric assessment includes:

For participants:

1. the BDI-II

2. the Hospital Anxiety Depression Scale / Snaith Irritability Scales (HADS/SIS) for depression, anxiety, and irritability,
3. the FrSBe,
4. the Baltimore apathy/irritability scale

For companions, if available (to be completed at home):

1. a companion version of the FrSBe
2. a companion version of the Baltimore apathy/irritability scale

List of Tests	Abbrev	Rating Type	Time (min)
Beck Depression Inventory – Version II	BDI-II	Self-rating	5
Hospital Anxiety and Depression Scale / Snaith Irritability Scale	HADS/SIS	Self rating	5
Frontal Systems Behaviour Inventory-Self Rating	FrSBe-self	Self rating	5
Frontal Systems Behaviour Inventory-Family Rating	FrSBe-other	Companion rating	Home 5
Baltimore apathy/irritability scale	BAIS	Self rating	5
Baltimore apathy/irritability scale	BAIS	Companion rating	Home 5

Table 4 Neuropsychiatric assessments

Rationale for test selection

The evidence base for selecting neuropsychiatric assessment tools is limited. Therefore, the design of this battery uses existing evidence from previous studies whenever possible, but relies strongly on expert input for selection of the majority of the tests. Self-rating scales have the great advantage that one can measure subjective mental events which are not apparent in outward behaviour. However, Chatterjee et al., (2005) compared patient and companion ratings of irritability in early HD and found only fair agreement. Surprisingly, patients with the most intact cognition had the lowest levels of agreement in irritability ratings with companions. Therefore, we will get both self and companion ratings using the FrSBe.

Scientific questions to be addressed by the neuropsychiatric battery

Compared to the cognitive and motor domains, much less is known about how neuropsychiatric signs and symptoms manifest and change in the course of progression from health to illness in HD. For example, when, relative to onset, do specific neuropsychiatric changes begin, and what is the nature and rate of change of these symptoms across time? What role do neuropsychiatric measures have in potential clinical trials of premanifest? 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 Track-HD 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 multiply influenced both by natural fluctuations as well as disease progression, and thus they may lack sufficient precision to sensitively reveal disease progression.

5.5.5.2. Information on specific instruments

Beck Depression Inventory II (BDI)

The goal of the BDI-II is to obtain self-ratings of the mood, somatic, and cognitive symptoms of depression. The BDI II is a 21-item self report questionnaire which is commercially available and very widely used. Participants are asked to rate each item on a 4-point scale (0, 1, 2, 3) reflecting severity of a symptom. Scores on individual items are summed to determine depression severity, with higher total scores indicating more severe depressive symptoms.

The BDI was selected on the basis of existing evidence for its sensitivity in premanifest and early HD. The BDI has been widely used in cross-sectional studies of HD. Using meta-analysis, ESs (Cohen's d) (Cohen, 1988) for premanifest and early HD are - 0.46 (p=0.002) and - 0.82 (p=0.006), respectively, which are medium to large effects. The Predict-HD study is collecting longitudinal data on the BDI-II which will eventually be useful in estimating the longitudinal sensitivity of the BDI-II however these results are not yet available. A potential limitation of the BDI-II for assessing depression in premanifest and early HD is that somatic items relating to, for example, fatigue and appetite, may be confounded by HD, and therefore the total score may not accurately reflect severity of depression in the Track-HD sample. To address this problem, BDI-II scores can be recomputed without these items, and as well, item and factor analyses may be undertaken to determine the most sensitive items and the inter-item relationships.

Hospital Anxiety/Depression Scale (HADS)

The goal of the HADS is to obtain a brief rating of depression and anxiety symptoms that reflects primarily mood rather than cognitive and somatic symptoms. This commercially available scale has 14 items, 7 measuring anxiety and 7 measuring depression producing separate anxiety and depression sub-scores. Each item is rated on a four-point scale. Individual item and global (summed) depression and anxiety sub-scores will be analyzed. In addition, the HADS and the BDI will be compared for their sensitivity in this population and therefore will be useful in informing future studies.

The HADS was selected on the basis of expert input from David Craufurd. He cites an advantage of the HADS over the BDI-II in that the HADS is less susceptible to confounds from the somatic symptoms in HD. At this stage, there is no evidence available in premanifest or early HD, although the scale is reportedly being used in a validation study (against the SCAN – Schedule for Clinical Assessment in Neuropsychiatry) by Jenny Keylock from Hugh Rickard's group at the Queen Elizabeth Psychiatric Hospital in Birmingham.Snaith Irritability Scale (SIS)

The goal of the SIS is to obtain brief ratings of irritability. The scale is composed of 8 items each rated on a four-point scale. Four items are focused on inwardly focused irritability and four items are focused on outward irritability. Individual item and global (summed) inward and outward irritability sub-scores will be analyzed as well as a total scale score.

The SIS was selected because it is the only measure of irritability we could identify that has been shown, in self-ratings only, to be sensitive to changes in premanifest HD. According to the report by Berrios et al. (2002), compared to controls, premanifest HD subjects rated themselves as more irritable than control subjects (both inward and outward irritability (Cohen's d ES= 0.67, p=0.002, medium). In addition, both subscales were correlated with estimated time to onset in premanifest HD (inward irritability ES=0.62 and outward irritability ES=0.89; (Berrios et al., 2001)). We found no longitudinal reports or companion rating studies using the SIS in HD.

Frontal Systems Behaviour Inventory (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. In addition, comparison of companion and participant ratings has yielded some specific

discrepancies which will be further analysed in the Track study. Hamilton et al. (2003) showed that this rating scale was sensitive to changes occurring between the premanifest period and early HD.

Baltimore apathy/irritability scale (BAIS)

The BAIS was designed to form a composite picture of an apathetic or irritability syndrome in HD. The apathy scale consists of 14 items regarding different dimensions of apathetic behaviour. The score for each item ranges from 0 (apathetic behaviour not present) to 3 (maximum intensity of apathetic behaviour). The range of all possible scores is from 0–42 (maximum). The irritability scale also consists of 14 items regarding various dimensions of irritable behaviour. The score for each item ranges from 0 (irritable behaviour not present) to 3 (maximum intensity of irritable behaviour).The range of all possible scores is from 0 – 42 (maximum).

The BAIS is useful for assessing inter-rater agreement between HD patients and caregivers impressions of apathy and irritability. A previous study has shown that apathy varies and that caregivers may be better at rating apathy than patients (Chatterjee et al 2005), particularly as cognitive performance in patients declines. In the same study the assessment of irritability by patient and caregiver was in fair agreement at regardless of cognitive status.

5.5.6. Biosample collection

All participants will be invited to donate up to 50ml of blood for biomarker analysis at every visit. These will be collected by the site neurologist, or other qualified staff, from all participants willing to donate blood. Biological specimens are donated with the understanding that all specimens are used for HD-related research, and that they are stored at a central bio-repository. Samples will be processed on-site without delay to extract good quality plasma and divide it into 500µL aliquots for freezing. All consumables will be provided by Biorep on a per-participant basis and samples will be shipped to a research facility selected for TRACK-HD (Biorep, Srl in Milan, Italy or such other facility designated by CHDI Foundation) on a monthly basis.

The sample for DNA and LB lines will be shipped at baseline on the day of collection at ambient temperature. All other samples will be collected locally, stored locally at -80C and shipped on dry ice to a research facility selected for TRACK-HD (BioRep, Srl in Milan, Italy, or such other facility designated by CHDI Foundation) at monthly intervals.

DNA and DNA derived from lymphoblastoid cell lines will be used (1) to confirm the presence and the size of the CAG expansion mutation within the HD gene for research purposes only and if not already available from previous TRACK-HD visits, and (2) to identify genetic modifiers of HD, in particular genetic modifiers of age of onset, rate of progression and phenotypic characteristics presentations. For this purpose, up to two tubes of ACD blood will be collected for the extraction of DNA, the generation of lymphoblastoid cell lines and the cryopreservation of lymphocytes.

Further blood samples (up to a total of 50ml) will be collected for plasma, PBMC in FicollGradient or Lymphopaqe/Histopaque tubes (≤3 × 10ml) for mutant htt quantification, proteomic, ELISA and meso-scale analysis, and/or PAXgene RNA tubes (≤2 × 2.5ml) for the isolation of RNA for microarray or other RNA biomarker analysis.

In addition to the blood sample, a buccal swab will also be taken to collect cheek cells to test for levels of the huntingtin protein. This will be required from all study participants including controls. This sample will also be sent to a research facility selected for TRACK-HD (BioRep, Srl in Milan, Italy, or such other facility designated by CHDI Foundation).

5.5.7. Cognitive assessment

5.5.7.1. Overview

Time required: up to an average of 60 minutes total

Summary

The goal of the Track-HD cognitive battery is briefly to assess cognitive function and relate findings to progression of disease and to motor/oculomotor, neuropsychiatric symptoms, functional/quality of life, imaging, and wet biomarker measures in the Track-HD study. To achieve this goal, the cognitive assessment includes assessments based on evidence for sensitivity to pre-manifest HD.

The cognitive battery will consist of tests designed to be good markers of cognitive decline based on a meta-analysis of previous studies (from the HD Toolkit, a project headed by Julie Stout), as well as the initial data from the Predict-HD study (Jane Paulsen, PI) and TRACK-HD (Tabrizi et al. 2009, Tabrizi et al 2011).

Test name *[Abbreviation]*	**Type of Test**	**Avg. Time (Min)**	**Longest Time (Min)**
Symbol Digit Modalities Test *[SMDT]*	Paper	3	3
Stroop Word	Paper	2	2
Self-Paced Tapping, 3Hz	Computer	3	5
IQ Covariate (NART [London], ANART [Vancouver], DART [Netherlands], Echelle de vocabulaire (Raven, J.C., Court, J.H., & Raven, J. (1986). (*Baseline only*)	Paper	2.5	4.5
Map Search	Paper/pencil	2	3
Shepard-Metzler Mental Rotation	Computer	5	8
Circle Tracing Task*	Computer	9*	13*
Circle Tracing with Counting Backwards	Computer	4	9
Visual Array Comparison Task (WM)	Computer	4	6
Cancellation Task	Computer	3	3
Virtual maze task	Computer	17	20

*** removed at V7**

Table 5 Cognitive battery

Rationale for test selection

Based on cross-sectional studies (such as Tabrizi et al 2009) there is now strong evidence that cognitive function starts to decline in CAG-expanded individuals in the period prior to clinical diagnosis of manifest HD (i.e., in Premanifest HD). Compared to other areas of clinical assessment, cognitive assessment for HD has been well studied and a large quantity of evidence can be brought to bear on test selection. Therefore, whenever possible, tests for the cognitive assessment protocol were selected based strong existing evidence. In the HD Toolkit project, we have evaluated all such evidence for cognitive tests published since the 1993 advent of the highly reliable polymerase chain reaction test for the mutant huntingtin gene. We have quantified the cross-sectional and longitudinal effect sizes for pre-manifest and early HD, and these findings have been carefully considered and have influenced test selection. Furthermore, we have now analysed 36-months of data from TRACK-HD and longitudinal performance of the original cognitive tests has been taken into account.

Scientific questions for the cognitive battery. When is the earliest time-point that cognitive changes can be detected? What is the most sensitive set of tests for tracking cognitive change in premanifest HD? What is the nature and rate of change in cognitive function across time? What role do cognitive measures have in potential clinical trials of premanifest HD? What is the unique predictive contribution of cognitive function beyond what is provided by other assessment domains (other clinical markers and biological markers)?

As described above, factors such as age, education, gender, fatigue, and mood are known to influence performance on many tests and need to be taken into account in data analyses. Since premorbid differences in IQ also affect cognitive performance (independent of disease progression) the inclusion of a brief IQ estimation test in the protocol is essential. Finally, practice effects are common in cognitive tests, and in some cases may reduce the sensitivity of tasks used longitudinally. Therefore, practice effects should be specifically considered in analyses and interpretation of these data.

5.5.7.2. Information on specific instruments

Symbol Digit Modalities Test (SDMT; Total Time Required: 3 minutes)

This is a test of visuomotor integration, involving visual scanning, tracking, and motor speed. The examinee is given 90 seconds to match symbols and digits as quickly as possible. The key (specifying which number corresponds to each symbol) is located at the top of the page (Smith, 1991).

Likely main variable for analysis: Total number of correct responses.

Rationale and strength of evidence for the selection of SDMT

HD Toolkit meta-analysis suggests that SDMT performance declines longitudinally in both Premanifest HD Near Onset and Early HD (Figure 4). In Premanifest HD, SDMT also provides unique ability to predict probability of onset within 5 years (Langbehn et al., 2004) beyond that of the other tasks in the proposed core cognitive battery. In TRACK-HD, the SDMT has showed differences in rates of change at both 12, 24 and 36 months in early HD, and also had one of the largest effect sizes in premanifest HD of 0.20 (95% CI: -0.03 to 0.43) over 12 months and 0.14 (95% CI: -0.11 to 0.38) over 24 months, although these were not significant.

Stroop Word Test (Total time required: 2 minutes)

The Stroop Test has three conditions that require visual scanning, cognitive control and processing speed. Because the Word Reading condition (the first condition normally presented) is the most sensitive in premanifest HD, it is the only Stroop condition that will be used in the Track Cognitive battery. Participants are given a card on which the names of colors are printed in black ink and must read as many words as they are able in 45 seconds.

Likely main variable for analysis: Number of words read correctly in 45 seconds

Rationale and Strength of Evidence

HD Toolkit meta-analysis suggests that performance on the Stroop Word Test deteriorates longitudinally in both premanifest (Predict-HD) and Early HD (Figure 4). Longitudinal studies are supported by a consistent pattern of results in cross-sectional studies of premanifest and early HD and sizeable correlations in premanifest HD with time to onset (ES=0.54, $p<.001$) and neuropathology/striatal volume (ES=0.35, $p<.001$); and in early HD with striatal volume (ES=-0.35, $p<.001$). Furthermore, in premanifest HD, Stroop Word provides unique ability to predict UHDRS motor score beyond that of the other tasks in the proposed core cognitive battery.

Self-Paced Tapping (Total time required: 4 minutes)

Self-paced tapping provides a measure of psychomotor functioning, including timing. The task begins with the repeated presentation of a tone at a constant rate (3Hz). The participant is instructed to begin to tap with alternating thumbs at the same rate as the tone, when the participant feels that he/she has a sense of the timing. Once the participant begins to tap, the tone continues for another 12 taps, but is then discontinued. The participant will then attempt to maintain the timing of the tap for another 31 taps. This sequence is repeated 4 times for a total of 5 trials.

Likely main variable for analysis: Mean intertap interval.

Rationale and strength of evidence for the selection of Self-Paced Tapping

Effect sizes from the Predict-HD longitudinal database indicate that decline is only at trend level for this measure in Premanifest HD Near onset. However, we include this measure because it was the only measure in Predict-HD that showed some evidence of sensitivity in those 9-15 years from onset.

IQ Covariate (Total time required: 3 minutes; Baseline only)

The American National Adult Reading Test (ANART) (Gladsjo et al., 1999) and the National Adult Reading Test (NART-2) (Nelson & Willison, 1991) were chosen as estimates of IQ. Both the ANART and the NART-2 are 50-word tests that examine the pronunciation of phonetically-irregular words of varied culturally appropriate frequency (26 of the same words are included on both tests and 24 words on each test are culturally unique), thought to provide an index of the size of a person's vocabulary (Lezak et al., 2004) and a reflection of their premorbid level of intelligence. For Dutch, IQ will be estimated using the Dutch Adult Reading Test (DART; Bouma, Lendeboom, & Mulder, 1996), which is modeled on the NART and also consists of 50 irregularly spelled words which have to be pronounced correctly. For French, word pronunciation-based IQ estimates do not suffice because there are no comparable sets of irregularly spelled words; instead, the Echelle de vocabulaire Mill Hill will be used. For this test, participants are asked to judge pairs of words to determine whether they are synonyms.

Likely main variable for analysis: Estimated IQ score
IQ affects performance on a wide range of cognitive tests (Diaz-Asper et al., 2004). Thus, to assess appropriately the impact of brain injury or disease on cognition, estimates of premorbid IQ should be taken into account in the analysis and interpretation of cognitive data obtained from neurological populations. Data from Predict-HD have demonstrated that the ANART is generally superior to the two-subtest version of the Wechsler Abbreviated Scale of Intelligence (WASI) for estimating pre-morbid IQ in pre-HD (Carlozzi et al., manuscript in progress). Specifically, these data showed that ANART was less related to indices of disease progression (proximity to clinical diagnosis, difference from parental age at diagnosis, diagnostic confidence level and motor score) compared to the WASI.

Circle Tracing Task (Total time required: 9 minutes) **(V1-6)***

The Circle Tracing Task is designed to measure precision of motor movements that require continuous error feedback control (Lemay et al., 2005). The participant traces a 90mm diameter circle on a horizontal computer tablet while trying to remain within a 5 mm error margin that is indicated by a white annulus on a grey background. The participant first completes the task while directly viewing hand and stylus movement (3 trials, 45 seconds each.) The participant then repeats the task while indirectly viewing stylus movement on a separate, vertical computer screen with hand and stylus movement occluded from view (3 trials, 45 seconds each.)

Data generated for this task: Speed and errors (e.g. number of deviations per rotation)

Rationale and strength of evidence

HD Toolkit meta-analysis suggests that tracing tasks and movement to target tasks have promising cross-sectional effect sizes. Note that Figure 5 illustrates the more traditional Cohen's *d* ES statistic. Because most of the studies of target tracing tasks have utilized relatively small sample sizes, however, we are also providing the ES statistic Hedges *g* in Table 6 which includes a correction for potential bias associated with small sample sizes.

	Cohen's *d*	**Hedge's *g***
Boulet et al., 2005	-2.64	-2.53
Georgiou et al., 1997	-3.14	-3.03

Lemay et al., 2001	-3.55	-3.43
Smith et al., 2000	-1.68	-1.63

Table 6 Effect size comparison: Cohen's *d* and Hedge's *g*

In TRACK-HD, circle tracing detected differences in rates of change at both 12 and 24 months in early HD, and, together with SDMT, had one of the largest effect sizes in premanifest HD of 0.23 (95% CI: -0.03 to 0.51) over 12 months and 0.19 (95% CI: -0.10 to 0.48) over 24 months, although these were not significant. The test has therefore been recalibrated with the aim of increasing sensitivity in the premanifest population.

Analysis of current longitudinal data for the Circle Tracing task shows that the indirect condition is showing evidence of plateauing in longitudinal performance for controls and convergence of the premanifest HD groups at visit 5, and accordingly, it has small effect sizes. The Circle Tracing task will therefore be discontinued at V7 in order to interrogate the data.

Circle Tracing with Counting Backwards (Total time required: 4 minutes)

This condition is added to the existing circle tracing task in which participants perform the circle tracing task while at the same time counting backwards condition (e.g. counting from 100 backwards by 3s). The participant traces a 90mm diameter circle on a horizontal computer tablet while trying to remain within a 5 mm error margin that is indicated by a white annulus on a grey background. The participant attempts the task while indirectly viewing stylus movement on a separate, vertical computer screen with hand and stylus movement occluded from view (3 trials, 45 seconds each). The counting backwards component of this task requires participants to count aloud backwards by some number while performing the Circle Tracing task. Prior to combining circle tracing and counting backwards, participants will practice counting backwards alone.

Data generated for this task: Speed and errors (e.g. number of deviations per rotation)

Visual Array Comparison Task (Total time required: 8 minutes)

This task assesses the ability to sustain object and location representations without the aid of rehearsal and chunking strategies, leaving a purer measure of attentional capacities. This task is thought to be sensitive to a critical bottleneck for executing perceptual and cognitive functions that occurs when it is necessary to extract and retain items in visual short-term memory (on average about 4 items).

On a given trial of the Visual Array Comparison Task (Cowan, 2001; Cowan et al., 2005; Luck & Vogel, 1997), an array of (4 or 8) coloured squares (Figure 2) is presented for 250 ms (short enough so that participants cannot verbally encode the items). After 1000 ms a similar array is presented with one of the squares encircled. Participants decide whether the square within the circle is the same as in the original array or has changed in colour.

Data generated for this task: discriminability and bias indices and Cowan's (2001) K formula for estimating the number of items encoded at each set size

Rationale and strength of evidence

Cowan et al. (2005) found that tasks of this type correlate well with other working memory (WM) measures, and with GF and other aptitude tests (r = 0.31 - 0.52). Cowan suggests that the task assesses individual differences in the flexibility of the scope of attention, such that higher WM individuals are able to "zoom out" to apprehend and sustain more items from the visual field. Unlike the greater activity typically observed in the lateral prefrontal areas during performance on traditional WM tasks, recent evidence suggests that neural activity associated with the capacity of sustaining conjunctive object/location information in this type of task is most strongly observed in the posterior parietal and lateral occipital areas (Todd & Marois, 2004, 2005; Vogel & Machizawa, 2004; Xu & Chun, 2006). Furthermore, the magnitude of this activity is predictive of individual differences in the number of items that can be retained (Todd & Marois, 2005; Vogel & Machizawa, 2004).

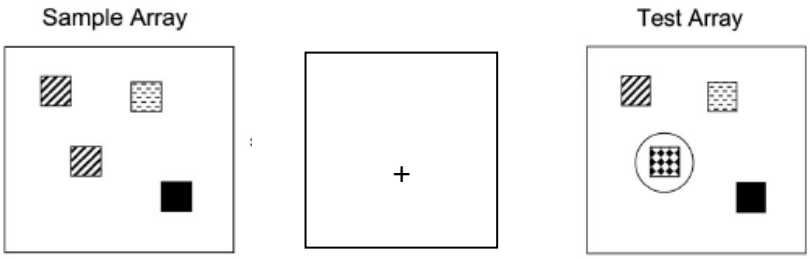


Figure 2 Sample trial for Visual Array Comparison Task

When comparing effect sizes between SPOT 7 and 5, cross-sectional effects sizes for controls versus gene-positive groups were generally larger for SPOT 5 than for SPOT 7. Due to these differences in effect sizes and the continuous use of SPOT 5 throughout the TrackHD study, a decision has been made to remove SPOT 7 from the V7 cognitive battery, leaving only the SPOT 5 condition.

Map Search (Total time required: 2 min)

Map Search is a measure of visuospatial selective attention that is part of the Test of Everyday Attention (TEA). Participants are required to search a visually cluttered display (a map of the Philadelphia area) to identify targets (symbol for restaurant, gas station, or garage) among distracters (other symbols and map characters). Testing time is two minutes. After one minute, participants are given a different coloured pen, to enable the tester to count the number of targets located in one minute versus the total for two minutes. The total score is the number of correctly identified target symbols (maximum of 80) at one minute and at two minutes.

Data generated for this task: Number of correct symbols found in one minute

Rationale and strength of evidence

Map search is a test of visual attention that is likely to be related to parieto-occipital function and which adds substantively to a cognitive ability area less well-represented in Track-HD. It has some potential to reveal cognitive deficits related to the imaging findings in the parieto-occipital lobe. Advantages are that the test is fast, engaging for the participant, and has very large effect sizes in one sample, albeit small, of early HD participants (ES = -2.92, nHD = 10, ncontrol = 17) (Murray & Stout, 1999). A related test of visual attention using visual search also suggests sensitivity in early HD (TEA Telephone Search cross sectional ES = -2.25 nHD = 9 ncontrol = 8) (Murray & Stout, 1999).

