Study protocol for the iMarkHD study in individuals with Huntington's disease

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Abstract

Background: Huntington's disease (HD) is still often defined by the onset of motor symptoms, inversely associated with the size of the CAG repeat expansion in the *huntingtin* gene. Although the cause of HD is known, much remains unknown about mechanisms underlying clinical symptom development, disease progression, and specific clinical subtypes/endophenotypes.

Objectives: In the iMarkHD study, we aim to investigate four discrete molecular positron emission tomography (PET) tracers and magnetic resonance imaging (MRI) markers as biomarkers for disease and symptom progression.

Methods: Following MRI optimisation in five healthy volunteers (cohort 1), we aim to recruit 108 participants of whom 72 are people with HD (PwHD) and 36 healthy volunteers (cohort 2). Pending interim analysis, these numbers could increase to 96 PwHD and 48 healthy controls. Participants will complete a total of 10 study visits, consisting of a screening visit followed by a clinical and MRI visit and PET visits at baseline, year 1, and year 2. PET targets include the cannabinoid 1, histamine 3, and serotonin 2A receptors, and phosphodiesterase 10A, whereas MRI will be multimodal, including, but not limited to, the assessment of cerebral blood flow, functional connectivity, and brain iron.

Results: Recruitment is currently active and started in September 2022.

Conclusions: By combining PET and multi-modal MRI assessments we expect to provide a comprehensive examination of the molecular, functional, and structural framework of HD progression. As such, the iMarkHD study will provide a solid base for the identification of treatment targets and novel outcome measures for future clinical trials.

Keywords: Huntington's disease, MRI, PET, neuroimaging, progression, biomarker.

Introduction

Huntington's disease (HD) is still often defined by the onset of motor symptoms, inversely associated with the size of the CAG repeat expansion in the *huntingtin* (*HTT*) gene. [1] Nevertheless, subclinical changes and pathological processes precede the initiation of clinical symptoms, often by many years. [2-4] The availability of genetic testing and full penetrance of the *HTT* mutation in people with ≥40 CAG expansions provide a unique window of opportunity to examine the pattern of signs, symptoms, and neurobiological changes as they emerge, and study the clinical course of HD before the development of overt symptoms. [5] Although the cause of HD is known, much remains unknown about the mechanisms underlying the development of clinical symptoms, disease progression, and specific clinical subtypes/endophenotypes. Moreover, the identification of easily obtainable, reliable, and robust biomarkers of HD progression is crucial for the development and evaluation of disease-modifying treatments.

Previous efforts to identify such biomarkers include the TRACK-HD study, which was a longitudinal study focusing on sensitive and reliable biomarkers with extensive annual assessments involving magnetic resonance imaging (MRI) and clinical measures in both premanifest and manifest people with HD (PwHD). [2-4,6] In addition to motor progression, clear progression in cognitive dysfunction was observed, [2] particularly in relation to progression in apathy. Moreover, baseline apathy scores were significant predictors of later functional decline, as well as other neuropsychiatric symptoms associated with frontal lobe function, including affect and irritability. [2] MRI measures showed significant total brain volume reduction, most prominent in caudate and putamen volume, as well as striatal, corpus callosum and posterior white matter tract changes, with a strong relation to total functional capacity decline. [3]

Although MRI provides useful information about structural and functional organisation of brain changes in HD, including brain volume, perfusion, iron levels, and structural and functional connectivity, this technique is less