Shepard and Metzler 3D Mental Rotation (Total time required: 5 min)

This computerised task assesses the accuracy and speed at which participants can mentally rotate 3D cube stimuli (Figure 3). On each trial a pair of cube stimuli is presented with one figure rotated with respect to the other. Participants respond as to whether the rotated figure is identical to ("same") or a mirror image of ("different") the comparison figure. Fifteen practice trials precede the 30 experimental trials.

Data generated for this task: Total number of correct responses and Normalised response time (ms/deg for correct responses on non-mirror trials)

Rationale and strength of evidence

Several MRI studies using cortical thinning or voxel-based morphometry methods have illustrated significant changes in posterior cortical regions of the brain in prodromal and early HD (Beste, et al., 2008; 2006; Rosas, et al., 2005; Rosas, et al., 2008). This task was selected as a measure that reliably recruits these regions, in

particular, the inferior and superior parietal lobes in healthy controls (Alivisatos & Petrides, 1997; Farah & Hammond, 1988; Harris, et al., 2000; Ng, et al., 2000; Zacks, 2008).

Very little is known about mental rotation abilities in participants presymptomatic for HD. One small cross sectional study using a mental rotation task with the Shepard and Metzler cube stimuli showed a respectable effect size in a small sample of PreHD near-to-onset participants (ES = -0.68 ; nHD = 11, ncontrol = 31) (Robins Wahlin, Lundin, & Dear, 2007). This task was introduced at Year 3 of TRACK-HD and cross-sectional data showed impaired accuracy in both early stage and premanifest participants.

Figure 3 A normal and mirror stimulus from the Mental Rotation 3D task

Cancellation Test (Total time required: 6 minutes)

This task measures selective attention. Across several trials (typically 3 90-sec trials), participants must locate particular stimuli or combinations of stimuli (for example small squares with a small line on a given side, or pictures of fruits) randomly distributed among distractors (squares with a line on another side, pictures of other fruits or other similar looking objects) on a tablet computer.

Data generated for this task: speed, correct identifications and errors

When comparing effect sizes using the available cross-sectional data, the digit condition was found to have larger effect sizes compared to the figure condition, and when comparing significant group differences from p-values, the digit condition showed more significant group differences than the figure condition. It has therefore been decided that the figure condition of the Cancellation task will be removed from the V7 cognitive battery, leaving only the digit condition,

Virtual Maze task (Total time required 20 mins)

This task measures spatial navigation using a virtual environment presented on a computer screen. Participants are required to navigate through the virtual environment in the shortest possible time to a particular target, from differing locations and through varied environments.

Data generated for this task: number of trials completed, choice accuracy, error rate, latency.

Rationale and strength of evidence

Spatial navigation strategies are thought to tap into hippocampal and caudate functions: brain regions thought to play a role in the possible compensatory mechanisms proposed in premanifest HD. When navigating virtual environments, participants utilise "spatial strategy" or "response strategy" (Iaria et al., 2003): *Spatial strategy* involves navigating relationships between environmental landmarks, leading to the development of cognitive maps; subserved by the hippocampus (Morris et al., 1982; Bohbot et al., 1998). Additionally, a virtual spatial memory task correlated with total hippocampus volume (Chen et al., 2010 & Head and Isom,

2010). In contrast, *response strategy* involves learning a series of stimulus-response associations, ie. a pattern of left and right turns; an inflexible strategy which relies on the striatum, including the caudate nucleus in humans (Packard & Knowlton, 2002; White and McDonald, 2002). Head & Ison, 2010 provides further evidence reporting a positive correlation between caudate nucleus volume and performance on a virtual route learning task; a task known to utilize response strategies.

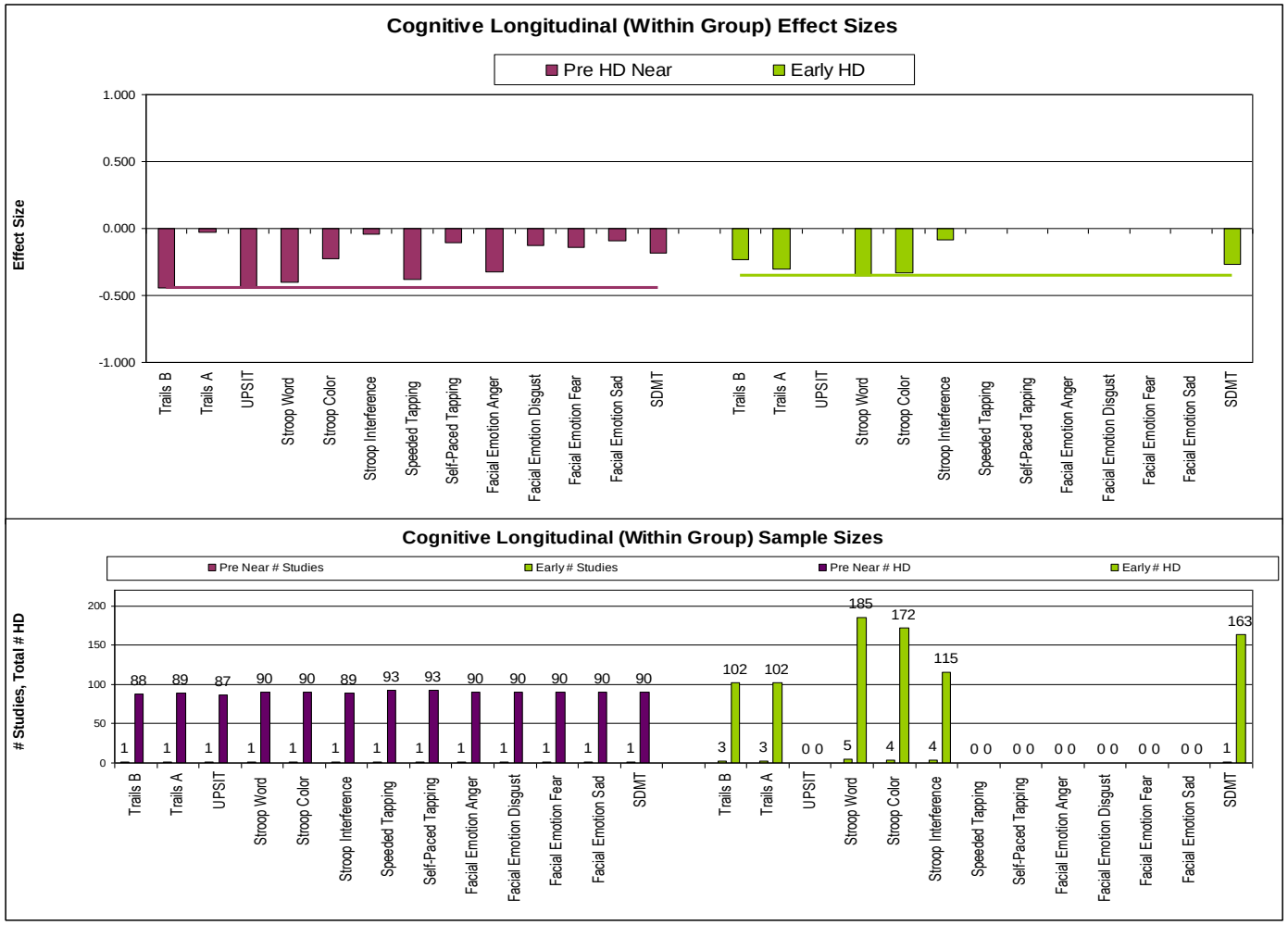


Figure 4 Cognitive Longitudinal Effect Sizes (and Sample Sizes) from HD Toolkit

(Star indicates ES based solely on Predict-HD data)

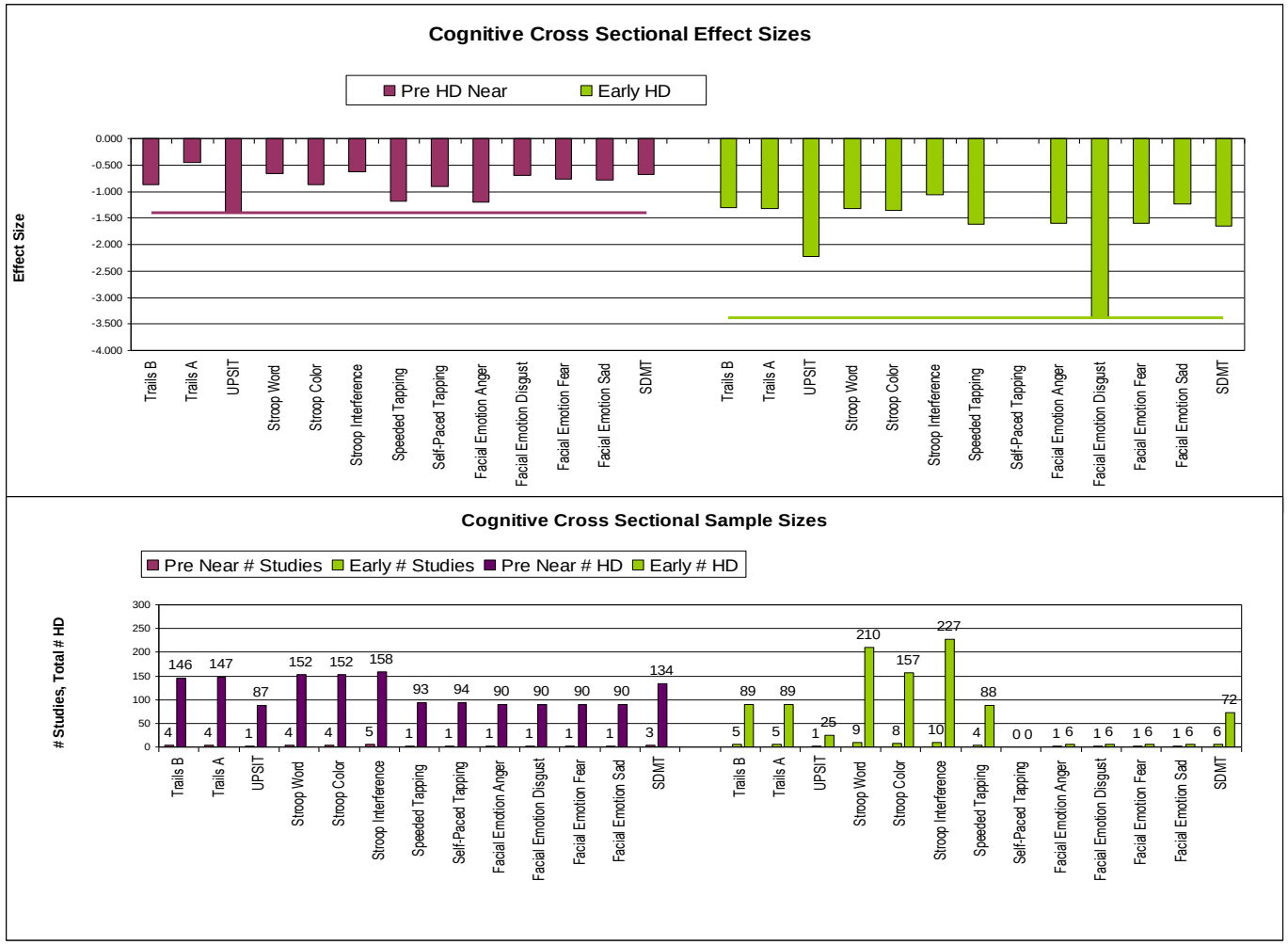


Figure 5 Cognitive Cross-sectional Effect Sizes (and Sample Sizes) from HD Toolkit

(Star indicates ES based solely on Predict-HD data)

5.5.8. Quantitative motor assessment

5.5.8.1. Overview

Motor dysfunction is a prominent sign of symptomatic HD, and evidence from TRACK-HD and PREDICT-HD demonstrates conclusively that motor signs begin to develop well in advance of disease diagnosis. In TRACK-HD, the first subtle deficits in motor coordination can be detected objectively in HD gene carriers up to 2 decades before clinical disease onset (Tabrizi et al, 2013). However, while cross-sectional deficits in premanifest gene carriers can be quantified in several quantitative motor assessments, compared to controls, progression after 24 months of follow-up was evident in speeded tapping only (Tabrizi et al, 2013). The ongoing goal of the quantified motor assessment component of Track-HD is to test a set of quantitative neurophysiological motor measures that use objective and precise measurement techniques, with the hope of both improved reliability and sensitivity, for tracking progression in this group.

Six quantified motor measures were selected, on the basis of expert input from Ralf Reilmann and the EHDN Motor Working Group, for inclusion in the Track-HD protocol, as well as a review of existing studies and TRACK-HD data. These measures allow for a multimodal motor assessment of the key motor systems using sophisticated gadgets. The goal of these assessments is to test a set of novel or modified paradigms tailored at the premanifest stage. Although data exists for some of these tasks in the TRACK-HD cohort, premanifest participants need to be observed over longer periods in order to measure detectable disease progression. In addition, measures such as speeded tapping and chorea position- and orientation-indices, may be used for correlation analyses with MRI, fMRI or TMS measures.

Test	**Duration (min)**	**Measurement / Analysis**
Glossometry*	5*	tongue force analysis
Manumotography	2.5	isometric grip force analysis
Force matching	10	Isometric grip force matching
Digitomotography	8	speeded, paced and alternative index finger tapping with isometric force transducer
Dysdiadochomotography		pronate/supinate alternation
Choreomotography	n/a	Position- and orientation-index
Pedomotography	5	Foot tapping / speeded
Posturography test	5	lower extremity motor coordination force plate
Go-No-Go task	6	Measure of the ability to withhold a tapping response

***Removed at V7**

Table 7 Quantitative motor measures

5.5.8.2. Equipment required per site

1. One Multimodal Force Assessment System (supplied by laboratory of Ralf Reilmann, MD) including:
   - Personal computer with monitor or laptop equipped with extension for three serial ports (DB-9 standard) running a Windows operating system and pre-installed data acquisition system ZOOM/SC for Windows (licensed to Ralf Reilmann for use in TRACK-HD).

- One pre-calibrated force transducer Mini-40 and amplifier (force transducer can be exchanged easily between tongue-force-, grip-force-, and tapping device by the investigator)
- One Polhemus 3D-position sensor system including one transmitter and one receiver
- One platform for tongue force measurement
- One two-finger grip device
- One finger tapping device
- One force matching device
- One foot-force device

2. One force-plate for posturography including software

5.5.8.3. Information on specific tests

Glossomotography (tongue force variability) (Total time required: max. 5 min) **(Visits 1-6)***

This task assesses the coordination of tongue protrusion forces (named "glossomotography"). Tongue force is measured using a specially designed setup (Figure 6): a force transducer is mounted on a height adjustable base located on a table. The force transducer is interfaced with a personal computer using the flexible data acquisition system ZOOM/SC (University of Umea, Sweden). The laboratory of Dr. Ralf Reilmann has obtained special limited licences for use in the set up of this study. Programs written in a special SC language for data acquisition are supplied by Dr. Reilmann's laboratory.

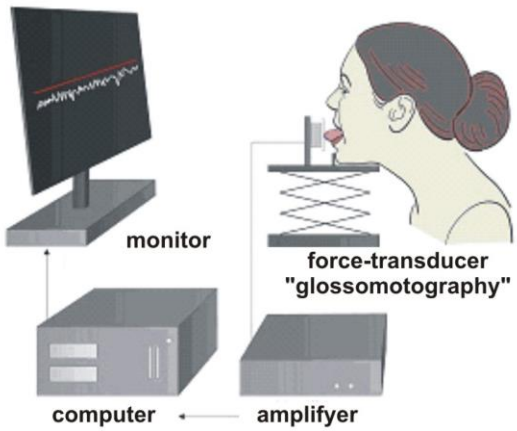


Figure 6 Tongue force apparatus

The primary outcome measure "tongue force variability" (TFV) was shown to be impaired in early stage and premanifest HD compared to controls (Reilmann et al 2010). TFV was correlated to UHDRS-TMS and the disease burden score (Tabrizi et al 2009) (Figure 7). In addition, the amplitude of TFV impairment was correlated to changes in VBM striatal volume and loss of cortical thickness in this TRACK-HD baseline data.

Participants are asked to place her/his chin on the base of the assessment system. Following a cueing tone, participants are instructed to protrude their tongue, press on the force transducer and generate a target force level presented as feedback on a monitor in front of them. Five 20 second trials are performed. Isometric tongue protrusion forces are recorded. The participants should be instructed not to bite on their tongue while protruding the tongue. If participants retract the tongue they should be asked to try to protrude it again and continue to press on the force transducer for as long as possible while the trial is running.

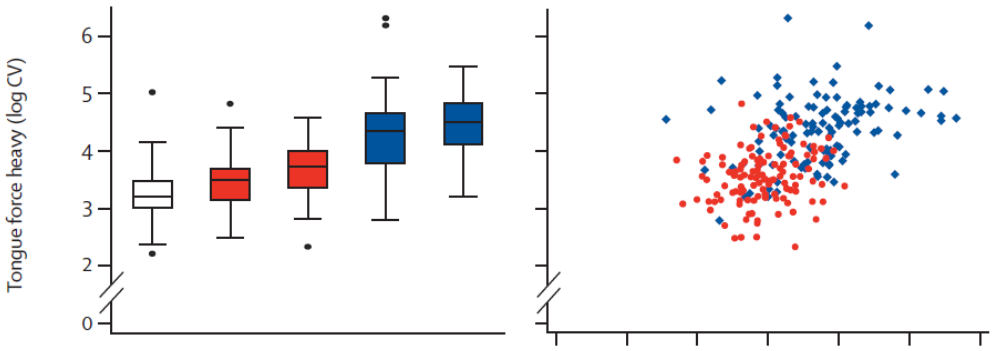


Figure 7 Tongue force variability (including a correlation to the disease burden score (right))

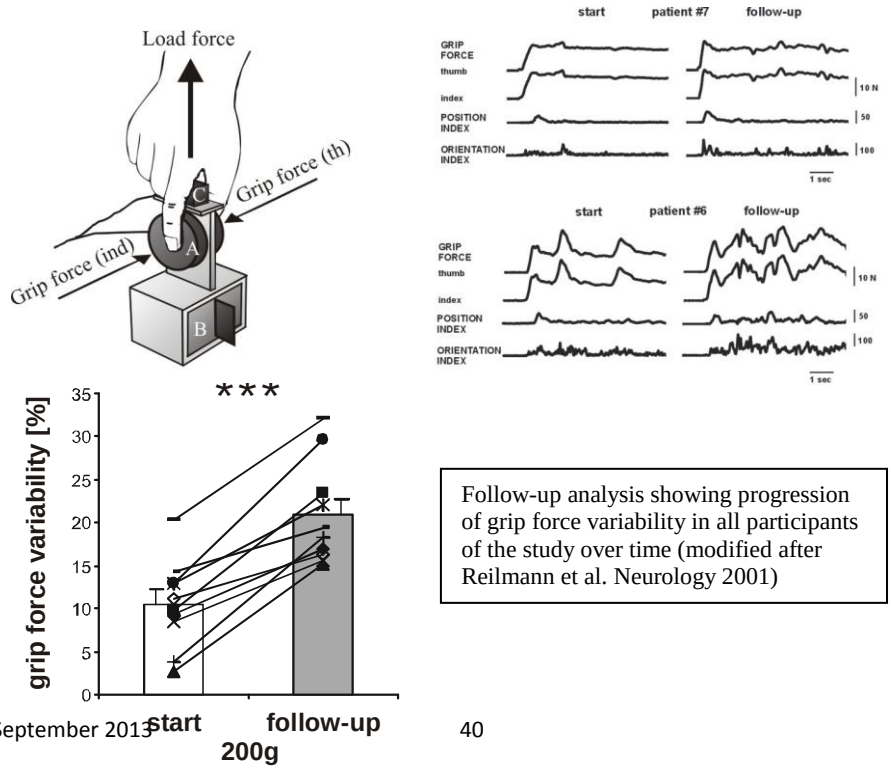
Data generated for this task includes, for each trial:

1. tongue force variability (primary outcome measure) [%]
2. mean tongue force [N]
3. mean contact time [sec]

Analysis of current longitudinal data provides sufficient evidence that glossomotography is sensitive in HD1, HD2, and preHD-B, although not in preHD-A. The glossomotography assessment will therefore be discontinued at V7 in order to interrogate the data.

Manumotography (isometric grip force) (Total time required: max 5 min).

This task assesses the coordination of isometric grip forces in the precision grip between the thumb and index finger during grip initiation, object transport and in a static holding phase. Grip forces and object position are measured using a grip device (Figure 8), equipped with a pre-calibrated force transducer measuring grip (normal) and lift (vertical) forces and a Polhemus 3D position sensor measuring x-, y-, z-position and roll-, pitch-, yaw-orientation of the object to assess object movement.



Follow-up analysis showing progression of grip force variability in all participants of the study over time (modified after Reilmann et al. Neurology 2001)

Figure 8 Grip force apparatus, sample recordings & follow up analysis

The force transducer and Polhemus are interfaced with a personal computer using the flexible data acquisition system ZOOM/SC (University of Umea, Sweden). The laboratory of Dr. Reilmann has obtained special limited licences for use in the setting of this study. Programs written in a special SC language for data acquisition are supplied by this laboratory. The coordination of grip forces including the timing and variability of force generation and amount and the impact of involuntary choreic movements (3D data – see Choreomotography) are measured and analysed (detailed list of variables see below).

Using the paradigm described in this protocol, "grip force variability" (GFV) was correlated to the UHDRS-TMS (Gordon et al. 2000), and showed progression in a follow-up study in manifest HD participants and correlation to the disease burden score in premanifest carriers of the Huntington gene (Reilmann et al., 2010). These findings were confirmed in the cross sectional and longitudinal analysis of TRACK-HD.

Participants are seated in front of a table with their wrist resting on the edge of the table and the grip-device placed 30 cm away from the edge of the table in front of them. Participants are instructed to grasp the grip device at a comfortable speed after a cueing tone signals the start of the trial. They are instructed to lift the device and hold it stable next to a marker made up by a wooden block, 10 cm high. A second cueing tone signals the end of the trial 30 seconds after the first tone, a which participants are instructed to replace the device at comfortable speed on the table, release the grip and return the wrist to the resting position before initiation of the next trial.

Due to the higher sensitivity of the heavy weight condition of the grip force variability measure in both cross-sectional and longitudinal analyses, the manumotography task with the light weight, dominant and non-dominant hand, is being discontinued at V7.

Data generated for this task includes, for each condition:

1. mean static grip force variability (primary outcome measure) [%]
2. mean static grip force [N]

Upper extremity force matching task (Total time required: 10 min)

This task assesses the coordination of isometric grip forces in the precision grip between the thumb and index finger and is assessed while participants are instructed to generate a target force presented on a monitor in front of them. Normal force of the thumb is recorded and variability of grip forces during the static force matching period is assessed.

5 trials of 25 seconds duration are performed with the right and left hand in 3 target force conditions – high, middle and low.

Data generated for this task includes, for each condition:

1. mean static grip force variability (primary outcome measure) [%]
2. mean static grip force [N]
3. maximal grip force [N]

Digitomotography/Dysdiadochomotography (speeded & paced/metronome & alternative index finger tapping with force transducer tasks (Total time required: 10 min)

For the following tasks, a force transducer is attached to a base located on the table 30 cm in front of the participant (Figure 9). Following from the design of the finger tapping test used in the Predict/Track core battery, and based on the findings from the TRACK-HD and Predict data showing that non-dominant hand finger tapping typically shows the greatest sensitivity, participants will be instructed to use the non-dominant hand for most finger tapping tasks. The exception is for speeded tapping, where preliminary data from TRACK has shown interesting findings in subgroup comparisons in dominant speeded tapping in certain stages of HD (Tabrizi et al 2009).

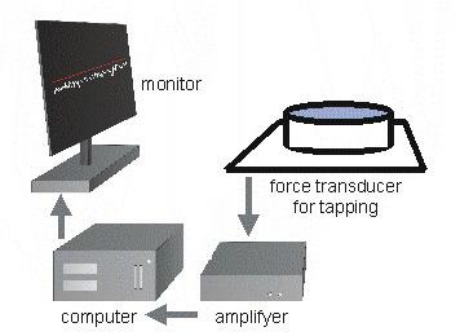


Figure 9 Finger tapping force apparatus

Speeded Tapping

Participants will be instructed to use both the dominant and non-dominant hand and tap as quickly as possible from the time a first auditory signal is sounded until a second one is sounded 10 seconds later. Each participant will complete five 10 second trials using each hand.

The Finger Tapping Test is sensitive in Premanifest HD near onset in the Predict-HD database, and has the largest longitudinal effect size for all measures in the cognitive battery for Predict-HD. The addition of the force-related variables may enhance the sensitivity of the test. Furthermore, the test can be modified to make it more sensitive by adding a cognitive load (see Speeded Tapping with Cognitive Load). In TRACK-HD, tapping paradigms have detected differences between premanifest, as well as early stage HD, compared to controls, and measures are correlated to both the UHDRS-TMS and the disease burden score across both premanifest and manifest participants (Bechtel et al., 2010).

Speeded Tapping with Cognitive Load (Visits 3 to 6)

A cognitive load is added to the speeded tapping task described above to increase difficulty and create a dual task condition in the hope of adding sensitivity to difficulties in multi-tasking thought to occur in Premanifest HD. In addition to tapping as fast as possible with the nondominant index finger, the participant will also perform the serial 2's task. In this task the participant starts at 100 and serially subtracts backwards by 2's, announcing each difference (e.g., 100, 98, 96, 94, 92,). Five trials will be included, each serial 2 sequence starting from a different number.

Likely main variable for analysis: Standard Deviation of the self-paced intertap interval (primary outcome measure), and Serial 2's number correct

Although executive function is posited to be affected in HD and Premanifest HD, measures that tap this deficit have been difficult to find. Simple or well practiced motor movements such as walking are not impaired in healthy individuals when accompanied by a cognitive load. However, populations with executive function impairments (e.g., elderly) appear to lose motor automaticity and, consequently, their motor performance suffers when they simultaneously perform an additional cognitive task (cognitive load, Springer et al., 2006.) For

example, gait variability in Parkinson Disease patients increases with cognitive load (Yogev et al., 2005) Therefore, adding a cognitive load to Self-Paced Tapping has the potential to improve the sensitivity of a task that is already somewhat sensitive to HD progression.

The Serial 2's Task, as currently performed, has not proved superior to other tapping paradigms; and contains a number of parameters that are not well controlled, meaning that comparability of results within and across participants is consequently not currently given. Also, propensity to practice effects means it may not be suitable as a longitudinal measure. It has therefore been decided to remove the task to provide space for new, possibly more promising tasks.

Metronome/Self-Paced Tapping

Paced tapping provides a measure of psychomotor functioning, including timing. The task begins with the repeated presentation of a tone at a constant rate. The participant is instructed to begin to tap at the same rate as the tone. After 5 seconds, the tone stops and the participant is asked to continue tapping at the same pace for another 10 seconds until the next tone sounds. The sequence is repeated five times with tones spaced every 180msec and five times with tones spaced every 50 msecs for a total of 10 trials. A fast and slow pace will be assessed with the non-dominant hand only.

Effect sizes from the Predict-HD longitudinal database indicate that decline is only at trend level for this measure in Premanifest HD near onset. However, preliminary data from TRACK-HD (Tabrizi et al 2009) indicates evidence of group differences in the precision of paced tapping. This task differs from the bilateral thumb task in the cognitive battery and used the index finger of the non-dominant hand only. The rate of 50 msec is intended to explore the ability to tap at slower frequency and to provide additional information to that derived from the higher frequencies used in the cognitive paced tapping task.

Bimanual Tapping

In addition, a task assessing bimanual motor coordination will be performed: participants will be asked to tap using alternating index fingers of the right and left hand. Each trial will be of 10 seconds duration and 5 will be completed.

Dysdiadochomotography

In "Dysdiadochomotography" we aim to investigate the regularity of alternating pronation and supination hand movements – see Figure 10. The participant is instructed to perform alternating pronation-supination movements and to tap on the force transducer with the palmar or dorsal hand surface as regular and fast as possible. The assessment duration and evaluation are equivalent to speeded tapping and the same setup is used.

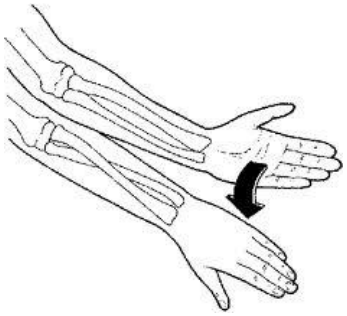


Figure 10 Dysdiadochomotography

Data generated for all Digitomotography/Dysdiadochomotography include, for each trial:

1. tapping rate [n]
2. tapping rate variability [%]
3. inter tap interval
4. inter peak interval
5. tapping intensity
a. normal force applied [N]
b. maximal force generation rate [N/s]

Pedomotography (speeded tapping foot) (Total time required 5 min)

For this task, a force transducer is attached to a foot force tapping device placed on the ground in front of the participant (Figure 11). Participants will be instructed to tap as quickly and regular as possible with the right and left foot from the time a first auditory signal is sounded until a second one is sounded 10 seconds later.

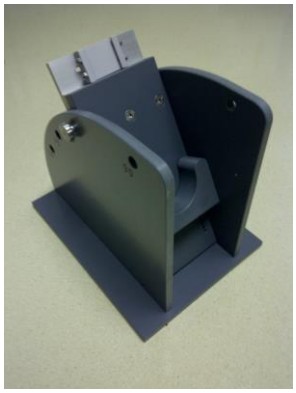


Figure 11 Foot tapping device

Data generated for this task includes, for each trial:

1. tapping rate [n]
2. tapping rate variability [%]
3. inter tap interval
4. inter peak interval
5. tapping intensity
c. normal force applied [N]
d. maximal force generation rate [N/s]

Posturography for lower extremity motor coordination test (balance assessment using force plate) (Total time required: 5 min)

This task assesses the balance of participants, which is dependent e.g. on lower extremity and trunk motor coordination. Position of the centre of mass is calculated using the input of three different integrated and pre-calibrated force transducers mounted in the force plate (Figure 12). Data acquisition, presentation and evaluation

are performed by a Windows based computer program called "SATEL" specifically designed for the force plate and installed on the assessment computer.

Assessment will be performed with the participant standing on both legs with visual feedback (eyes-open). The force plate should be placed next to a table or wall and the examiner should be next to the participant to prevent falls. Applicability of the force plate in HD has been demonstrated previously (Tian et al. 1991). Correlation of variables to the UHDRS-TMS was seen recently (Reilmann, pers. comm.)

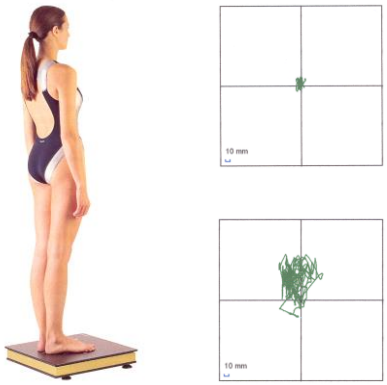


Figure 12 Posturography apparatus

Participants stand in front of the force plate bare feet. They are instructed to step on the force plate and place their feet in a marked position. Investigators verify that participants are in the right position prior to starting the assessment. They will be instructed to stand still as well as they can for a period of thirty seconds. Recording is initiated after a verbal instruction ("start"). A bar on the computer screen indicates the remaining time until the end of the trial. The investigator will indicate to the participant that the recording time is finished by saying aloud ("end") and verify the participant's position before initiating the next trial. The participant should perform the task 3 times standing on both legs eyes open.

Data generated for this task includes, for each trial:

1. surface area [mm2] (primary outcome measure)
2. distance moved [mm]
3. velocity [m/sec]

In addition to the unpublished work from Ralf Reilman's laboratory, the HD Toolkit project found 3 published articles reporting cross sectional data on posturography in HD populations. One article is still being retrieved. The Tian (1991) article had insufficient data to compute accurate effect sizes, but approximate cross sectional effect sizes suggest controls performed 0.6 to 0.8 standard deviations better than HD patients of unspecified extremity. In addition, a Tian (1992) article reports controls performing 1.6 to 2.1 standard deviations better than 20 late HD patients.

Cross sectional data from TRACK-HD indicated that first changes in posturography can be found in premanifest gene-carriers (Tabrizi et al 2009), and these changes observed are correlated to atrophy in the striatum as assessed by VBM. Interestingly, the results show that withdrawal of visual feedback does not increase the sensitivity of the assessment in premanifest HD. Therefore assessments without visual feedback are discontinued.

Choreomotography (Neurophysiological analysis of involuntary choreatic movements) (acquired from grip force task – no additional time needed)

During the upper extremity motor coordination test with the grip device, 3D position (x, y, z) and orientation (roll, pitch, yaw) are recorded objectively and quantitatively. Participants are instructed to hold the object stable next to a marker and involuntary choreatic movements interfering with this task are recorded. Mathematical analyses of the deviations occurring during the static holding phase provide the derived measures "position-index" (sum of absolute values of first derivatives of x-, y-, and z-channels) and "orientation-index" (sum of absolute values of first derivatives of roll-, pitch-, and yaw-channels) (Figure 13). The analysis was used to objectively assess the impact of chorea on other motor tasks and both measures were shown to be correlated to UHDRS-TMS chorea scores and the disease burden score in TRACK-HD (Tabrizi et al 2009).

Data generated for this task includes:

1. position-index
2. orientation-index

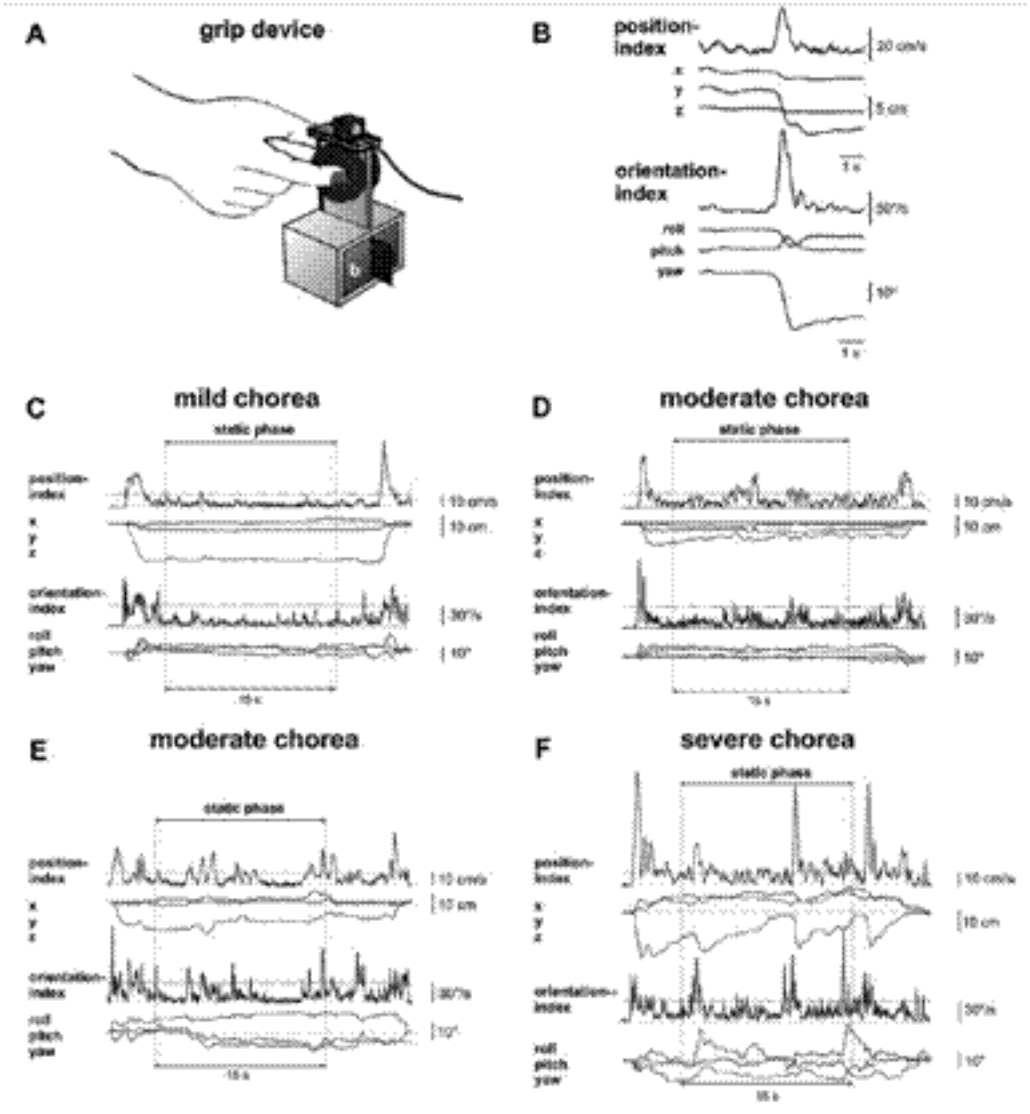


Figure 13 Objective quantitative analysis of chorea

Go-No-Go task (Total time 6 min)

For this task, two easily discriminable auditory stimuli (tone R (react), and tone I (inhibit)) ,with similar intensity (i.e. loudness and length), will be presented at 50/50 chance in pseudo-randomised order; with consistent time interval between the two stimuli at 1500 msec. Participants are instructed to execute a tap on the tapping device as soon as they hear tone R (the higher tone) and withold response if tone I (the lower tone) is presented.

The task will contain 100 trials, resulting in 50 repetitions per condition (go/no-go); and trials presented in one single block after a 10 trial practice block. The task will be done with the dominant and non-dominant hand, starting with the dominant hand. Practice will be done with the dominant hand only.

Versions of the Go/No-Go Task are widely used in cognitive psychology as a measure of the ability to withhold a response (e.g. Falkenstein et al. 1999). In basic versions of this psychomotor task, participants are usually presented with two stimuli (e.g. two differently pitched tones) and asked to respond to only one while displaying no reaction to the other stimulus. The response inhibition process required for the latter is part of a set of cognitive functions subsumed under the notion "cognitive control" (Ridderinkhoff et al. 2004), and is thought to be mediated by frontostriatal circuits which are distorted by striatal pathology in HD patients (Aron et al. 2003).

In previous studies comparing HD patients' performance to controls or to other patient samples, HD patients displayed significantly more false alarms, i.e. reactions to the inhibitory stimulus, than the other groups (Beste et al. 2008a; Beste et al. 2010). This was even the case for the pre-HD group included in one of these studies (Beste et al. 2010). Futher, Beste et al. (2008b) found an attenuation in an inhibition related EEG-component, the No-Go-P3, that was correlated to CAG-repeat length as well as a CAG-index derived from age and duration since motor diagnosis.

Overall, previous findings suggest that a Go/No-Go paradigm may be a sensitive measure for psychomotor and cognitive impairment in HD. Its inclusion in Track-HD potentially also provides detailed insights into the tasks ability to distinguish between different disease stages, and how performance in response inhibition develops over time.

Evaluation will be by:
response times for the go and no-go condition
error rates for the go and no-go condition
response times for go stimuli presented after a no-go stimulus
error rates for no-go stimuli presented after a go stimulus

5.5.9. Imaging assessment

All participants undergo structural (T1- T2- and diffusion weighted) and functional (resting state and activation) MRI at every visit on 3T scanners. In addition, magnetic resonance spectroscopy (MRS) is performed at 2 clinical sites (Vancouver and Leiden). At visit 7 participants will undergo Amide Chemical Exchange Saturation Transfer (CEST) and Magnetisation Transfer Ratio (MTR) at Leiden and Vancouver, and Neurite Orientation Dispersion and Density Imaging (NODDI) at London and Paris. Protocol details will be provided in the Track-HD SOP documents. T1-2, MRS and fMRI modalities were chosen because they 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, Klöppel 2009). CEST, MTR and NODDI are exploratory techniques which have been added to provide complementary assessments of hallmarks of HD, such as protein accumulation and subtle changes in tissue microstructure (eg. Jones et al., 2006; Zhou et al, 2003; Filippi et al., 1998; van den Boggaard et al.,2012; Zhang et al, 2012). Only one fMRI task will now be performed at each site (working memory in London and Paris; motor task in Leiden and Vancouver) to ensure that the total scanning

time will remain at 100 minutes, with breaks in between.. Ultimately, all imaging data will be collected, quality-controlled, stored, distributed and analysed through the imaging CRO.

3T MRI scanners have been chosen for Track-HD, 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 from TRACK-HD will be invaluable for further analyses in the future, in addition to the morphometric studies planned for TRACK-HD.
- 3T imaging represents technology that will become dominant, which means that TRACK-HD 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.

The following image analysis will be performed by specified experts upon successful application to the Track-HD Steering committee:

1) Whole brain, ventricular and caudate volumes, and rates of atrophy using the boundary shift integral (BSSI) (BBSI) (Freeborough & Fox, 1997)

2) Assessment of diffusion metrics and fibre tracking from Diffusion Weighted Imaging (DWI)

3) 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 (London and Paris only)

4) Grey- and white-matter density using voxel-based morphometry (VBM) (Ashburner & Friston, 2000)

5) Automated segmentation of regions of interest including caudate and putamen using the Brain Research: Analysis of Images, Networks, and Systems (BRAINS, Magnotta et al. 2002) software which integrates reliable and validated image analysis tools for large neuroimaging studies.

6) Resting state fMRI region analyses and principal component analyses.

7) FMRI activation data, correlating the onset of a movement with the changes in blood oxygenation caused by it, and analyses of functional and effective connectivity. The analysis of fMRI data is well established and uses SPM. Two well established fMRI activation studies will be performed. One task involves finger movements following visual and auditory stimuli. Movements differ in speed and complexity, and some may require the participant to withhold a movement. The second task focuses on memory and participants need to memorise numbers or objects and recall them later.

8) Magnetic resonance spectroscopy to detect changes in metabolites indicative of neuronal health (Vancouver and Leiden only)

9) CEST chemical exchange rate analysis to indirectly image protein accumulation in its soluble form, and in particular HTT, thought to be the earliest characteristic of HD (Vancouver and Leiden only)

10) MTR to detect levels of aggregated mutant HTT and disease-related microstructural change. (Vancouver and Leiden only).

In addition, Track-HD and CHDI support the widest possible use of these data for scientific purposes. The full imaging datasets will therefore be made available for legitimate research purposes on request subject to agreement of the steering committee. For full details on Data sharing, see section 5.19.2.

5.5.10. Transcranial Magnectic Stimulation (TMS)

5.5.10.1. Overview

TMS is a non-invasive in-vivo motor cortex stimulation, which provides an insight into the electrophysiological properties of corticospinal neurons and the trans-synaptic regulation of inhibitory and facilitatory circuits within the motor cortex. It has previously been used to demonstrate abnormalities in motor cortex excitability in heterogeneous, manifest and premanifest populations (Schippling et al, 2009). These abnormalities include prolonged cortical silent periods, reduced short interval intra-cortical inhibition and enhanced or normal intra-cortical facilitation.

It is also well-known that somatosensory evoked potentials (SEPs) become abnormal as HD progresses. This might reflect a disease-related influence on the sensory input part of sensory-motor integration pathways. One such pathway that can be tested easily in humans is short latency afferent inhibition (SAI in which a transient sensory input leads to a rapid and short-lasting inhibition of the motor cortex). Since SAI is thought to reflect changes within the somatosensory-motor pathways in HD, it may also be worthwhile to consider comparisons with cognitive tasks that include aspects of motor integration.

The aim is to focus on the primary sensory-afferent inhibition (SAI) variable, and perform comparisons with a wider range of quantitative motor and imaging methods. The TRACK-HD premanifest cohort is sufficient to meet sample size estimates for the SAI and over 24 months would have the additional advantage of being able to compare not only those near and far from onset, but also potentially fast and slow progressors. The cohort also benefits from the existing 36 months of data to inform these new cross-sectional and longitudinal studies.

Test/Method	Duration (min)	Measurement / Analysis
Core protocol		
Transcranial Magnetic Stimulation	15	Hot-spot determination and motor thresholds (rest and active)
	8	Cortical silent periods and input-output curves at 110, 130 and 150% of RMT at rest, and 130, 150 and 175% of AMT with pre-activation.
	7	Short latency afferent inhibition*
Evoked potentials	3	Sensory evoked potentials
	3	Long-latency reflexes
Visual Evoked potentials (VEP)[1]	15	VEP P2 latency, N1/P2 and P2/N2 amplitude
TMS Evoked potential (TMS-EEG)[2]	20	Baseline EEG; EEG with TMS. Differences between baseline and TMS EEG
Optional components		

Transcranial magnetic stimulation	20	Rapid paired associative stimulation

1. Leiden only 2. London & Vancouver * Removed at V7

Table 8 TMS assessments

5.5.10.2. Equipment per site

- Hand-held figure-of-eight coil
- High Power Magstim 200 stimulator or newer
- Silver/silver-chloride disc surface electrodes (1 cm diameter)
- Digitimer D150 amplifier
- Peripheral nerve stimulator
- Signal software (or similar)

5.5.10.3. Experimental set up

Electromyography recordings and Transcranial Magnetic Stimulation

Surface electromyograms (EMG) are recorded from the right first dorsal interossoeus (FDI), right abductor policies brevis (APB) and right abductor digiti minimi (ADM) muscle using silver/silver-chloride disc surface electrodes (1 cm diameter) in a belly tendon montage. The EMG signal is amplified and analogue filtered (30Hz to 1kHz) with a Digitimer D150 amplifier (Digitimer Ltd., Welwyn Garden City, UK). Data (sampling rate 4kHz) is digitised for off-line analysis using Signal software (Cambridge Electronic Devices, Cambridge, UK).

Partcipants are seated in a comfortable chair. They are asked to relax as much as possible. Magnetic stimuli are given with a hand-held figure-of-eight coil (outer winding diameter 9cm) connected to a High Power Magstim

200 stimulator or newer (Magstim Co., Whitland, Dyfed, UK). These stimulators generate a magnetic pulse with monophasic waveform that induces a current in the brain with posterior-anterior flow when the coil handle is positioned at an angle of 45° pointing backwards.

5.5.10.4. Information on specific tests

Hot spot determination, motor thresholds, input-output (I/O) curves at rest
The optimal spot for right APB stimulation (largest MEP recorded from APB) is marked with a felt pen. Resting motor threshold (RMT) is defined as the minimum intensity sufficient for a motor evoked potential MEP of >50µV in 5 out of 10 consecutive trials in the relaxed APB. Active motor threshold (AMT) is defined as the minimum intensity (in % of maximum stimulator output) needed to evoke a MEP of >200µV in 5 out of 10 trials in the tonically active APB (~20% of maximal contraction as assessed visually on an oscilloscope). Thresholds are approached from above threshold in steps of 1% stimulator output. Once no MEPs can be elicited the intensity is increased in steps of 1% stimulator output until a minimal MEP is observed. This intensity is taken as motor threshold.

Input-output (I/O) curves are examined by measuring MEP size of MEPs elicited at stimulus intensities of 110, 130 and 150%RMT. Ten trials are recorded, and the average MEP area is taken as MEP size.

Cortical silent periods (CSP)
CSPs are recorded from the tonically active right APB with the participants abducting the thumb at around 20-30% of maximum force output. Ten trials at fixed test stimulus intensities of 125, 150 and 175% AMT are collected in each participant with an interval of 5-6 seconds between trials. In each individual trial the duration of the silent period is measured from the visually identified beginning of the MEP evoked by the test stimulus to the resumption of (any level of) sustained EMG activity. In addition, the area under the MEP is determined and a ratio of silent period duration/MEP area calculated because silent period duration and the size of the preceding MEP correlate so that a ratio represents an additional measure of the inhibitory circuits underlying the CSP (Orth and Rothwell, 2004). The gain of the recordings is set to 1mV/V in order to measure the end of the silent period, and in a second channel is set to 10mV/V in order to measure the size of the MEP. Gain settings are the same for all experiments.

Somatosensory evoked potentials
After stimulation of the median nerve at sensory threshold for median nerve, at threshold intensities for median nerve motor stimulation and at 150% median nerve motor threshold somatosensory evoked potentials are recorded with a surface electrode over the somatosensory cortex (2cm posterior of C3 in the international eletroencephalography (EEG) 10-20 system) referenced against the opposite ear lobe and Fz. Stimulation is given at a frequency of 3 Hz; a total of at least 300 stimuli are averaged.

Short latency afferent inhibition (SAI) by somatosensory input from the median nerve (V5&6)*
Short latency afferent inhibition (SAI) of the motor cortex is examined as previously described (Tokimura et al., 2000). In brief, a MEP of ~1mV peak-to-peak amplitude is elicited in the FDI, APB and ADM by TMS. A paired pulse paradigm examines the influence on MEP size of a supra-threshold electrical stimulus given to the median nerve through bipolar electrodes. The electrical stimulus to the median nerve precedes the TMS pulse to the APB hot spot in relation to the N20+2 component of somatosensory evoked potentials (N20+2 and N20+4 for inhibition; N20+14, N20+16 for facilitation). The median nerve electrical stimulus is delivered at an intensity to evoke a visible contraction in the thenar muscles (above motor threshold). Twenty trials of the MEP elicited by TMS alone and 10 trials of conditioned MEPs for each ISI are collected. The amplitude of the MEP in the FDI/ADM/APB is measured with in-house software. The average amplitude of the conditioned MEP is expressed in percent of the average amplitude of the unconditioned MEP alone.

Contrary to expectations, data analysis has shown there were no differences between controls and any of the HD groups using SAI. It is unlikely that such differences will emerge given the variability of the data and therefore SAI will be discontinued.

Long-latency reflexes
The method follows the description by Deuschl & Eisen (Deuschl and Eisen, 1999). Reflexes are elicited in the contracted right abductor pollicis brevis muscle by electrical stimulation of the median nerve at the wrist. Participants sit with their pronated forearm supported before them on a table and contract the APB muscle isometrically to approximately 40 % of maximum by abducting the thumb against a force transducer with reference to a visual display before them. The median nerve will be stimulated just above the intensity needed to evoke a visible twitch in the APB using surface electrodes with the cathode proximal to the anode (stimulus duration, 1.0 ms; random rate from 0.9 to 1.1 Hz; constant current source). The reflexes following electrical nerve stimulation are visually inspected on average records of full-wave rectified EMG activity. Then the end of the short latency and beginning of the long latency reflex is determined when the average surface rectified EMG increases abruptly at a latency of between 45 and 55 ms. First, the duration of the short and long latency components of the EMG responses is determined. Then, the integral of the rectified EMG activity is calculated as the size of the reflexes. Stimulation is given at a frequency of 3 Hz; a total of 300 stimuli are averaged.

Visual Evoked Potentials (VEP)
Before genetic testing for HD was available reduced amplitudes of VEPS have been reported in manifest HD and in a proportion of at risk individuals (Hennerici, Homberg & Lange, 1985); additionally TRACK-HD data have indicated early involvement of the occipital lobe in HD.

The participants will be seated in front of a checkerboard at a distance of 1m. With each eye separately, while the other eye is covered, the participants will fixate on a cross at the center of the screen while the checkerboard flashes at a frequency of 2Hz. A total of 100-200 trials will be recorded. The EEG signal over the occipital lobe will be recorded from Oz and referenced to Fz, according to the 10-20-system. Data analysis will be performed using appropriate software. Trails containing artefacts will be excluded. The latencies and amplitudes of different components of the VEP will be analyzed.

TMS evoked potential (TMS-EEG)
Participants will be stimulated at resting motor thresholds at the motor cortex (M1) and at the premotor cortex. For stimulation of the premotor cortex, the coil is placed 2cm anterior to the motor hotspot. Single pulse TMS-evoked EEG responses are recorded by using a TMS-compatible amplifier. For each stimulation site 50 pulses will be administered. The EEG signals from 32 electrodes are referenced to an additional electrode on the forehead, and two extra sensors record the electrooculogram. EEG will be recorded while 60 TMS single pulses are given every 4-5 seconds as described above. Data analysis will be performed using appropriate software. EEG trials containing artefacts, such as muscle activity or eye movements, will be excluded. Source modelling analysis will then identify the TMS induced EEG scalp potentials followed by statistical analysis of differences between, and the timing of, resting EEG activity and TMS-induced potentials.

5.5.10.5. Optional component

Using the same experimental set-up the following optional components may be added in a subset of participants and if sufficient time is available and the participant gives consent:

Rapid paired associative stimulation (PAS)
The experimental set-up is the same as described above. The method is that described by Quartarone and colleagues (Quartarone etal., 2008). In brief it consists of 2 minutes of median nerve stimulation (600 pairs of pulses at a frequency of 5 Hz) and 15 to 20 minutes of follow-up with recordings of 10 MEPs immediately after rapid PAS, and then every 5 minutes until 20 minutes after rapid PAS. MEPs will be recorded from 2 muscles (APB and ADM).

5.5.11. Oculomotor assessment (Visits 1-6 only)

5.5.11.1. Overview

Changes in parameters of rapid eye movements (saccades), such as inability to suppress reflexive saccades and delayed initiation of voluntary saccades, are one of the earliest markers of Huntington's disease (Lakse & Zee, 1997). The cortico-basal systems that underpin voluntary eye movement significantly overlap the neural degeneration associated with HD, and it is not surprising that eye movements are a sensitive indicator of the associated cognitive and motor impairments.

There have been a number of recent publications correlating saccadic parameters such as latency, velocity and error rate with numerical indicators of HD progression (UHDRS score, or as a function of CAG repeat length x Age). The Track-HD oculomotor test battery incorporated the effective components of these studies.

Ali et al. (2006) reported a significant increase in mean saccadic latency for a reflexive task, and upon further analysis showed a significant increase in the proportion of early saccades in Early HD patients compared to premanifest HD gene carriers. The strength of this result was sufficient to permit a 75% accurate prediction of HD status. This strength was due to the high number of repeated trials (300) and a sophisticated latency analysis technique called LATER modelling (Reddi & Carpenter, 2000).

Various voluntary saccade paradigms have been reported as having good correlations with HD progression. Blekher et al. (2004) & (2006) showed an increase in mean saccadic latency in voluntary saccade tasks. Golding et al. (2006) supported this finding and reported an increase in latency variance that correlated linearly with HD progression. In TRACK-HD, baseline antisaccade error rates relative to controls were greater in premanifest individuals furthest from predicted clinical onset and those with early-HD (Tabrizi et al. 2009). Higher error rates were also associated with higher disease burden scores. Over 12-months, longitudinal change was detected only in early stage participants with stage 2 disease (Tabrizi et al. 2011).

The anti-saccade paradigm is a successful predictor of HD progression, with saccade latency and error rate correlating positively with HD progression (Blekher et al., 2004, 2006). Recently, Rivaud-Pechouxet al. (2007) reported that tests with mixed pro and anti saccades have even greater power when compared to performance in single tasks (of either pro or anti saccades). The mixed paradigms increase cognitive demand by requiring the participant to switch between rules. However, mixed paradigms are significantly longer in duration and require more complex instructions, and in TRACK-HD many participants found the task difficult and performed it in a variety of ways resulting in highly variable data. A refocused task that tests specific oculomotor abilities address this and should permit participants to respond more similarly which may allow us to better quantify group differences and longitudinal changes, particularly in the premanifest participants.

List of tests Total time required: 15 minutes	**Saccade types**	**Dependent variables and comparisons**
2 x blocks of 50 antisaccades*	anti-saccades	Latency, velocity and errors*

*removed at V7

Table 9 Oculomotor assessments

5.5.11.2. Equipment per site

- Personal computer with monitor, or laptop
- Saccadometer Advanced Eye Tracker (Ober Consulting)
- Software
- (2) AA batteries (spare)
- Desk & chair

5.5.11.3. Information on specific tasks

Antisaccade Reflexive Task

Participants perform a brief reflexivesaccadic task where they must look rapidly from using the Saccadometer, which has a set of lasers to present visual targets on a nearby wall. The participant begins a trial by staring at a central cue to red target. A new target suddenly appears 10 degrees to the left or right in and the participant must look as quickly as possible to the mirror opposite position. For example, if a target appears at 10 degrees to the left, the opposite direction from a peripheral target whichparticipant must look at a point 10 degrees to the right.

These trials will appear left or right at random. This provides a measure of antisaccade to be repeated 50 times in a block, and the block will be repeated after a short rest-break. The total number of 100 antisaccades is required to get a reliable estimate of the individual's mean latency, error rate and saccade velocity.

Overview and rationale for oculomotor assessment session

The reduction in number of necessary conditions makes for a much simpler testing paradigm, and the large number of repeats is necessary to perform LATER model analysis.

The Track-HD battery was composed of a mixed pro/anti-saccade battery that tested both the oculomotor functions of the individual as well as their cognitive abililties. While this battery produced highly significant cross-sectional differences between sub-groups, each group had a high degree of internal variation (due possibly to increased cognitive demands) which precluded the detection of longitudinal changes. When analysed separately, the antisaccades in the Track-HD battery correlated very well with striatal volume loss, pre-frontal fibre track integrity, and cortical thickness. Hence for Track-HD a pure antisaccade battery was run to focus solely on the oculomotor deficits in premanifest HD gene carriers and further the investigations into the functional consequences of neuronal loss. The new antisaccade test was included at Visits 5 and 6 to provide 12-months of longitudinal data for analysis before any further testing.

5.5.11.4. Overview and rationale for selection of eye tracking hardware

The Saccadometer Advanced (Ober Consulting Poland) was selected for its easae of use and low cost. It is 1/10th the price of the standard lab-based eye trackers, (such as the Eyelink II), and has very high temporal resolution (1000 Hz) and an equivalent spatial resolution (0.1 degree). It is battery operated and hand held, with lasers mounted onto the front to project visual targets on almost any surface. This makes the Saccadometer free to be used in practically any room, as long as there is a clear wall available. Furthermore, because the visual targets are also head mounted, there is no need to compensate for head movement. In all, the Saccadometer is a far simpler solution to multi-site eye tracking, and at a fraction of the price it is the best choice for providing a systematic data collection environment.

The Saccadometer has been used successfully in an HD study (Ali et al., 2006) and other studies (Reddi & Carpenter, 2000). We used Saccadometers extensively since 2007 and site staff in the TRACK-HD study have found it reliable and easy to use. The data analysis software that comes included is sophisticated and efficient. Blinks and other erroneous eye movements are automatically excluded, and the onsite experimenter needs only to enter the Participant and Clinic ID before transmitting the recordings to the database. The data files produced by the Saccadometer are incredibly compact, < 500 kilobytes per test, which facilitates the transmission of data between Track-HD testing sites and the eCRF.

5.5.12. Observation schedule

The observation schedule for Track-HD is as follows and assessments are represented in detail in Table 10 below:

PROCEDURE / CRF form	Year 5 48Months[1]	Year 6 60 Months[1]	Year 7 72 Months[1]
General			
Informed Consent/In-Exclusion	X		
History (medical, disease, psychiatric)	X	X	X
Invariable Demographic Data	X		
Co-morbid Conditions	X	X	X
Concomitant Medication	X	X	X
Family History	X	X	X
CAG[2]	X		
Variable Demographic Data	X	X	X
End of Trial			X
Clinical History and Ratings			
UHDRS '99 TFC	X	X	X
UHDRS '99 Motor	X	X	X
Function	X	X	X
HD-specific Quality of Life	X		
Physical Activity Questionnaire (PAQ)	X		
Quality of Life Index	X		
Neuropsychiatric Assessment			
Hospital Anxiety/Depression Scale - HADS	X	X	X
Snaith Irritability Scale - SIS	X	X	X
Becks Depression Inventory Version II - BDI II	X	X	X
Frontal Systems Behaviour Inventory - FrSBe	X	X	X
Baltimore apathy/irritability scale (BAIS)	X	X	X
Biosample Collection			
Samples (DNA and LB lines at Baseline)	X		
ACD tubes for DNA & lymphoblastoid cell line	X	X	X
EDTA tube for peripheral blood mononuclear cells (PBMC)	X	X	
Plasma & PBMC from FicollGradient or Lymphopaqe/Histopaque	X	X	X
Buccal swab	X	X	X
PAXgene RNA tube(s) for the isolation of RNA for microarray/biomarker analysis.			X
Cognitive Assessments			
Core Cognitive Battery			
Symbol Digit Modalities Test	X	X	X
Stroop Word Test	X	X	X
IQ Covariate[2]	X		
Visual Array Comparison Task (SPOT-5)	X	X	X

PROCEDURE / CRF form	Year 5 48Months[1]	Year 6 60 Months[1]	Year 7 72 Months[1]
Map search	X	X	X
Shepard and Metzler 3D Mental Rotation	X	X	X
Circle Tracing Task	X	X	
Paced Tapping Task (3Hz)	X	X	X
Circle Tracing with Counting Backwards condition	X	X	X
Cancellation Task	X	X	X
Virtual maze task			X
Quantitative Motor Assessments			
Glossometry	X	X	
Manumotography	X	X	X
Force matching	X	X	X
Digitomotography	X	X	X
Dysdiadochomotography	X	X	X
Choreomotography	X	X	X
Pedomotography	X	X	X
Posturography test	X	X	X
Go-No-Go task			X
Imaging			
3T MRI scans (T1)	X	X	X
1 T2 Scan	X	X	X
Resting fMRI	X	X	X
MRS (Vancouver and Leiden only)	X	X	X
Task related fMRI	X	X	X
DWI	X	X	X
Amide CEST/MTR (Vancouver/Leiden)			X
NODDI (London/Paris)			X
Transcranial Magenetic Stimulation			
Hot spot determination	X	X	X
Cortical silent periods	X	X	X
Short latency afferent inhibition	X	X	
Sensory evoked potentials°	X	X	X
Long-latency reflexes	X	X	X
TMS- EEG (London & Vancouver)			X
Visual Evoked Potentials (Leiden)			X
Oculomotor Assessment			
Antisaccade Latency and Velocity	X	X	-

* Year 5 is equivalent to 48-month follow-up for TRACK-HD participants recruited in Year 1 of the study. For new premanifest participants recruited at Visit3 it is equivalent to 24-month follow-up and is the baseline visit for any new participants recruited at Visit 5. # new premanifest participants only. °Leiden will only collect SEPs and VEP data

Table 10 Detailed assessment plan

5.6. Study Schedule

The planned study start date was 1st March 2012 at all sites. New clinical staff were in post by 1st January 2012 with full training for all study site staff at UCL from 13-15th February 2012. Training for clinical sites was held via webinar in 2013 and face-to-face training is planned for 2014 .

5.6.1. Ethical/IRB approvals for study sites

All IRB votes will be filed locally for each site. IRB approval at each site is needed for transfer of data to Ulm and LONI and biosamples to Biorep in Milan or other central repositories. Full detailed participant and companion information sheets and consent forms (section 6) are incorporated into this protocol for the local site IRB applications. The clinical trial manager, investigator leads for each section and central coordination will answer any queries from the site PIs regarding their IRB applications.

5.6.2. Staff recruitment

Many existing staff are already place and have undergone annual training for TRACK-HD assessments. New staff were in place in sufficient time to join the existing staff for the training meeting which took place at UCL in February 2012. Minimal staff recruitment is anticipated for V7.

5.6.3. Staff training

The site neurologist and psychologist were in post 2 months before the first participant visit was scheduled. They trained for two months prior to the first participant visit in March 2012, learning the relevant assessments and practising on normal volunteers. The new site neurologist also observed and rated HD patients in a clinical setting, under supervision. The full 3-day intensive training session in mid-February 2012 at UCL included motor, oculomotor, cognitive, neuropsychiatric and clinical assessments for all staff. Imaging and Biosample collection training was also performed locally with the site PIs, clinical trial manager and local lab staff. The psychologist will be trained on site to administer the fMRI activation tasks. After training the staff were assessed for their competence on each of the batteries by the relevant expert or key investigator. Clinical site training was delivered by webinar in 2013 and face-to-face training is planned for 2014.

5.6.4. Participant recruitment

PM and control participants from TRACK-HD were invited to continue their participation at V5, V6 & V7. Where fewer than 30 participants in each group were available at each clinical site at V5, additional participants were recruited to meet this target, resulting in 33 new controls and 30 new PM participants to the study across sites. Previous experience has shown that booking participants approximately 2 months prior to assessment means that participants have good availability and allows booking of a steady rate of participants.

5.7. The TRACK-HD Day

Each participant will see a site neurologist and psychologist (P). Ideally, each assessment should take place at the same time of day in each centre. In practice this may not be possible owing to staff and scanner availability. Timings will be recorded as meta-data to allow analysis of the effect of time of day on outcomes. Timings allow for time to move between departments, and short comfort breaks. Participants are offered more refreshments at the beginning of the assessments as well, as required. A timetable for a participant visit is shown in Table 11.

Time	Assessment Type	Details	Duration
In advance of visit		Neurologist prepares consent form, information sheet, MRI checklist, DPA form Psychologist collates necessary testing forms, sets up relevant computer programs Neurologist ensures adequate sample tubes, equipment, questionnaires etc.	
09.30	General	UHDRS, medical history, co-morbidity, demographics, biosamples, buccal swab	30 min
10.30	Imaging	Positioning	2-10 min*
		1 x T1 scans (if imaging quality poor a repeat T1 may replace MRS)	12 min
		Resting fMRI	15 min
		Field map	2 min
		Amide CEST and MTR (Vancouver and Leiden)	15 min
		DWI (London and Paris)	10 min
		MRS (Leiden and Vancouver)	
		Break	10 min
		Training for activation fMRI tasks (outside scanner)	5-10 min
		Positioning	2 -10 min*
		Task-related fMRI: (Working memory – London and Paris; Motor task – Vancouver and Leiden)	15 min
		Field map	2 min
		NODDI (London and Paris)	25 min
		DWI (Vancovuer and Leiden only)	5 min
		1 x T2 scan	12 min
12:30	LUNCH		
13:30	Cognitive	SDMT Stroop IQ covariate Visual array task Map search Mental rotation Paced tapping (3Hz) Circle tracing with backward counting Cancellation task Virtual maze task	50 min
14:30	Quantitative Motor	Manumotography Force matching Digitomotography Dysdiadochomotography Choreomotography Pedomotography Posturography Go-No-Go task	45 min

15:30	Neuropsychiatric & Functional	HADS BDI FrSBE# BAIS#	20 min
16:15	TMS	Transcranial Magnetic Stimulation/Electromyography Hot spot determination, Cortical silent periods Somatosensory evoked potentials,Long-latency reflexes, Visual evoked potentials, TMS evoked potential,	45 min
Post visit		Member of research team: 1. enters remaining data onto electronic database 2. files questionnaires 3. copies data from laptop to central system 4. copies cognitive results for filing in participants' clinical notes 5. transfers scans once available on server 6. brief check for gross problems 7. enters participant scan details in scan log and into electronic database Local check on scan to ensure no clinical abnormalities via local neuroradiologist and then uploads scan to LONI for transfer to Imaging CRO.	

* Positioning in Philips scanners is achieved using scan shot from previous scan to ensure longitudinal consistency and can take anywhere from 2-10 minutes depending on the participant #If not already completed at home

Table 11 TRACK-HD visit timetable

5.8. Study Population

5.8.1. Population

The standard cohort for each Track-HD centre is 30 premanifest individuals and 30 control participants. Sites recruited additional participants as necessary at V5 to replace withdrawals from the existing TRACK-HD cohort.

5.8.2. Inclusion criteria

Written informed consent must be obtained from the participant, who must agree to all the assessments. In addition:

1. All participants should be able to tolerate MRI and sample donation
2. Participants will be either
   - **a. Control participant**
     - i. An existing control participant previously enrolled in TRACK-HD
     - ii. A newly recruited control participant who is either
       - Partner/spouse of a participant, not at risk of HD (note these participants will not have CAG repeat testing)
       - HD Normal repeat length sibling or HD normal repeat length control volunteer
   - **b. Premanifest gene carrier**
     - i. An existing premanifest gene carrier previously enrolled in TRACK-HD
     - ii. A newly recruited premanifest gene carrier with:
       - Positive genetic test with CAG repeat length ≥ 40 and
       - Burden of pathology score (CAG-35.5) × age >250 and

Control participants will be age and gender frequency matched to the PM group.

5.8.3. Exclusion criteria

1. Stage 1 (UHDRS diagnostic confidence score of 4) or greater at time of enrolment, unless previously enrolled as a premanifest participant in TRACK-HD
2. Less than 18 years of age
3. More than 65 years of age (unless previously enrolled in TRACK-HD)
4. Major psychiatric disorder at time of enrolment
5. Concomitant significant neurological disorder
6. Concomitant significant medical illness
7. Unsuitability for MRI, e.g. claustrophobia, metal implants
8. Unwillingness to donate blood
9. History of significant head injury
10. Predictable non–compliance by drug and/or alcohol abuse
11. Significant hand injuries that preclude either writing or rapid computerized responding
12. Participant in Predict-HD
13. Currently participating in a clinical drug trial

5.8.4. Recruitment and screening

New participants recruited were under the care of the HD clinical service at each centre. Prior to being invited to join Track-HD, participants were screened based on already known clinical information. This was especially important in allocating participants to the premanifest group, and in rigorously excluding participants in clinical stage 1 or beyond (although any participant who was originally enrolled as a PM participant or who progresses to Stage 1 during the course of this extension to the study will be allowed to continue participation if they wish to do so).

5.9. Personnel

The following staff will be required at each study site:

Staff	Generic duties
Site neurologist	Clinical, neuropsychiatric, motor and oculomotor assessments, take blood, process blood; clinical analysis; check scans locally and transfer scans, electronic data transfer; motor, clinical, genetic and imaging data analysis dependant on research interests
Research assistant	Recruitment and booking of participants, processing expenses for participants, associated record-keeping; electronic data transfer; data analysis
Psychology research assistant	Cognitive, quantitative motor, oculomotor and neuropsychiatric assessments, scoring of cognitive tasks; data transfer; cognitive analysis;

These duties are generic and it is anticipated that duties will be shared so two personnel are trained for the assessments in the event of sickness etc. The site PI will cover the site neurologist in the event of sickness.

5.10. Funding

Track-HD is sponsored by the CHDI Foundation Inc., (formerly CHDI/High Q Foundation Inc.) a private philanthropic foundation that was established in 2002 with the mission of bringing together academia, industry, governmental agencies, and other funding organizations in the search for Huntington's disease (HD) treatments.

5.11. Data storage and security

In Track-HD, collected data is stored in three different central databases:

- the phenotypical data in CTMS, hosted at the EHDN in Ulm,
- the imaging data in LONI at the UCLA in Los Angeles, and
- the bio samples with resp. data at Biorep in Milan

All data related to study participants will be stored only in pseudonymised manner. Identifying data such as names or contact details will never be stored electronically at any time. Data entered by investigators are only entered via a modern web browser into secure web interfaces (https/SSL). Any data transmission, from the investigator's web browser to one of the systems or among the systems, is done in a secure and encrypted manner (https/SSL). Data obtained during the course of the study can also be stored, on behalf of the Sponsor, at secure central databases other than those listed above.

The participant's pseudonym is created based on unchanging information (date of birth, birth name, place of birth and mother's maiden name) after the inclusion of the study participant. Technically the pseudonym - a nine digit number – is automatically computed by using a secure one-way well-accepted cryptographic algorithm (MD5 or SHA1). The pseudonym creation can only be done by a very limited group of persons (the site staff) and only via the CTMS web system. It is unique, duplicate-free and not reversible. For example the data "Christine Mustermann, Date of Birth: 13 April 1964, Place of Birth: Berlin, Birth name: Maier; Mother's maiden name: Schmidt" produces the pseudonym: 344-259-192.

All sites should have local ethical and data protection approval, particularly for the transfer of pseudonymised data overseas. Since the identifying data is never stored electronically, the investigator will store the original data and the pseudonym in the source documents (participant file) and in the investigator file.

For the protection of each database containing pseudonymised data against unauthorised access several precautions are in place to ensure integrity, confidentiality and security of the database. The servers are managed by full-time system administrators. All network traffic is encrypted via network hubs using SSL/TLS with a key length of 128 or more. Servers have been customized to run the bare minimum of network services in order to minimize potential 'back door' attacks, and are updated on a regular basis with the latest vendor recommended software fixes. In addition, other security software runs continuously minimizing other potential attacks. All accounts are password protected.

All phenotypical data will be stored in PostgreSQL, a relational database management system, which resides on a Linux Server running the Linux Operating Environment. The server resides inside a locked computer room that is physically accessible only by the authorized personal. This room is located in the central coordination suite of EHDN at Ulm that is also locked. Different keys are required for both the computer room and the suite. The computer room is temperature controlled. It is equipped with smoke/fire detection sensors. To ensure high system availability the server is equipped with dual power supplies, hot-swappable RAID 5 disk drives, and an APC uninterruptible power supply. Every 12 hours the system is backed up to a second, mirrored server in a similarly protected environment located at a physically distant (> 50 km) site. All electronic data are fully audit trail enabled so that all changes to the data can be monitored and/or recovered. The CTMS implements a permission-based security methodology that limits access to study data based on the particular study, user ID, and user roles using access control lists (ACL). Permissions are carefully maintained to allow only the required level of access to study data. The operating environment requires username/password authentication, and implements its own permissions structure based on ACLs. Files and directories are carefully set with only the required level of access. Users are required to change password on a regular basis. The password must have a

length of at least 8 characters including 2 special ones. Every precaution has been taken to assure that computer confidentiality is maintained.

The secure data capturing of the phenotypical data is summarized in the diagram below (Figure 14). The workflow for entering data with use to all distributed databases is shown in the diagram below (Figure 15).

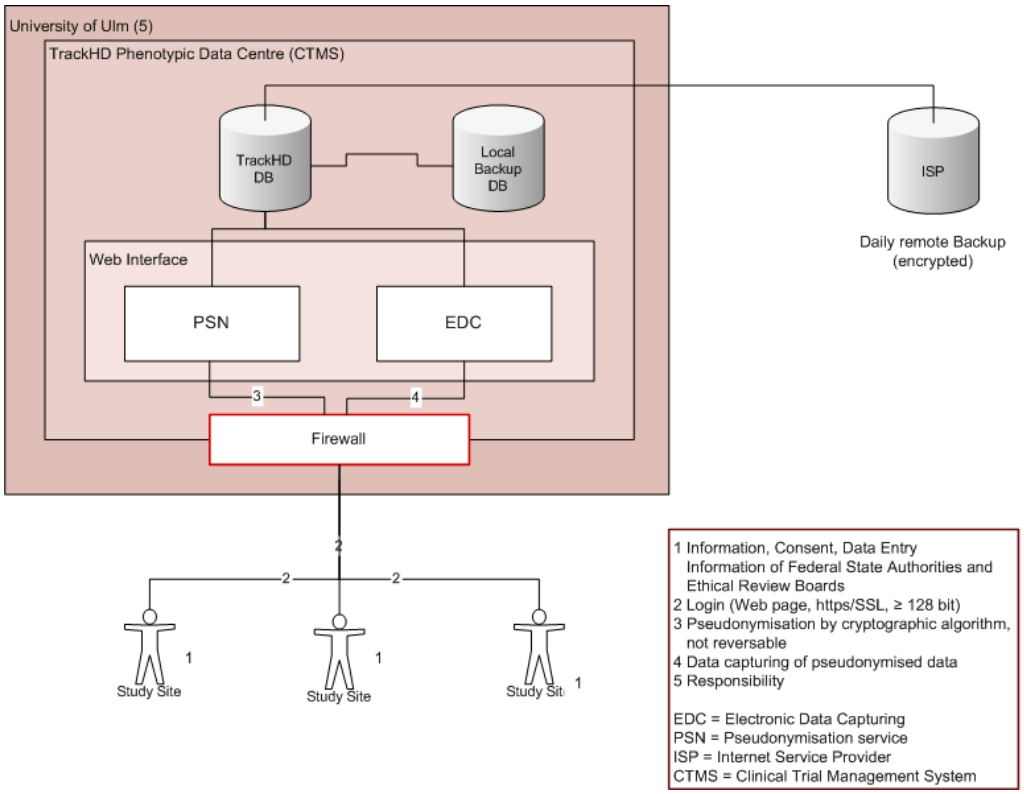


Figure 14 Secure capturing of phenotypical data in CTMS

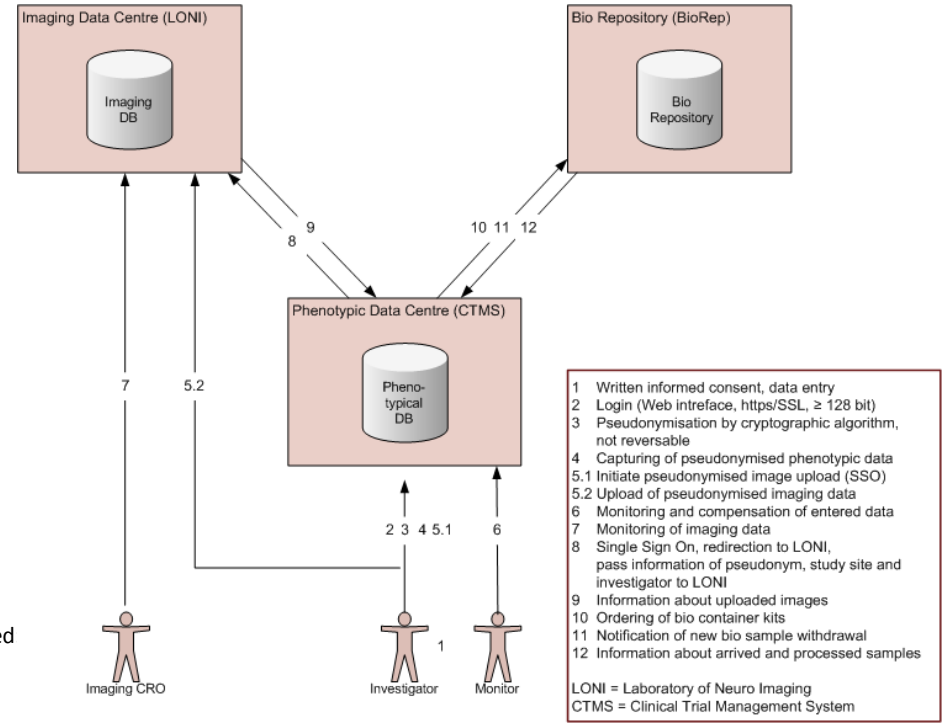


Figure 15 Data entry workflow and databases

5.12. Ethical considerations

5.12.1. IRB and R&D submission

IRB approval for the study and for international data transfer to Ulm and LONI and other central repositories was sought in each country along with local R&D approval. Central coordination works with site PIs to obtain ethical approval at each site.

The Track-HD protocol will be subject to amendments for clinical tests to be added or removed as new data becomes available during the study's progression. It is imperative at each study site that local IRB amendments for minor protocol alterations can be approved quickly.

5.12.2. Participant costs and expenses

Participants will incur no cost for participation in this study. Participants will receive no payment for participation in this study but will receive full compensation for their travel expenses and the cost of lunch during study visits. Expense refunds will be handled locally by each study centre. Where local ethics committees allow it, control participants may be offered an honorarium for participating.

5.12.3. Participant risk

Since Track-HD is an observational study, participants do not undergo specific risks by participating: therefore no medical insurance is provided unless the respective national law requires one.

Participants may experience anxiety while completing clinical, cognitive and neuropsychiatric assessments and MRI scans. Site staff should refer to the "Stopping the protocol" SOP developed to advise assessors when to withdraw a participant from a given task or tasks due to fatigue or anxiety. If as a result of a participant's response to any of the questionnaires, or if for any other reason the researcher develops concerns that the participant is at risk of harm to themself or to others, the risk assessment protocol will be followed.

Some people experience claustrophobia when having an MRI scan, but the research team will do whatever possible to help them relax before and during the scan.

There are additional potential risks associated with phlebotomy. A minor amount of pain inevitable accompanies phlebotomy. The collection of blood specimens may cause bruising at the site where blood is drawn. Fainting or feeling light-headed may occur during or shortly after having blood drawn. If a participant experiences this, the participant will be instructed to lie down immediately to avoid possible injuries. Localized clot formation and infections may occur, but this is very rare. Only experienced staff (e.g. site neurologists) will draw blood for this study. In order to ensure the confidentiality of donors contributing to the central repository, Biorep will never receive identifying data along with the biosamples sent for storage. Instead, Biorep will receive the biosamples from study sites with only the pseudonym as identifier.

In addition, despite best efforts, it is not humanly possible to exclude with 100% certainty a breach of confidentiality by unauthorized people obtaining access to information in medical files and records thus resulting in a loss of confidentiality. All reasonable safeguards to prevent such an occurrence will be undertaken. For instance, all data entered into the electronic database of Track-HD will be stored under a numerical pseudonym rather than name or other identifying data. At all times, only the local site investigators are aware of the identifying data associated with the pseudonym.

All users of the database outside the local study site will work exclusively with pseudonymised data. The database is secured as detailed in section 5.11.

5.12.4. Potential benefit

Participants will receive no immediate benefit from participation in this study. The only potential benefit is a better understanding of HD and the possibility that the information obtained in this study will lead to potential treatments and to plan future research studies of experimental drugs aimed at slowing disease progression or postponing the onset of HD.

5.12.5. Alternatives to participation

The only alternative to participation in this study is not to participate.

5.12.6. Withdrawal from participation

If a participant does not want to continue, the participant can leave the study at any time. Participants do not have to disclose their reasons for withdrawal of consent. On the participant's request, all information obtained so far will be anonymised (identifying data discarded). Similarly, on the participant's request, all biosamples collected and stored at the central Biorep repository may be destroyed. However, samples that have already been distributed for the purposes of HD research cannot be destroyed. Participants have to be aware that an 'End of Study form' must be completed by the investigator, detailing the reasons for withdrawal (e.g. marking "participant request").

Participants may be withdrawn from the study for the following reasons:
1. Failure to complete the required study procedures, regardless of reason.
2. The site investigator feels that it is in the best interest of the participant.

5.13. Quality assurance and quality control

5.13.1. Rationale

Quality assurance refers to the procedures put in place to ensure quality, whereas quality control refers to the evaluation of the effectiveness of those procedures. The key distinction is between preparing for quality in the study (quality assurance) and checking for quality of data collected (quality control).

The ultimate aim of Track-HD's QA/QC measures is to ensure that, to the maximum reasonable extent, data that are analysed and published are as true a reflection as possible of the neurobiological state of each participant. Robust measures will be required to ensure a reliable "chain of evidence" from the participant to the point of publication.

As far as possible, QA/QC will be centralised to ensure consistency between all sites and across time. Low tolerance for deviation from protocol and rapid feedback to sites and raters will be essential. The emphasis will be on central QA to ensure consistency. QA will be handled by EHDN-appointed data monitors with site visits to ensure that procedures are being followed, including checking on-site assessments, giving feedback to sites and ensuring up-to-date training and accreditation. Central checking of data for completeness and plausibility at the level of the data repository will also be needed. Sites will be evaluated at least annually.

5.13.2. Monitoring of database entries

To obtain optimal data quality and reach the highest standards of reliability, Track-HD will be monitored on the basis of the rules of ICH-GCP. After initiation of the respective study site, an independent monitor associated with EHDN will visit the centres in predefined intervals (following the enrolment of the first three participants – premanifest + control -, and thereafter every four weeks) to make sure that the centre complies with the Track-HD protocol and the principles of the Declaration of Helsinki.

The first visit (**initiation visit**) will be performed by the clinical trial manager/monitor to make sure that all study site personal involved with Track-HD are not only familiar with the protocol, the respective SOPs and the EDC methods used within Track-HD but intensively trained in the application of the so-called test batteries. At the initiation visit the Investigator's Study File (a binder with all study related documents, e.g. protocol, IRB approval and insurance certificates, distributed at the training meeting) will be updated and completed by signed CV, the protocol signatures, the financial agreements, copies of the training certificates and the quality certificates of the equipment employed in the study (Freezer, balance etc) – a short visit at the department of radiology may be necessary).

The quality of phenotypical data collected in Track-HD is ensured through three mechanisms:

- internal plausibility checks provided within the eCRFs
- online monitoring
- regular on-site monitoring visit for source data verification

The plausibility checks of the electronic data capture system will make sure that not only omissions and obviously erroneous entries were identified as such but it will also alert for unusual values and will be able to cross-check the contents of the single CRFs and across visits. Specifically, the in/exclusion criteria are compared with the basic information given e.g. in the participants' history or the report of the genetic laboratory. Incongruence's were highlighted and contradictions will lead to mandatory queries.

The online monitoring provides the feedback for the investigator/data entry personnel within 24 hours after the documentation. Problems, e.g. violations of the inclusion criteria will be discussed and –after consultation with the external experts – waivers will be given, if necessary. The online monitoring also makes sure that, in addition to the automatic reminder system, the time schedule of the visits and tests are followed.

Through the instrument of on-line monitoring the identity of the HD rater and data entry person can be followed assuring that only qualified raters or their designated deputies using with their unique passwords are entering data thus minimising errors in data entry.

The regular onsite monitoring is intended, as defined by ICH-GCP (5.18), to verify

- that the rights and well-being of the participants are protected
- the reported trial data are accurate, complete and verifiable from source documents
- the conduct of the trial is in compliance with the currently approved protocol,

GCP and the regulatory requirements.

In accordance with the international guidelines, the burden of onsite monitoring in Track-HD is to some extent reduced by the above mentioned e-tools. Nevertheless, on-site source data verification is still necessary.

100% verification of source data will be required for

- Identity (birth date, sex, pseudonym) of the participant
- Informed consent

- In/Exclusion criteria (as defined in 5.8)
- Concomitant medication at the time of inclusion

In Track-HD, the documentation of most test results will be performed electronically; source data are therefore available as part of the data base.

During the onsite monitoring, the monitor has also to verify that the facilities (including the department of radiology), the equipment and the staff are adequate to safely and properly conduct Track-HD by checking:

- The freezers used for storing biosamples (temperature logs have to be written)
- Test certificates of the equipment (MRI, Saccadometer)
- The successful transfer of videotapes, MRI images and bio samples to the external experts, the specialized CRO and Biorep

Furthermore, the completeness and up-to-dateness of the investigator's File has to be checked at each visit and the training of new staff has to be ensured.

5.13.3. QA/QC systems for clinical assessment

Quality assurance

UHDRS motor assessment is an important core assessment but also a major potential source of experimental variability. All clinical raters will be trained and assessed in UHDRS motor assessment according to the standards of the EHDN Motor working group including annually repeated video motor ratings on which a permanent record is kept (see video rating in Track-HD SOP documents). Data will be checked locally for missing or erroneous data. Central automated checking will highlight data anomalies which can be raised with the study site within 24 hours.

Quality Control

We recommend periodic direct observation of the clinical assessment (including the demographic and medical interview) by QC personnel appointed by central coordination, to ensure that the clinical battery is being administered by each rater in accordance with the SOP.

5.13.4. QA/QC systems for functional, QoL and neuropsychiatric assessment

Quality Assurance

BDI-II, HADS/SIS, BAIS and FrSBe data acquisition: BDI-II and HADS/SIS are given to the participant to fill out during the study visit and no special examiner training is required. All other questionnaires and companion versions are mailed with detailed written instructions in due/appropriate time prior to the assessment date. Instructions will emphasize the need to complete the questionnaire without outside help. A phone number will be provided if an individual has a question. A reminder phone call will be made the day prior to the appointment. If the participant fails to return the completed forms on or before the assessment date the forms will be completed during the assessment day If the companion fails to return the completed forms on or before the assessment date, an additional copy will be provided. Completed rating forms will be forwarded to Manchester for final scoring and quality control.Translation of all questionnaires will be undertaken according to the standard procedures employed in Track-HD.

Quality Control

Data will be monitored centrally for completeness and range of values. Any anomalies found in the data will be followed up by further training at the sites.

5.13.5. QA/QC systems for biosample collection

Quality Assurance

All personnel will be trained and observed in collecting and processing blood from volunteer participants to ensure protocol compliance.

All consumables (except for dry ice) will be provided by Biorep to ensure consistency between sites and minimise potential processing error. Tubes will be labelled and colour-coded to prevent confusion. On-site QC includes a visual check for plasma quality to prevent the inclusion of haemolysed samples.

To prevent sample misidentification, participant samples will be processed immediately on-site one at a time and stored in labelled, bar-coded tubes with immediate recording of identifying information. Biorep has in place robust QC measures to ensure continuing integrity of samples and metadata.

5.13.5.1. Quality Control

Biorep will analyse incoming samples for quality. Plasma will be analysed for haemoglobin contamination due to haemolysis using a HemoCue plasma Haemoglogin analyser. Creatine phosphokinase, lactate dehydrogenase and C-reactive protein levels will be measured spectrophotometrically as markers of plasma protein integrity. Periodically samples will also be assessed by Prof Elaine Holmes (Imperial College) using NMR analysis.

5.13.6. QA/QC systems for cognitive assessment

Quality Assurance

Training – Cognitive Examiners must be trained at a face to face, or webinar training session and certified by an approved cognitive trainer prior to running any participants. Prior to face to face training, the trainee should review the operating procedures manual.

During the training, the following occurs:

- Trainer instructs trainees on optimizing participant test performance
- Trainer instructs trainees on importance of and methods for standardized administration
- Trainer orients trainees to the test battery
- Trainer demonstrates test battery administration
- Trainees practice full test battery administration on each other with informal feedback from trainer
- Trainee administers full test battery to trainer
- Trainer provides written feedback to trainee and either
    - Certifies the trainee to test participants with the caveat that the initial administration must be videotaped and sent in for evaluation, OR
    - Requires trainee to do additional work, including
        - practice at the home site to address shortcomings highlighted in the written training session feedback
        - video recording of a practice administration of the test battery
        - Certification based on the trainer's rating of the videotaped practice administration

This training and certification process is executed each time the test battery is changed in any way that alters the test battery administration. In addition to initial certification, cognitive examiners may be required to submit a videotape of at least one test administration per year e.g. new rater. This tape is reviewed by a cognitive trainer and written feedback is provided to the examiner. In some cases, corrective action will be required and the trainer may request a second videotape of a battery administration that demonstrates the cognitive examiner has addressed the trainer's concerns.

Equipment - Standard operating procedures require calibration all relevant hardware before each administration. The touch surface of the tablet PC needs to be calibrated to increase precision of spatial measurements. Document scanners require setup to be done consistently to maintain quality of scanning from site to site. For audio recordings, the hardware and the software need to be setup properly to ensure a useful recording in every circumstance.

Quality Control

All paper/pencil tasks will be scored by the examiner at the home site and then rescored by a certified scorer. Discrepancies will require a third scoring. A cognitive trainer will provide feedback to the examiners who produce score that are consistently discrepant from those of the secondary and tertiary scorers.

Electronic Data Collection Quality Control – Data generated by electronic means must be collected, recorded and reported, as consistently, accurately and precisely as possible.

- Collection refers to the administration of the tasks by cognitive examiners.
    - Collection must be done in a consistent and reliable way such that the data are not affected by individualized administration practices.
    - Cognitive examiners will be trained on proper administration of the tasks to reduce collection errors as much as possible.
- Recording refers to the dependent variables of the computerized task, such as response time, or inter-tap-interval variance.
    - As the tasks are written, each program undergoes a strict QA process to verify that the program is measuring what it claims to measure within the required specifications and this reduces recording errors. All data measured by the tasks must be as accurate and precise as possible for all hardware.
- Reporting refers to the association of data with the correct administration in the database.
    - Each participant number from each site for each visit must be correctly linked to the proper and complete set of administration data for the tasks. Time and date stamps are taken for each task administration, and additionally participant number, site and visit numbers are all recorded for each battery administration. This information, along with notes about the administration from the cognitive examiner, are used to link the electronically collected data to the official list of administrations. This reduces reporting errors.

5.13.7. QA/QC systems for quantified motor assessment

Quality Assurance

Validation of the hardware and software – All equipment will be assembled and tested at the laboratory of Dr. Ralf Reilmann in Muenster and shipped to the study sites. After the equipment is installed at individual sites, 1-2 mock participants will be assessed using the complete protocol. These data will then be sent to Dr. Reilmann for inspection to ensure that the equipment has been correctly installed at the sites, is working, and is producing usable and accurate data.

Calibration of equipment

The force transducer, force-plate, and Polhemus 3D-position-sensor used for the assessments are industrial standard pre-calibrated systems at factory delivery and do not require recalibration. They are used in robotics in industrial processing and calibration is assured by electronic circuits including adjustments for temperature.

Training, certification, and re-certification of examiners

All examiners must be trained in a face-to-face setting by a certified trainer. As part of the training, the trainer will observe the examiner administering the tasks, provide written feedback to the examiner regarding the administration of each of the tasks, and, when the examiner is considered to have the skills for standard task administration, the trainer will add the examiner to the list of certified Track-HD quantitative motor examiners. Re-certification is required on an annual basis and can be accomplished either in person or by videotapes available via the EHDN motor training website (again, reviewed by a certified trainer for approval of recertification). In addition, videotaped sample assessments of all paradigms used will be made available on the EHDN website.

Quality Control

Data Quality will be monitored by Dr. Reilmann and colleagues in Muenster by conducting intensive review of the data from 1-2 mock participants at each site before the start of the study, and then by reviewing data from participants from all sites within four weeks of delivery. Specifically, this data review will include an inspection of the data for completeness, and range to check for range violations. Any anomalies found in the data will be followed up by either further training at the sites or checking and repair of the equipment as necessary.

5.13.8. QA/QC systems for imaging assessment

Quality Assurance

The Imaging CRO will put in place QA procedures to ensure that scans obtained at different times in different centres using different hardware/software combinations are as comparable as possible. This will include standardisation of scanning protocols and inter-scanner comparisons using phantoms or human volunteers. The Image Acquisition preparatory phase work for Track-HD was led by Dr Hans Johnson, and Dr Stefan Klöppel and IXICO Ltd for Visits 5 and 6, and Professor Xavier Golay for visit 7.

Quality Control

The local site PI is responsible for ensuring clinical assessment of the scans by a local radiologist within five working days of the scan and will be responsible for clinical follow-up if any abnormalities detected.

Imaging QC and feedback to sites will ultimately be carried out centrally by the Imaging CRO. This is detailed in the Imaging Track-HD SOP document.

5.13.9. QA/QC systems Transcranial Magnetic Stimulation (TMS)

Quality Assurance

TMS stimulators, associated equipment and settings will be standardised between sites. All sites will use MagStim 200 stimulators, or newer, and figure-of-eight coils with 90mm outer winding diameter with posterior to anterior current flow. Data acquisition will follow a standard protocol provided by Dr Michael Orth on behalf of the TMS advisory group. Other QA procedures will include a standard operating procedure for TMS and a check-list to ensure that equipment and software settings, and data recording, follow the TMS protocol. Performance of each TMS lab and inter-investigator variability will be assessed by Dr Michael Orth with at least one human volunteer being tested at all sites before data collection begins. All preparatory work for TMS is being led by Dr Michael Orth on behalf of the TRACK-HD TMS advisory committee.

Quality Control

All TMS data uploaded onto the TRACK-HD data repository will be monitored within a week by Dr Michael Orth and his team. Data will be examined for completeness, for any potential data entry errors and for potential

equipment problems. Any query will be fed back to the site to ensure acquisition of data is of the best possible quality.

5.13.10. QA/QC systems for oculomotor assessment

Quality Assurance

All testing sites will be provided with a Saccadometer and experimenters will be trained and assessed in the administration of the oculomotor test battery. All oculomotor examiners must be trained at a face to face training session, and certified by an approved oculomotor trainer prior to running with any participants. Prior to face to face training, the trainee should review the operating procedures manual. During the face to face training:

1. Trainer instructs trainees on optimizing participant test performance
2. Trainer instructs trainees on importance of and methods for standardized administration
3. Trainer orients trainees to the test battery.
4. Trainer demonstrates test battery administration
5. Trainees practice full test battery administration on each other with feeback from the trainer
6. Trainer certifies the trainee to test participants with the caveat that the initial administration must be videotaped and sent for evaluation

The major elements of the oculomotor test battery are easily visible at a distance and the quality of the data can be assessed via the CTMS. A physical trip a clinical site will be undertaken if significant problems are revealed in the video recordings, or if sites experience systematic problems.

Quality Control

Once the oculomotor test battery is running as planned, QC becomes focussed on data management. The primary QC will be weekly checks via the CTMS to ensure that the data is being stored correctly. This will be assessed in the following ways:

1. Light levels will be regularly monitored in the testing environment and reported on a monthly basis to ensure they remain within the specified boundaries for the LCSLC
2. The number of data files added to the database per week will be compared with the expected amount.
3. Data files for that week will be downloaded and checked to ensure that they can be opened with the program LatencyMeter (Ober Consulting), that the Clinic, Experimenter and Participant IDs are all entered correctly, and that the data itself fits the expected profile.

5.14. General statistical principles for TRACK-HD

5.14.1. Underlying assumptions: relationship between TRACK-HD study design & goals

To quote section 4.8: *The primary aim of the study - to provide essential methodological advances needed for optimizing neuroprotective clinical trials in HD – has already yielded a range of quantitiative outcome measures suitable for use in potential clinical trials in the early stages of the disease. Track-HD will now focus on individual and combined clinical and biological outcome measures for tracking progression in the premanifest stages of the disease, in which despite significant progressive regional and whole-brain atrophy and clear cross-sectional deficits compared with controls, there has so far been limited detectable cognitive or motor decline (Tabrizi et al. 2011).*

A number of assumptions are implicit in the marriage of this objective to the study design of Track-HD. We here attempt to make these assumptions explicit with the goal of clearly linking the planned data analysis to the study objective.

Longitudinal changes in the outcome measures (candidate markers) are the primary objective of study.

For the premanifest group, naturalistic observation of longitudinal change mimics the course expected of future clinical trial participants receiving either a placebo or a completely ineffective treatment. This assumption seems logical such that it can be made with some confidence. (However, it does not allow for the possibility of true placebo effects in future trials.)

For the non-expanded control group, longitudinal change mimics the course that would be expected in a completely effective treatment of HD under the additional assumption that the characteristic in question is a valid surrogate outcome. This assumption is clearly less certain. For example, it is conceivable that a completely effective treatment could either lead to reversal of previous HD-induced change or fail to immediately arrest the "momentum" built into longitudinal changes that are occurring at the time effective treatment is introduced. Nonetheless, for study planning, sample size estimation, etc. it seems difficult to preferentially choose and quantify either of these alternatives.

Change that is observed in the control group will reflect a number of other phenomena associated with longitudinal observation: (a) imperfect measurement reliability (b) short-term within-participant fluctuation in the true phenomenon being measured—which, along with (a) leads to regression towards the mean)—(c) background long-term causative effects such as aging, and (d) practice effects. These phenomena are also assumed present in the CAG-expanded groups, along with the potential longitudinal effect of evolving HD.

Given the prior assumptions, the differences in longitudinal marker change between the premanifest group and the control group are estimates of the maximum possible surrogate treatment effect achievable via that marker in a clinical trial. Since hypothesized treatment effects will typically be considerably less than 100%, these longitudinal differences will need to be notable if the marker is to pass this criterion for candidate surrogacy.

None of the above is meant to distract from the other considerable hurdles that a marker must overcome in order to reach surrogate-outcome status.

Even markers that do not qualify as surrogate outcomes can be quite valuable as risk stratifiers. It can be shown that various strategies related to stratification in recruitment and/or analysis lead to substantial improvement in the power of clinical trials. Thus we do not underestimate the importance of a secondary goal of the study, cross-sectional comparison of groups on the basis of candidate markers.

5.14.2. General principles of statistical analysis

We will strive to always distinguish between a priori and post hoc hypotheses, with a priori hypotheses defined as comprehensively as possible before data analysis begins. An honest distinction is critical to true progress and optimal allocation of future resources in HD research, since the nominal statistical significance for a post-hoc hypothesis, suggested only by preliminary exploration of the data, is typically inflated in ways that are often impossible to quantify (Good & Hardin, 2006).

We will rely on mixed effect linear models to assess most longitudinal change (Verbeke & Molenberghs, 2000; Littell et al., 2006). Fixed effects will typically include group (diagnosed, premanifest, or control), age, gender, time-since-first-measurement ("time", which will be 0 at baseline), and interactions between group and time. Substantial interactions between group and time will usually be the main parameters of interest. Other fixed covariates will be determined by anticipated confounders for the specific outcomes. For example, most psychometric tests require adjustment for years of education.

Depending on the year of enrolment and study visit at which an assessment was introduced for the first time, the number of existing measurements for each TRACK-HD assessment will range from 2 to 4 measurements over 1 to 3 years. Additional data collected for these assessments will contribute to the overall ongoing longitudinal analyses. For new assessments, e.g. fRMI, and TMS, and/or new participants there will be 3 measurements over 2 years; and Viritual Maze task, Go-No-Go task, VEPs, TMS-EEG, CEST, NODDI and MTR there will be cross-sectional data only.

Our primary hypotheses will treat time as a linear effect unless there is a compelling a priori reason to use varying degrees of freedom in estimating separate effects for specific participants and/or assessments. Secondary analyses will typically explore whether longitudinal change is related to estimated prognosis within the premanifest group (based on CAG and age). Random effects will always include a within-participant term. Our sample size will be such that we will generally model unstructured within-participant covariance over visits. Other candidate random effects will include variance components for study site and examiner, as appropriate.

Certain longitudinal outcomes will not be amendable to the methods described above in 2. Categorical outcomes or counts will instead be modelled using mixed generalized linear models (e.g. logistic, Poisson), with other considerations identical to those above (Agresti, 2002; Littell et al., 2006). Conversions of status (i.e., diagnosis) will be analyzed by survival analysis. Multivariate-outcome imaging data present special longitudinal challenges. Changes over a single time interval can usually be approached using methods designed primarily for cross-sectional analysis. (The one set of changes is the single "cross-sectional" measurement.) In other instances, the multivariate data are reducible to one or a few summary statistics that can be handled by standard longitudinal methods. These limited generalizations notwithstanding, analysis plans for the imaging component will be described separately below.

Cross-sectional analyses will typically be by linear model with the fixed effects described in 2 above as the predictor variables. Scientific interest will generally focus on group effects between controls and premanifest.

Longitudinal studies inevitably entail loss to follow-up. We will monitor drop-out rates and, prior to final analyses, we will investigate whether probability of drop out is predictable on the basis of information known about the participants, including research data acquired at previous visits. If there is notable drop-out, that appears unpredictable ("completely at random" in statistical parlance), and there is no basis for suspecting that it is related to outcomes of interest, then we will proceed with the previously described methods without modification. For our analyses using mixed modelling theory, we will proceed if the data missingness appears predictable based on known data regarding the participants ("missing at random" but not "missing completely at random"), as these methods handle such missing data appropriately. In other analyses with substantial, plausibly "missing-at-random" data, we will employ multiple imputation methods to assess the effect of such drop-out on our analyses. If there is substantial drop-out and strong reason to think that it is not missing at random, then our analyses will be subject to sensitivity analyses across a range of assumptions regarding the relationship between drop-out and outcomes. All principles and procedures mentioned in this paragraph are reviewed in greater detail by Verbeke and Molenberghs (2000) and Molenberghs and Kenward (2007).

5.15. Data analysis

5.15.1. Key questions for TRACK-HD across assessment domains

1. A key question for Track-HD was "What individual or composite measure within each assessment domain shows the greatest change (largest effect sizes) over a one year time period?" Based on previous results from TRACK-HD, it is now possible to recommend a range of objective, quantifiable outcome measures for clinical trials in early stage HD participants over a realistic timeframe and with practical sample sizes (Tabrizi et al. 2011, Tabrizi et al under review Lancet Neurology). However, this question remains for potential disease modifying treatments in premanifest HD.

2. For those measures with the largest longitudinal effect sizes, what sample sizes are needed over what intervals to detect disease modifying effects?

3. How do domain specific effect sizes per interval compare and how might data be combined across domains to reduce redundancy, enhance the variance for which we can account, and ultimately increase efficiency of clinical trials?

Analytical comments:

a) Within the bounds set by recruitment guidelines, participants in Track-HD are at arbitrary points in the HD development trajectory. Therefore, the annual slope estimated by ongoing modelling longitudinal change provides an estimate, statistical significance, and implicit power information regarding rates of one year change. However, it must be recognized that repeated measurement effects such as learning effects may have an impact on some of the measurements. This is known to be an issue for many cognitive measures. (See 5.15.7 below.) Therefore, we will also conduct secondary analyses looking specifically at observed effects over only the first year of any new assessment in order to test the consistency of inference from future observations. Longitudinal analysis will proceed along guidelines outlined in 5.14**Error! Reference source not found.** even in this case, with the results obviously directly applicable to the one-year hypothesis.

b) The general strategy outlined in 5.14 will provide estimates and standard errors of longitudinal change, variance, and within-participant covariance for each outcome. These will be readily combinable with hypothesized treatment effects to estimate sample size/durations, with confidence intervals, for future clinical trials. The approach is the same that Langbehn used (section 5.16.2) to advise on sample size for Track-HD.

c) The relevant effect sizes for outcome comparisons are defined by change per unit of time divided by the mean within-participant residual standard deviations. This information is a product of the general analytic approach described above. The combination of longitudinal outcomes is subject to strong caveats discussed above in 5.14. Bearing these limitations in mind, we will attempt to maximize longitudinal variance explained via principal component analysis of the set of standardized change scores, adjusted for background covariates, and via canonical correlation analysis of these change scores and separate, well-validated prognostic variables (at a minimum, with CAG-age estimated probability of onset).

5.15.2. Secondary questions for TRACK-HD

1. What is the earliest point during the premanifest disease process at which longitudinal effect sizes for any measure become sufficient to useful in a clinical trial?

2. What are the key demographic and clinical variables that must be taken into account to maximize measure sensitivity, such as age, education level, IQ, sex, etc.?

Analytical comments:

a) It will be impossible to know the true time from diagnosis for premanifest participants who do not show substantial signs of developing illness during the course of the study. Therefore, estimates of earliest detectable longitudinal changes will rely partly on comparisons to separate, already validated prognostic indicators. For example, we will test relationships between longitudinal change and CAG-age based estimated time until diagnosis and striatal volumes.

b) As discussed in 5.14, potential demographic and clinical confounders, as identified by the investigators, will be controlled as statistical covariates in the longitudinal and other analytical models.

5.15.3. Functional and QoL data analysis

Data will include a variety of summary scores from ratings scales (clinician rated and self-report).

Ancillary questions and analysis plans specific to functional and QOL assessments:

1. What are the relationships between functional and quality of life assessments and other phenotypic and imaging measures described above?

2. These measurements are of special interest, as they attempt to measure a quality that has some face validity as an outcome for clinical trials. We analyzed longitudinal change in functional assessments as a function of group status and validated prognostic indicators using the methods outlined in 5.14 and 5.15.1. If measurable changes can be shown for these measures, potential associations with other Track-HD outcomes will be investigated using adjusted correlation analysis and canonical correlation methods similar to those discussed above in 5.15.1.c.

5.15.4. Neuropsychiatric data analysis

Data will include summary values from individual neuropsychiatric ratings scales (self report and clinician rated).

Ancillary questions and analysis plans specific to neuropsychiatric data

1. Are individual or composite neuropsychiatric measures best used as covariates or outcome measures in clinical trials?

2. What neuropsychiatric characteristics alter the relationships between estimated proximity to onset and variables from other domains, i.e., cognitive, quantitative motor?

3. Longitudinal analyses of neuropsychiatric variables as outcomes will be by principles set out in 5.14 and 5.15.1. Since (potentially fluctuating) psychiatric status may confound other measurements, especially certain aspects of cognitive and physical examination, selected neuropsychiatric measures such as depression scores and others specified by the investigators will be tested as potential confounders using methods addressed in 5.14 and 5.15.2.

5.15.5. Biomarkers in plasma data analysis

Proteomic, neuroinflammatory, transcriptomic, and metabolic markers identified from our current ongoing biomarker research initiatives will be further validated using the Track-HD plasma and RNA samples. Specific *a priori* candidates will be analyzed using the general longitudinal approach described in 5.14. Any additional screening for new candidate markers will be subject to multiple comparison corrections (e.g. false discovery rate) in assessing the probable statistical and scientific significance of the results. Changes in each of the laboratory biomarkers identified will be correlated with the clinical and imaging phenotypic data using multi-variate assessments.

5.15.6. Genetic modifier data analysis studies

In collaboration with the EHDN Registry, the EHDN Genetic Modifiers working group, the HSG COHORT study and Professor James Gusella (Harvard) we will use lymphoblastoid cell line DNA together with clinical and family history data for identification of genetic modifiers of age of onset and the different clinical phenotypic presentations of HD. Identification of genes that modify the pathogenic process in HD offers a direct route to validate targets for development of HD experimental therapeutics. Track-HD will provide a wide range of HD-associated phenotypes by which to identify modifier genes. Initially, the phenotypes available will be

derived from clinical assessments (UHDRS), but the collection of biological samples will also permit the study of additional phenotypes at the levels of RNA, protein, metabolites and cultured cells. The combination of phenotypic and genotypic information will permit analysis of relationships between individual polymorphisms and genes and the effect they have on modifying the phenotypic presentation, rate of progression and response to treatment of HD using genetic linkage and genome-wide association strategies.

5.15.7. Cognitive data analysis

Data will include summary variables from individual cognitive tasks, generally indicating either response times and number of correct items. In addition, we will use factor analyses (or other means of creating composite scores from multiple variables) and examine factor scores as dependent variables.

Ancillary questions and analysis plans specific to cognitive data

1. What are the key demographic and clinical variables, and the practice effects, that must be taken into account to maximize the sensitivity of the cognitive data, such as age, education level, IQ, sex, etc.?

2. For individual tests, how do practice effects need to be taken into account to maximize utility in the context of a clinical trial?

3. Longitudinal analyses will be by principles set out in 5.14 and 5.15.1. Potential confounding is addressed in 5.15.2. The use of controls to study practice effects is addressed above in 5.14.

5.15.8. Quantitative motor data analysis

Data will include summary variables from individual motor tasks, generally indicating either response times and number of correct items. In addition, we will use factor analyses (or other means of creating composite scores from multiple variables) and examine factor scores as dependent variables.

Ancillary questions and analysis plans specific to quantitative motor analysis:

1. What are the key demographic and clinical variables, and the practice effects, that must be taken into account to maximize the sensitivity of the quantified motor data, such as age, education level, IQ, sex, etc.?

2. For individual tests, how do practice effects need to be taken into account to maximize utility in the context of a clinical trial?

3. Longitudinal analyses will be by principles set out in 5.14 and 5.15.1. Potential confounding is addressed in 5.15.2. The use of controls to study practice effects is addressed above in 5.14.

5.15.9. Imaging data analysis

T1- and T2- weighted images

Measures will include: VBM (Ashburner & Friston, 2000); BSI-derived rates for whole brain and caudate, atrophy and ventricular expansion (Freeborough & Fox, 1997); volumes from automated segmentation of striatal structures (Magnotta et al. 2002);. Cross-comparisons between each of these image-analysis methods will be undertaken.

Diffusion weighted imaging and Neurite Orientation and Dispersion Diffusion Imaging (NODDI)

We plan to perform well established compound measures of white matter integrity (e.g. fractional anisotropy, mean diffusivity, axial and radial diffusion, etc.). In addition, we plan to employ probabilistic fibre tracking to

determine white matter connection in the brain. We will also investigate neurite orientation and dispersion using the novel NODDI image acquisition.

Resting state fMRI analyses

We plan to perform seed region analyses and well as principal component analyses.

Activation fMRI analyses

fMRI activation data will be processed by correlating the onset of a movement with the changes in blood oxygenation caused by it. In addition, we plan to perform analyses of functional and effective connectivity. The analysis of fMRI data is well established and uses SPM .

Amide CEST and MTR

Levels of soluble and aggregated mutant HTT will be assessed using CEST and MTR image acquisitions respectively.

Each imaging measure will be assessed individually by determining the sample sizes needed in order to detect (with sufficient power) disease-modifying effects of various magnitudes. This will allow imaging measures to be compared, both with each other and with the other clinical phenotypic parameters. Secondary analyses will look at the associations between imaging measures and other measures (clinical assessments, CAG length, CAG-age prognosis, cognitive profile, neuropsychiatric scores, motor assessment, functional measures, wet biomarker profiling etc.). Note that, in addition to the usual CAG-age prognosis, CAG length is of separate interest for imaging and other biological markers, as modification of the huntingtin protein's biological effect is a function of CAG length.

The additional aim of the fMRI data is to look into disease heterogeneity and compensatory mechanisms that are in place at the pre-symptomatic stage of the disease. Network analyses, using e.g. Dynamic Causal Modelling [Friston et al, 2003) should help to identify the underlying networks.

Finally (as with all measures) models will be fitted to see whether a combination of measures (either within a modality or across modalities) can reduce the sample sizes needed to detect a disease-modifying effect.

5.15.10. Transcranial magnetic stimulation data analysis

Measures of the core protocol will include: somatosensory evoked potentials (SEP), long-loop reflexes, resting and active motor thresholds (RMT and AMT), input/output (I/O) curves, short afferent inhibition (SAI; 2 inhibitory interstimulus intervals (ISI), 2 facilitatory ISIs). Optional components consist of 5Hz paired associative stimulation (PAS) and EEG recording with individual TMS pulses

The core protocol will result in the following metrics:

MEP: latency (1 value)

SEP: latencies (N20) (1 value), amplitude/area under the curve of N20/P45 (1 value)

Long-loop reflexes (LLR): latencies (1 value for LLRI and LLRII), area under curve LLRII (1 value)

Cortical Relay Time (LLRII = MEP lat + SEP N20 + CRT)

RMT, AMT: 1 value each (%stimulator output)

I/O curve: slope from MEP size at three different stimulation intensities (110, 130, 150%RMT)

SAI: one value (% unconditioned) for inhibitory ISIs, one value (% unconditioned) for facilitatory ISIs

Each TMS measure will be assessed individually. This will allow TMS measures to be compared with each other and with the other clinical phenotypic parameters. Secondary analyses will look at the associations between TMS measures and other measures (clinical assessments, CAG length, CAG-age prognosis, cognitive profile, neuropsychiatric scores, oculomotor measures, motor assessment, functional measures, wet biomarker profiling etc.).

5.15.11. Oculomotor data analysis

The oculomotor data will be preprocessed from the raw data by the Kennard group to extract the following variables:

1. The median latency of correct antisaccades (ms)
2. Antisaccade Error Rate (%)
3. Peak saccade velocity (deg/s)
4. Saccade velocity function (the slope of velocity/amplitude for correct antisaccades)
5. Saccade latency variability for pooled correct and incorrect antisaccades (quantified as the standard deviation of the mean latency, and as the slope of the the saccadic latency "LATER plot" (Reddi & Carpenter, 2000)

These measures are all established standard variables in the oculomotor literature. Statistical analyses will be based on methods and principles generally outlined in 5.14 and 5.15.1 Post hoc approaches will follow guidelines discussed in 5.15.2.

5.16. Sample size considerations

While sample size estimates can be calculated from the literature for individual measures for desired power, significance and effect sizes, this exercise can only be performed reliably over the interval originally studied. Extrapolated sample size estimates for other observation intervals or longer follow-up periods may be notably more inaccurate. Moreover, one of our aims is to generate multivariate, multimodal data with high temporal resolution. Until the desired measures have been made simultaneously, calculations cannot be performed to determine the required sample size for reliably identifying the optimum multimodal battery of measures in premanifest and early HD, or calculating the cohort size required for such a battery to be able to detect the effect of a disease modifying intervention.

Sample size estimates for TRACK-HD visits 5, 6 and 7 are based on the number of eligible PM and control participants available at the time of the calculation, which is approximately 123 PM and 102 controls. For potential longitudinal follow-up we assume a 10% loss in each of the groups, yielding 110 CAG-expanded and 92 controls. However, additional participants will be recruited in each group as specified above.

As of visit 4, few convincing longitudinal differences between PM participants and controls have been detectable. For measures continued since the study's inception, the additional 12 to 36 months of follow-up gained by visits 5, 6 and 7 will provide substantially greater power to detect meaningful longitudinal separation of the disease path. The precise increase in power depends on extrapolation of several assumptions about how within-participant measures continue to correlate over time. The nature of this continuing correlation may vary, depending on the measure, and is difficult to predict on the basis of the currently available observations. However, within a realistic range of continuing assumptions, visit 5 follow-up should allow us equivalent power to detect changes that are between 75% and 89% the effect detectable after visit 4 of the study of the study. At

visit 6, when total follow-up is 60 months, we will be able to detect effects between 58% and 80% of those detectable at 36 months of follow-up.

Based on published cross-sectional TMS data in premanifest HD, this proposed sample size would yield >99% power to detect equivalent published standardized effect size differences, even with a type 1 error rate of 0.1%. Thus, with a considerable margin of error, TRACK-HD has adequate power to detect cross-sectional TMS effects of the magnitude previously reported; there are no known longitudinal TMS studies on which to base sample size calculations.

For fMRI, cross-sectional effect sizes reported in the literature are between 1.0 and 1.5 for fMRI in early HD subjects but again there is a lack of longitudinal data. For visit 7, even splitting the different fMRI tasks across the two scanner types and thereby reducing the sample by a half, we still retain 80% power to detect cross-sectional effect sizes of 0.525. This allows a substantial safety margin for cross-sectional power, including allowing for publication bias which might reduce the reporting of false negatives and possibly smaller true effects in a predominantly preHD sample. Therefore, based on the literature reviewed, after making a range of assumptions about measurement reliability and the time course for the evolution of HD effects, we have judged it plausible that the proposed sample size would yield adequate power to detect longitudinal changes over 36 months of observation in visits 5, 6 and 7.

The novel amide CEST and NODDI imaging techniques have never been applied in Huntington's disease to date. Although cross-sectional and longitudinal data from a previous small single site MTR study did not provide conclusive information regarding sensitivity to premanifest disease (van den Bogaard et al, 2012 ; van den Bogaard et al 2013), we are employing this technique in unique combination with amide CEST imaging. MTR is acquired simultaneously during amide CEST imaging, requiring no additional acquisition time. We anticipate that this combined sequence will provide complementary information on both aggregated and mutant huntingtin buildup. These novel imaging techniques are necessarily exploratory and consequently we do not have data to estimate appropriate sample sizes.

5.17. Modifications of the protocol

Any modification of the protocol which may have an impact on the conduct of the study, including study objectives, study design, participant population, study procedures or significant administrative aspects, will require a formal amendment to the protocol. The organising group and the 4 local IRBs will agree upon such amendments during the course of the study.

5.18. Administrative responsibilities

The investigator must follow national guidelines for good clinical practice and is responsible for the safety and the medical care of the participant.

A contract will be issued to regulate the obligations and rights of the investigator and the responsibilities of the Track-HD trial coordination including the sponsors; the contract will be signed between authorised representatives of the respective institutions with which the investigators are affiliated and Track-HD trial coordination.

The Steering committee of Track-HD is responsible for overseeing the monitoring and data quality control procedures. EHDN Central Coordination is responsible for the execution of monitoring according to the principles of Good Clinical Practice and for supplying trained personal for this purpose.

The Steering committee of Track-HD is responsible for promoting inclusion into Track-HD and for developing the protocol of the Track-HD study.

5.19. Publications and data access

5.19.1. Data analysis by Track-HD investigators

The Track-HD outcome data will be authored and published by the Track-HD investigators, and all publications will be finally ratified by the Steering committee and biostatisticians.

5.19.2. Data access and data sharing

Sharing data and other biomedical research resources (including biological specimens) reinforces open scientific inquiry, encourages diversity of analysis and opinion, promotes new research, makes possible the testing of new or alternative hypotheses and methods of analysis, supports studies on data collection methods and measurement, facilitates the education of new scientists, enables the exploration of topics not envisioned by the initial investigators, and permits the creation of new datasets when data from multiple sources are combined.

There will be an Access to Data Policy that follows the guidelines of the EHDN (see full details at https://www.euro-hd.net/html/network/project/constitution/docs).. The members of the TRACK-HD Steering Committee will serve as the Track-HD SRB. Researchers interested in obtaining data for further analysis will submit brief outlines of their HD related research project to the Track-HD Scientific Review Board (SRB). The SRB will assess whether the proposed project falls within the subject area to which participants gave their informed consent (i.e. studies establish and validate biological markers for HD) and whether the proposal is ethically and scientifically sound. Once a project is approved by the SRB, the proposer must confirm in writing to comply with the data access and publication policy. Researchers conducting an approved project will then be granted access to explore a recoded excerpt of the clinical database for selection of appropriate samples based on phenotypic characteristics as well as Biorep's database to explore availability of samples.

The database to which the researchers conducting an approved project is granted access is recoded in order to (1) control for double publication of the same data sets and (2) avoid researchers recognising data sets as their own contribution. In parallel and prior to the release of samples, confirmation will be sought from the respective leading national Ethical Review Board that no objections are raised against the assessment by the SRB that the proposed research project falls within the subject area to which participants gave their informed consent.

The following common language for Clinical Data, Biological Specimens, and Imaging Data must be included in every IRB and Ethics applications:

Clinical Data is renewable therefore scientists will have open access to such de-identified data by request to the Track-HD Steering committee. Family History Data is renewable therefore scientists will have open access to such de-identified data by request to the Track-HD Steering committee through the Clinical Trial Manager.

Biological Specimens may be renewable (e.g., DNA, cell lines) or limited (e.g., plasma, urine). Scientists will have open access to de-identified renewable biological specimens subject to compliance with reasonable material transfer procedures. The use of limited biological specimens will be subject to scientific review by the Track-HD Steering committee and the EHDN Registry steering committee to ensure that these scarce resources are put to their best use.

Imaging Data is renewable therefore scientists will have open access to such de-identified data by request to the Track-HD Steering committee.

The goal of the project is analysis of large, longitudinal datasets collected by multiple investigators. Each participant will be assigned pseudonymised research identifier. The link between the research identifier and the original participant identifier will be held at the individual study centres with the usual safeguards that are applied to all confidential information. The original participant identifier will never be known to external investigators.

The datasets will not include the participant's name, their street address, phone/fax numbers, email address, medical record number, account numbers, certificate/license numbers, vehicle identifiers including license plates, device identifiers and serial numbers, URLs, internet protocol addresses, and biometric identifiers. Additionally, any regional or cultural specific identification mechanisms (Social Security number, health plan beneficiary numbers, in the USA; NHS Numbers in UK, France, Netherlands, Canada; etc.) will also not be included. However, the date of the research scan will be included to maintain longitudinal information in the data.

All scientists requesting access to existing Track-HD data or biological specimens will be required to submit the following according to the EHDN data access policy.

- the investigator's biographical sketch
- a synopsis of the proposed study
- evidence of IRB approval or an IRB approved waiver for the proposed study
- statement of Research Intent and Assurance

5.19.3. Data access by the study sponsor

CHDI may **use** and make available for use by other service providers or researchers the **coded** clinical information collected for the following purposes:

- To check the quality of the clinical information, biological samples and brain images we collect from you.
- To better understand HD or other diseases being studied.
- To better understand how new treatments may influence HD or other diseases being studied.
- To improve the design of future research studies.
- To support scientific discussion and research that furthers the development of treatments for HD and other disorders.

CHDI may use and make available for use by other service providers or researchers the coded biological materials collected from you for the following purposes:

- To look at the DNA and see if there are special "markers" that help explain things about HD.
- To measure the amount of proteins and other molecules found in the biological samples that also might help explain things about HD.

CHDI may share coded clinical information, coded biological samples and coded brain images with the following third parties:

- Representatives of organizations providing services to CHDI in connection with TRACK-HD, such as laboratories and data and sample repositories, the organizations contracted for TRACK-HD to collect, maintain, manage, and monitor the information collected in the study.
- Representatives of the United States, Canada and other governmental and regulatory agencies, such as the United States Food and Drug Administration (FDA), Health Canada and the European Medicines Agency (EMA).
- Doctors at other sites that are taking part in TRACK-HD and the ethical review boards at those sites.
- Third parties working with or providing services to CHDI as part of scientific discussions. For example, CHDI may share coded information from TRACK-HD about the progression of a specific symptom of HD in order to discuss the best way to design a study to treat that symptom.

- Researchers (including researchers at companies) that wish to use the coded clinical information and coded biological samples for research that furthers the development of treatments of HD or other disorders.

5.20. TRACK-HD translation and coordination

All assessments will be translated and standardised across language areas. EHDN language area coordinators or other qualified translators will oversee this process and central study coordination will liaise between the translators and study centres within each language area.

Translation and cross-language validation will be overseen by central study coordination.

Steps for Translation of Tests

1. For each test, it is essential to ensure that appropriate permissions and contracts are in place to make the translations. Thus, before starting the translation process, it is necessary to determine where to purchase any commercially available tests, and also what intellectual property rights and copyrights exist and therefore must be respected.
2. If dealing with a measure that involves verbal stimuli (e.g., list learning tasks), review manuals & literature that describe how the task was developed – additional procedures may be needed to select stimuli with comparable frequency, etc.
3. Identify an appropriate translator, ideally someone that is familiar with the material to be translated (e.g., neuropsychologist, psychiatrist)
4. Translator makes an initial translation; it is useful at this stage to have this initial translation reviewed briefly by one or more native speakers of the translated material, and to revise as needed.
5. The measure should be piloted in 5-10 mock participants. Pilot participants should be asked for feedback on the test. Before proceeding, mock participant performance should be reviewed for range and to ensure it is close to what would be expected. The translation should be revised as needed based on feedback and data from mock participants.
6. Final pilot testing on the instrument should occur to check for clarity of the language and cultural sensitivity and to assess similarity of norms and other psychometric issues.
7. The translated test should be sent to the source (i.e., company that sells the test, investigator who originated the test) so that the test translation can be formally documented.

6. Participant information and consent

6.1. Study information sheet for participants

I. TITLE: TRACK-HD

II. PROTOCOL REFERENCE: 07/H0716/47 (version 5.0) London Queen Square

III. INTRODUCTION

We would like to invite you to take part in our ongoing research study, TRACK-HD. Before you decide whether to participate we would like you to understand why the research is being done and what it would involve for you. Before you agree to participate you should talk to others about the study if you wish. A member of the research team will go through the information sheet with you and answer any questions you have, which should take about 30 minutes. Part 1 of this information sheet tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Please don't hesitate to ask us if there is anything that is not clear.

INFORMATION ABOUT THE RESEARCH (PART 1)

IV. PURPOSE OF THE STUDY

You are being asked to participate in a research study named "TRACK-HD". TRACK-HD is an ongoing study which you may or may not have participated in previously. The purpose of this ongoing study is to collect clinical information about you and your health as well as biological samples, such as blood, DNA and brain images. Researchers will use this information and samples to learn more about HD and to try to find new treatments for the disease. The research study is funded by CHDI Foundation, Inc., a not-for-profit foundation that only works on HD.

We are asking you to participate in TRACK-HD because you have inherited the HD genetic mutation, and have had this confirmed by a predictive genetic test, or because you are a "control participant". For the purposes of this study, a control participant is a person who is not at risk of HD.

You are completely free to choose whether or not to participate in this research study. If you decide to participate, you can change your mind and withdraw from the research study at any time; you are not required to give any reasons for your decision. Deciding not to participate will not affect you or your family's current or future clinical care.

We will not put your name, address or any other information that could directly identify you on the clinical information, biological samples or brain images you allow us to collect from you. Only the site research staff will be aware of your identity and able to link the information collected from your study visits to you. All information collected about you will be coded with a numeric identifier used to

protect your identity and connect your clinical information to other HD studies in which you may participate. If you do not already have an identifier through previous participation in TRACK-HD, an identifier will be assigned to you at the initial visit.

All information collected for the study will be stored in secure databases and repositories where they will be available now and in the future to researchers who are trying to develop new tests for, and ways to treat HD and similar diseases.

Because TRACK-HD is a research study you will not be told the results of any of the tests performed in the study. If you would like to see one of your brain scans this can usually be arranged on the day with a member of the research staff. We will not usually tell you whether your results have changed from one visit to the next. If any aspect of the assessment worries you, we can arrange for you to be referred to an appropriate specialist to investigate this further. We would also like your agreement that we may inform you and your GP in the unlikely event that one of the scans revealed something unexpected and important, such as a brain haemorrhage. Once the study is finished, you will be told about the overall results of the study, which will be about the group as a whole rather than individuals.

About 240 people will take part in this phase of the ongoing study and approximately half of these will be people at risk of HD and half will be control participants. Many of these people are already participating in TRACK-HD, but other eligible people will be invited to take part if there are fewer than 240 TRACK-HD participants available.

V. WHAT WILL I BE ASKED TO DO IF I DECIDE TO TAKE PART?

TRACK-HD is an ongoing longitudinal study. That means we will ask you to undergo the research procedures about once a year for up to 3 years or as long as you are willing to take part. The study includes several research procedures that are carried out by experienced professionals and it has been reviewed and approved by National Research Ethics Service London Queen Square. Each study visit starts at 9am and is expected to take a full day. There will be plenty of time for refreshments, lunch and breaks.

If you choose to take part, it is important that you follow the research procedures closely. Because alcohol and certain medications may affect your ability to follow these procedures, you will be asked to abstain from alcohol and certain medicines, such as those that help you to sleep, for 48 hours before each study visit and during the day of the visit. The research staff will be able to tell you which medicines should be avoided. Any other prescription or over-the-counter medicines that are not on this list can be taken as usual.

If you consent to participate in TRACK-HD we will:

1. Conduct a clinical evaluation of your current medical status and wellbeing.
2. Collect a sample of your blood to study your DNA.
3. Collect brain images from an MRI scan.

4. Measure movement-related brain activity using transcranial magnetic stimulation
5. Include any data, samples and brain images collected from you previously if you participated previously in the TRACK-HD study, along with the data collected in these study visits (as described in Part 2).
6. Store the data, biological materials and brain images we collect from you (including any data, samples and brain images already collected as part of TRACK-HD) in a secure place and make them available for future research (as described in Part 2).

With your **optional** consent we will also:

1. Link clinical information about you collected in research studies other than TRACK-HD to the clinical information about you collected in this study.
2. Contact you between study visits to provide information about future HD research studies or find out information about your health status or wellbeing.

In order to participate in TRACK-HD you must agree to take part in all parts of the study. Each of the parts of TRACK-HD is described in more detail below.

1. CLINICAL EVALUATIONS

At the initial visit as well as at your follow-up visit(s) (about every 12 months), we will ask questions about your medical history, your current health and treatments (including medications) you are then taking, and we will measure your height and weight. We also will conduct the following tests to see how well you move, think, remember things, perform daily tasks, and behave – all behaviours which may be affected by HD.

- A brief neurological examination will be performed, which is designed to show up and help us measure any physical effects of the disease.

- You will be asked to perform a number of automated tests to see how well you move, such as repeatedly pressing a button, and measurement of your strength, such as your tongue and grip. Lightweight sensors that detect very small movements will also be attached to your wrists and leg to measure any small involuntary movements while you are resting. This will take about 30 minutes in total.

- You will spend about an hour doing some thinking tasks, which will include some pencil and paper tasks and some automated tasks. These are measures of how you think, and the tasks are different to assess different areas of thinking. Some of the tasks are performed using a computer but you do not need to have any knowledge of computers in order to do them.

- Your eye movements will be assessed using a special set of goggles that track your eyes as they move in response to computer-controlled targets projected onto a screen. The test will take approximately 10 minutes to complete once the goggles are in position.

- You will be asked to complete questionnaires asking about your mood and other aspects of how HD may affect your behaviour.

We hope that this examination will help give a better understanding of HD symptoms and the factors that determine how fast or how slow HD progresses. If you have inherited the HD genetic mutation and bring a companion to the research study visits, we may ask that person, with your permission, questions about your ability to do day-to-day activities and your behaviours.

2. COLLECTING BIOLOGICAL MATERIALS (INCLUDING BLOOD TO STUDY YOUR DNA)

At the initial visit as well as at your follow-up visits (about every 12 months), we will collect up to 50 ml of blood (which is equivalent to approximately 3 tablespoons) from a vein in your arm**.** This will take about 5 minutes. Your sample will be sent to a research facility selected for TRACK-HD (BioRep, Srl in Milan, Italy or such other facility designated by CHDI Foundation from time to time) where it will be used to obtain your DNA (the genetic material in your blood). The DNA will then be analyzed or "genotyped". If you have inherited the HD genetic mutation the number of CAG repeats in your HD gene will be counted as part of this genotyping. This is the genetic mutation that determines whether or not you have HD. Genotype information will also be used and shared as described in Part 2, Section V under the heading "WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL". Because this genotyping is being done as a part of this research study, the results are experimental data and normally the results will not be reported to you or to anyone at your research centre.

Note – If you are already a participant in the TRACK-HD research study and you already had your DNA genotyped, you will not need to do that again for this part of the study. If you have inherited the HD genetic mutation and have had HD genetic testing in the past, other than as part of TRACK-HD, and your CAG number is entered into the TRACK-HD database, that CAG number and the one determined as part of the research genotyping will be compared. If there is a difference in the two numbers and gives cause for concern, the research staff and your doctor may be contacted. Your doctor may talk to you about this difference depending on your medical condition.

In addition to the blood sample, a cheek swab will also be taken to collect cheek cells to test for levels of the protein huntingtin, which is made by the body using instructions from the huntingtin gene. This will be required from all study participants including controls. The cells are collected by rolling the collection swab on the inside of the cheek for approximately 30 seconds while at the same time moving the swab up and down-once on each side of the mouth. The procedure does not pose any risk and is painless. This sample will also be sent to a research facility selected for TRACK-HD (BioRep, Srl in Milan, Italy or such other facility designated by CHDI Foundation from time to time).

3. COLLECTING BRAIN IMAGES THROUGH CONDUCT OF MRI

At the initial visit as well as at your follow-up visit(s) (about every 12 months), you will be required to undergo magnetic resonance imaging (MRI), which is a painless and safe technique that can obtain detailed pictures of the brain. It uses magnetic fields to generate the pictures and, unlike X-ray

techniques, there is no ionising radiation. There are no known risks to you or others. However, MRI scans are not done on people with certain metal implants (such as pacemakers). Prior to the scan we will ask questions to find out whether there are any reasons why you should not have a brain scan, for example if you have a pacemaker or metal implants or if you are claustrophobic. If you are able and happy to be scanned, the scan will take about 90 minutes in total and you will be able to take a break if you need one. During the scan you will be required to complete automated tests of your thinking or hand movements that will take approximately 15 minutes in total. You will be showed how to carry out these tests before the scan starts.

4. MEASURING MOVEMENT-RELATED BRAIN ACTIVITY USING TRANSCRANIAL MAGNETIC STIMULATION

At the initial visit as well as at your follow-up visit(s) (about every 12 months), you will undergo a procedure called transcranial magnetic stimulation (TMS). This technique uses a magnetic field and is a painless and safe way to measure activity in the area of your brain responsible for controlling movements. By activating the area responsible for hand movements, the size of the magnetic field required to cause a small twitch in your hand can be measured. Activation is measured using small metal disks called electrodes that are placed on your hands and scalp using a sticky paste. We also stimulate a nerve at the wrist using a small electric pulse and measure the activation of the brain. The skin where each electrode is to be placed will be cleaned with an exfoliating gel to ensure that the electrical contact is good enough to allow the signals from the brain to be recorded properly. The research staff are very skilled at this procedure and it is not uncomfortable. When everything is ready, the technician will ask you to sit or lie in as relaxed a state as you can. During the activation you may experience a tingling sensation in your hand as well as the small twitch. The whole procedure, including the placement of the electrodes will take approximately 40 minutes.

VI. WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

If you have inherited the HD genetic mutation, there is a chance that you may develop clinical signs of HD during the course of this research study. If at any time you feel you could benefit from treatment or support, you may request to be referred for appropriate care, if you are not already receiving this.

If, as a result of your responses to questionnaires asking about your mood, we develop concerns that you could be at risk of harm to yourself or to others, we may need to disclose information about you without your consent to protect you or others around you. If possible, we will discuss this with you before making such disclosures.

You may experience anxiety or psychological discomfort while completing the clinical evaluation and family history questions. If at any time you feel you could benefit from treatment or support, you may request to be referred for appropriate care, if you are not already receiving this.

During the collection of blood samples you may experience pain and/or bruising at the site where blood is taken. Localized clot formation and infections may occur, but this is very rare. Fainting or feeling

lightheaded may occur during or shortly after having blood drawn. If you experience this, you should lie down immediately to avoid possible injuries and notify study personnel.

Some people experience claustrophobia when having an MRI scan, but we will do whatever we can to help you relax before and during the scan.

In the course of doing questionnaires or tests you may feel tired and/or irritable. If this happens please tell your doctor or a member of the research staff and ask them to allow you time to rest or stop the testing all together.

As with the collection of any personal (private) information, there is also a slight risk of accidental disclosure of information or breach of computer security. Loss of confidentiality could have a negative impact on you, your family, or other individuals or groups, including insurability, employability and/or family relationships. Safeguards are in place to minimize this potential risk.

VII. WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

You will not receive direct health benefit from participating in the TRACK-HD study. However, your participation may provide information that is useful to our understanding of HD and our efforts to find treatments for HD.

VIII. WHAT ARE THE POSSIBLE ALTERNATIVES TO TAKING PART?

You do not have to participate in TRACK-HD, and choosing not to participate will not affect your current or future medical care at the National Hospital for Neurology and Neurosurgery.

IX. EXPENSES AND PAYMENTS

You will not receive payment for participating in TRACK-HD. You will receive reimbursement for travel expenses and other costs resulting directly from your participation in TRACK-HD. Please discuss reimbursement with a member of the staff at the research centre.

X. WHAT WILL HAPPEN IF I DON'T WANT TO CARRY ON WITH THE STUDY?

Your participation in this research study is completely voluntary. You are free not to participate or to withdraw at any time, for whatever reason, without risking loss of present or future care you would otherwise expect to receive. In the event that you do withdraw from this research study, the information you have already provided that can identify you will be kept in a confidential manner.

XI. CONTACT PERSONS

For more information concerning this research please contact: [INSERT NAME AND PHONE NUMBER OF CONTACT PERSON FOR STUDY INFORMATION *Note: this person is usually the site's PI.]

If you have questions about your rights as a research participant, you may call [INSERT NAME AND PHONE NUMBER OF CONTACT PERSON FOR PARTICIPANT'S RIGHTS].

INFORMATION ABOUT THE RESEARCH (PART 2)

I. WHAT IF THERE IS A PROBLEM?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. If you believe that you have suffered a research related injury, please contact: [INSERT NAME AND PHONE NUMBER OF CONTACT PERSON FOR STUDY INFORMATION *Note: this person is usually the site's PI.] Regardless of this, if you wish to complain, or have any concerns of this study, the normal National Health Service complaints mechanisms should be available to you.

II. EARLY DISCONTINUATION OF THE STUDY

You may be withdrawn from TRACK-HD if you do not follow the directions of this research study or if your medical condition changes so that staying in this research study might risk your health or this research. You participation in the study may also end if CHDI Foundation discontinues funding for the study.

III. SPONSOR SUPPORT

TRACK-HD and the storage of coded clinical information and coded biological materials collected in the course of TRACK-HD are supported by CHDI Foundation, Inc., a not-for-profit foundation that funds a variety of research activities aimed at finding treatments for HD.

IV. INCLUDING YOUR INFORMATION AND SAMPLES FROM TRACK-HD

If you are already a participant in the TRACK-HD research study your consent to continue in the study will enable us to combine the information collected about you from previous visits, including any biological samples and brain images, with the new information being collected about you in this continuation study.

V. WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

The clinical information collected about you will be entered via secure internet connections into a confidential computer database that is located at a data storage facility selected for TRACK-HD (the University of Ulm in Ulm, Germany, the Huntington's Disease Neuro-Imaging Initiative in Los Angeles, USA or such other facility designated by CHDI Foundation from time to time). This facility, called a hosting facility, follows security procedures to make sure the information is safe and secure. **The clinical information that is entered into the database will not be associated with, or identified by your name or other information that could identify you.**

Only the site research staff will be aware of your identity and have the key to the code that links your clinical information, biological samples and brain images to you. However, to meet regulations or monitor correct data entry, site research staff may share a copy of this consent form and records that identify you with CHDI, its auditors or monitors, regulatory authorities or the NHS Trust.

CHDI may **use** and make available for use by other service providers or researchers the **coded** clinical information collected about you for the following purposes:

- To check the quality of the clinical information, biological samples and brain images we collect from you.
- To better understand HD or other diseases being studied.
- To better understand how new treatments may influence HD or other diseases being studied.
- To improve the design of future research studies.
- To support scientific discussion and research that furthers the development of treatments for HD and other disorders.

CHDI may **use** and make available for use by other service providers or researchers the **coded** biological materials collected from you for the following purposes:

- To look at the DNA and see if there are special "markers" that help explain things about HD.
- To measure the amount of proteins and other molecules found in the biological samples that also might help explain things about HD.

CHDI may **share coded** clinical information, **coded** biological samples and **coded brain** images with the following third parties:

- Representatives of organizations providing services to CHDI in connection with TRACK-HD, such as laboratories and data and sample repositories, the organizations contracted for TRACK-HD to collect, maintain, manage, and monitor the information collected in the study.
- Representatives of the United States, Canada and other governmental and regulatory agencies, such as the United States Food and Drug Administration (FDA), Health Canada and the European Medicines Agency (EMA).
- Doctors at other sites that are taking part in TRACK-HD and the ethical review boards at those sites.
- Third parties working with or providing services to CHDI as part of scientific discussions. For example, CHDI may share coded information from TRACK-HD about the progression of a specific symptom of HD in order to discuss the best way to design a study to treat that symptom.
- Researchers (including researchers at companies) that wish to use the coded clinical information and coded biological samples for research that furthers the development of treatments of HD or other disorders.

CHDI or these third parties, including TRACK-HD investigators, may publish the results of their research, including coded clinical data, in medical journals or present such results at meetings. Because your name, address or other identifying information are never given to CHDI and these third parties, this information will not be disclosed.

CHDI may also submit coded clinical information to be included in one or more other electronic databases for use by researchers conducting studies to further the development of treatments for HD, other disorders or the purposes of other bio-medical research.

The biological materials collected from you will be used only for research purposes and will not be sold. You can change your mind at any time about the storage and use of your biological materials. Just contact the site investigator and let him or her know that you no longer want your biological materials stored and they will be removed and destroyed. If your biological materials have already been distributed to a researcher for use, we may not be able to locate and destroy those biological materials.

Any of the uses and activities described above may involve sending coded clinical data, coded biological samples and coded brain images to other countries that may not have the same privacy laws as this country. However, given that only coded clinical data, coded biological samples and coded brain images are sent, the risk of unintended disclosure of identifying information is low.

VI. WILL MY DATA BE USED COMMERCIALLY?

Successful research by CHDI and other organizations using your coded clinical information and coded biological samples collected in the course of TRACK-HD could result in a commercial therapeutic product with significant value, such as a product for the treatment of HD. You will not receive any financial benefit from such a result.

VII. CONSENT FOR LINKING CLINICAL INFORMATION FROM PREVIOUS STUDIES TO TRACK-HD – OPTIONAL (NOT APPLICABLE TO CONTROLS)

An **optional** part of TRACK-HD involves linking clinical information about you collected in other HD studies (other than the TRACK-HD study) to the clinical information collected about you in TRACK-HD.

The purpose of this part of TRACK-HD is to give us the opportunity to track the progression of your Huntington's disease over an extended period of time or through different tests that are conducted in other HD studies. If you agree to participate in this part of TRACK-HD you will be asked to provide the name(s) of other HD studies you have participated in. If you cannot remember the study name, you can provide any information that may help to identify it such as the approximate year(s) you took part in the study(s), your participant identifier associated with the study(s) and/or the name of the drug under study. We will use this information to request that the clinical information collected about you in other HD studies be linked into the TRACK-HD database. This information will be captured electronically and will provide the opportunity to track your data across multiple studies.

VIII. CONTACTING YOU

A member of the site research staff will need to contact you to schedule your yearly study visits for as long as you are willing to participate. We will work with you to determine the best way to contact you to set up these visits. We recognize that there may be reasons you are not able to come in for a study visit. If this happens, you can still continue your participation in the research study and we will make arrangements to reschedule your appointment.

Contact Between Study Visits

Although we will contact you regarding your study appointments, you have the **option** of being contacted between visits to clarify questions (e.g. concerning your answers in TRACK-HD questionnaires), collect additional information or to provide you with study updates. If you allow us to do this, we will not identify ourselves as having any connection with a medical facility, so as to preserve your privacy.

6.2. Study information sheet for companions

I. TITLE: TRACK-HD

II. PROTOCOL REFERENCE: 07/H0716/47 (version 5.2) London Queen Square

III. INTRODUCTION

We would like to invite you to take part in our ongoing research study, TRACK-HD. Before you decide whether to participate we would like you to understand why the research is being done and what it would involve for you. Before you agree to participate you should talk to others about the study if you wish. A member of the research team will answer any questions you have. Please don't hesitate to ask us if there is anything that is not clear.

IV. PURPOSE OF THE STUDY

You are being asked to participate in a research study named "TRACK-HD". TRACK-HD is an ongoing study which you may or may not have participated in previously. The purpose of this ongoing study is to collect information about HD that researchers can use to learn more about HD and to try to find new treatments for the disease. The research study is funded by CHDI Foundation, Inc., a not-for-profit foundation that only works on HD.

We are asking you to participate in TRACK-HD because you are the companion of a person who has inherited the HD genetic mutation (and has had this confirmed by a predictive genetic test), and who is participating in the TRACK-HD research study. A copy of the information sheet provided to your companion is enclosed.

You are completely free to choose whether or not to participate in this research study. If you decide to participate, you can change your mind and withdraw from the research study at any time; you are not required to give any reasons for your decision. Deciding not to participate will not affect you or your companion's current or future clinical care.

We will not put your name, address or any other information that could directly identify you on the information you allow us to collect from you. Only the site research staff will be aware of your identity and able to link the information collected from your study visits to you and your companion. All information collected from you will be coded with a numeric identifier used to protect your identity and connect this information to other HD studies in which you may participate. If you do not already have an identifier through previous participation in TRACK-HD, an identifier will be assigned to you at the initial visit.

All information collected for the study will be stored in secure databases and repositories where they will be available now and in the future to researchers who are trying to develop new tests for, and ways to treat HD and similar diseases.

V. WHAT WILL I BE ASKED TO DO IF I DECIDE TO TAKE PART?

Since the symptoms of HD are noticed differently by companions and the affected persons themselves, and the disease of a close one also has an impact on those around them, we would like to ask you to complete 3 questionnaires about your companion at each visit. These questionnaires ask about any mood symptoms that your companion may suffer from.

VI. INCLUDING YOUR INFORMATION FROM TRACK-HD

If you are already a participant in the TRACK-HD research study your consent to continue will enable us to combine the information collected from you at previous visits with the new information being collected from you in this continuation study.

VII. WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

The information collected from you will be entered via secure internet connections into a confidential computer database that is located at a data storage facility selected for TRACK-HD (the University of Ulm in Ulm, Germany or such other facility designated by CHDI Foundation from time to time). This facility, called a hosting facility, follows security procedures to make sure the information is safe and secure. **The information that is entered into the database will not be associated with, or identified by your name or other information that could identify you or your companion.**

Only the site research staff will be aware of your identity and have the key to the code that links your information to you and your companion.

CHDI may **use** and make available for use by other service providers or researchers the **coded** information collected about you for the following purposes:

- To check the quality of the information we collect from you.
- To better understand HD or other diseases being studied.
- To better understand how new treatments may influence HD or other diseases being studied.
- To improve the design of future research studies.
- To support scientific discussion and research that furthers the development of treatments for HD and other disorders.

CHDI may **share coded** clinical information with the following third parties:

- Representatives of organisations providing services to CHDI in connection with TRACK-HD, such as data repositories, the organisations contracted for TRACK-HD to collect, maintain, manage, and monitor the information collected in the study.

- Representatives of the United States, Canada and other governmental and regulatory agencies, such as the United States Food and Drug Administration (FDA), Health Canada and the European Medicines Agency (EMA).
- Doctors at other sites that are taking part in TRACK-HD and the ethical review boards at those sites.
- Third parties working with or providing services to CHDI as part of scientific discussions. For example, CHDI may share coded information from TRACK-HD about the progression of a specific symptom of HD in order to discuss the best way to design a study to treat that symptom.
- Researchers (including researchers at companies) that wish to use the coded information for research that furthers the development of treatments of HD or other disorders.

CHDI or these third parties, including TRACK-HD investigators, may publish the results of their research, including coded clinical data, in medical journals or present such results at meetings. Because your name, address or other identifying information are never given to CHDI and these third parties, this information will not be disclosed.

CHDI may also submit coded information to be included in one or more other electronic databases for use by researchers conducting studies to further the development of treatments for HD, other disorders or the purposes of other bio-medical research.

Any of the uses and activities described above may involve sending coded data to other countries that may not have the same privacy laws as this country. However, given that only coded clinical data are sent, the risk of unintended disclosure of identifying information is low.

IV. CONTACT PERSONS

For more information concerning this research please contact: [INSERT NAME AND PHONE NUMBER OF CONTACT PERSON FOR STUDY INFORMATION *Note: this person is usually the site's PI.]

If you have questions about your rights as a research participant, you may call [INSERT NAME AND PHONE NUMBER OF CONTACT PERSON FOR PARTICIPANT'S RIGHTS].

V. CONTACTING YOU

A member of the site research staff will need to contact you to schedule your yearly study visits for as long as you are willing to participate. We will work with you to determine the best way to contact you to set up these visits.

6.3. Study information sheet for scan volunteers

Dear Participant,

Track-HD is a study being run at several centres throughout the world, which aims to understand Huntington's disease better and to improve the tools we can use to follow the course of the disease. We hope this will help us design future clinical trials of therapies for HD.

There are rigorous quality control processes in place to ensure that the study procedures are consistent across sites and over time. An important part of the quality control process involves comparing images from MRI brain scans to ensure that the parameters used by the scanner have not changed.

You are being invited to participate in the study to assist with these quality control processes and if you agree to take part an MRI brain scan will be performed at least 4 times over the next 3 years. We would also like your permission to contact you if additional scans are required, for example, if the MRI scanner undergoes an upgrade and we need check for consistency after the upgrade is complete. Each scan will last about 90 minutes and will be performed by experienced professionals.

What is an MRI brain scan?

Magnetic resonance imaging (MRI) is a painless and safe technique that can obtain detailed pictures of the brain. It uses magnetic fields to generate the pictures and, unlike X-ray techniques, there is no ionising radiation. MRI scans are not done on people with certain metal implants (such as pacemakers). There are no known risks to you or others. The entire process will take about 2 hours of your time and you won't be in the scanner for longer than about 90 minutes.

We will ask you questions to find out whether there are any reasons why you should not have a brain scan, for example if you have a pacemaker or metal implants or if you are claustrophobic. If you are able and happy to be scanned then we will continue with the study; if you are not able to be scanned for any reason then you will not have to participate any further.

What will happen to my results?

The results of these examinations will be entered onto an electronic database.

Your confidentiality is very important to us. Your name, address or any other information which could allow personal identification will never be recorded in the electronic database. Your data will recorded under a code-number (or 'pseudonym'). Therefore, nobody but the local study team knows your identity or can trace your code-number back to your real name.

By signing the consent form you are authorizing the use of your data for quality control of a large scale, multi-centre studies that will combine data from similar populations. These multi-centre studies are being conducted by the Huntington's Disease Neuroimaging Initiative (HDNI), a neuroscience consortium of universities and research institutes. Your data will be stored with a coded research identifier to protect your identity. Only pseudonymised data, which does not include anything that might directly identify you, will be shared with HDNI members and the general scientific community for purposes relating to quality control of this research. This data will be entered into linked databases at the University of California, Los Angeles and the University of Ulm, Germany to be used from this date and going forward.

Data entry and the use of the Track-HD database will be carried out over the internet using secure connections. The database is held at EHDN Central Coordination, Ulm University Hospital, Ulm, Germany. Your scan will also be held in approved, secure storage databases elsewhere in Europe and the USA.

Evaluation and publication of study results will be carried out anonymously and in the form of statistics. None of your personal data will ever be made public.

Who is running and funding Track-HD?

Track-HD is funded by the CHDI Foundation, Inc., an American charity founded in 2002 with the aim of finding treatments for HD. In Europe, Track-HD is coordinated by the European Huntington's Disease Network (EHDN). EHDN is a scientific network of doctors and scientists committed to HD research.

Are there any risks involved?

No treatments will be given, and there are no specific risks involved.

Some people experience claustrophobia when having an MRI scan, but we will do whatever we can to help you relax before and during the scan.

Will taking part cost me anything?

All travel expenses will be refunded.

Will I profit from participating?

There is no personal financial gain to yourself now or in the future should this research result in a biomarker being developed for use in HD therapy trials, even if this involves collaboration with a commercial company.

Do I have to take part?

No. Participation in the study is entirely voluntary and you are free to withdraw at any time without giving a reason. Your legal rights are not affected by participating in the study and the study is indemnified.

Will I be told the results of my scans?

If you would like to see one of your brain scans this can usually be arranged on the day with the radiographer. We will not usually tell you whether your results have changed from one scan to the next. If any aspect of the assessment worries you, we can arrange for you to be referred to an appropriate specialist to investigate this further. We would also like your agreement that we would inform you and your GP in the unlikely event that one of the scans revealed something unexpected and important, such as a brain haemorrhage.

Are there any restrictions on what I can eat or do?

We ask that you do not drink any alcohol during the day or evening before a scan. Otherwise, there are no restrictions.

Will the study team contact me?

We will ask your permission to contact you as required, to clarify any questions with you, to provide you with updates and to arrange your next scan. We will ask how and when you would like to be contacted.

Who can I contact for more information?

You may contact (name of investigator) on (telephone number).

Ethical review statement

This research project has been reviewed and approved by the London Queen Square Ethics Committee.

Compensation arrangements

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns of this study, the normal National Health Service complaints mechanisms should be available to you.

Confidentiality and data protection statement

All staff involved in looking after you during your participation in TRACK-HD are bound by medical confidentiality and are obliged to comply with data protection legislation. Research results relating to this study are intended for use in an anonymous form in scientific publications.

(Name of the site director)

(Place, date)

6.4. Consent forms for participants

I. TITLE: TRACK-HD

II. NAME OF RESEARCHER: Professor Sarah Tabrizi

TRACK-HD is a continuation of the TRACK-HD research study. The study is being done to help us understand Huntington's disease (HD) better and to improve the tools available to follow the course of the disease and to help in future to try to find new treatments for the disease. The research study is funded by CHDI Foundation, Inc., a not-for-profit foundation that only works on HD.

Please initial the boxes below if you consent to the following:

1. I confirm that I have read and understand the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical records and data that can identify me that is collected during the study may be looked at by (i) individuals from CHDI Foundation, Inc., its auditors and monitors, (ii) regulatory authorities or (iii) the NHS Trust, where required to meet regulations or to monitor correct data entry. I give permission for these individuals to have access to my records. ☐

Please initial the boxes below if you additionally consent to:

1. I consent to have clinical information collected from other HD studies that I have knowledge of participating in linked to this research study (not applicable to controls). ☐
2. I consent to being contacted between visits to provide additional information or receive study updates. ☐

III. CONSENT TO PARTICIPATE IN THIS RESEARCH STUDY

For Participants:

I have read (or have had read to me) the contents of this consent form and have been encouraged to ask questions. I have received answers to my questions. I agree to participate in this research study. I have received (or will receive) a signed copy of this form for my records and future reference.

Signature of Research Participant	Printed Name	Date

For Study Staff:

Person Obtaining Consent

I have read this form to the participant and/or the participant has read this form. An explanation of the research was given and questions from the participant were solicited and answered to the participant's satisfaction. In my judgment, the participant has demonstrated comprehension of the information.

Signature of Person Obtaining Consent	Printed Name and Title	Date

6.5. Consent form for companions

I. TITLE: TRACK-HD

II. NAME OF RESEARCHER: Professor Sarah Tabrizi

TRACK-HD is a continuation of the TRACK-HD research study. The study is being done to help us understand Huntington's disease (HD) better and to improve the tools available to follow the course of the disease and to help in future to try to find new treatments for the disease. The research study is funded by CHDI Foundation, Inc., a not-for-profit foundation that only works on HD.

Please initial box

1. I confirm that I have read and understand the information sheet dated..... (version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that data collected during the study may be looked at by individuals from CHDI Foundation, Inc., from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

III. CONSENT TO PARTICIPATE IN THIS RESEARCH STUDY

For Participants:

I have read (the contents of this consent form and have been encouraged to ask questions. I have received answers to my questions. I agree to participate in this research study. I have received (or will receive) a signed copy of this form for my records and future reference.

Signature of Research Participant	Printed Name	Date

For Study Staff:

Person Obtaining Consent

I have read this form to the participant and/or the participant has read this form. An explanation of the research was given and questions from the participant were solicited and answered to the participant's satisfaction. In my judgment, the participant has demonstrated comprehension of the information.

Signature of Person Obtaining Consent	Printed Name and Title	Date

6.6. Consent form for scan volunteers

Name of study: Track-HD

Initial each box

Study information

The content, procedures, risks and aims of the research project named above as well as the procedures for handling my data have been explained to me in detail by the researcher named below.

I have had the opportunity to ask questions and obtained answers which I felt were satisfactory. ☐

I have had sufficient time to decide whether or not I want to participate in the project.

My participation is entirely voluntary and participation will not affect my legal rights.

I have received a copy of the study information sheet.

Data protection and Data Sharing

I agree that data obtained during the course of this study can be recorded in electronic form, processed without providing personal identity and stored in pseudonymised form at a secure server located at the University of Ulm, Germany.

I agree to the storage of imaging data derived from MRI scans at the central repository of the Huntington's Disease Neuro-Imaging Initiative (HDNI) server in Los Angeles, USA.

I agree that the imaging data derived from MRI scans and pseudonymised data will be shared with the Sponsor.

By signing the consent form I am authorizing the use of my data for quality control of a large scale, multi-centre studies that will combine data from similar populations. These multi-centre studies are being conducted by the Huntington's Disease Neuroimaging Initiative (HDNI), a neuroscience consortium of universities and research institutes. My data will be stored with a coded research identifier to protect your identity. Only pseudonymised data, which does not include anything that might directly identify me, will be shared with HDNI members and the general scientific community for purposes relating to quality control of this research. This data will be entered into linked databases at the University of California, Los Angeles and the University of Ulm, Germany to be used from this date and going forward. ☐

I agree that authorised persons bound by confidentiality can view the personal data recorded as far as it is necessary or legally required for data control. **For this purpose only**, I exempt the clinician from the obligation to ensure medical confidentiality at all times.

Contact between visits

I give my permission for my study site team to contact me between visits: ☐

- to clarify any questions
- to provide me with updates on Track-HD; or
- to arrange future scans

I agree to take part in this study. ☐

Name of participant	**Signature of participant**	**Date**
.....	.....	.....
Name of researcher	**Signature of researcher**	**Date**

6.7. Risk assessment for harm to self or others

All personnel involved in the Track HD assessments will be informed of the risk indicators and protocols outlined below.

Self harm/Suicide

Any of the following occurrences will initiate the Suicide Risk Assessment Protocol outlined below:

1. A total score of >= 24 on the BDI II
2. Endorsement of the suicide item on the BDI II: at the level 3 ("I would like to kill myself") or level 4 ("I would kill myself if I had the chance).
3. Endorsement of the self harm item of the HADS: at the level 1 (I definitely feel like harming myself) or level 2 (I sometimes feel like harming myself).
4. Mention of suicide plans during any part of the Track-HD assessment day
5. Reports of concern regarding significant depressive symptoms from a care-giver/partner

Harm To Others

Any of the following occurrences will initiate the harm to others assessment protocol outlined below:

1. Report by partner/caregiver that they fear for their safety as a result of the HD participant's irritability and aggression.
2. Endorsement of the harm to others item of the HADS at level 1 (I sometimes feel I might lose control and hit or hurt someone) or level 2 (I occasionally feel I might lose control and hit or hurt someone).

Suicide/Harm to Others Risk Assessment Protocol

1. Each site will designate a primary licensed professional (e.g. neurologist, psychiatrist, clinical psychologist, HD nurse specialist, psychiatric nurse or clinical social worker) to further assess suicide risk or potential risk to others identified during screening. If unavailable, a suitable back-up must be provided. For most sites this should be the site PI and back-up support must be organised during periods of absence.
2. Further actions will depend on the discretion of the clinician but may include but are not limited to one or more of the following:
    - Determination that no further action is required
    - Follow-up phone contact
    - Referral to mental health services for further assessments
    - Follow-up at local HD clinic
    - Consultation with a family member
    - Immediate inpatient or outpatient treatment
    - Notification of law enforcement
3. Any initiation of this protocol must be documented and enforced by the site PI. Whenever this protocol is initiated it will be documented by the data monitors in the onsite monitoring reports and if any Track-HD participant requires urgent inpatient treatment or notification of law enforcement, the Track HD clinical trial manager and the Track-HD study PI (SJT) must be notified. This, as well as other reportable events, will be reviewed by clinicians on the Track HD executive and steering committee on a semi-annual basis to ensure continuing effectiveness of screening procedures and assessment/treatment protocols.

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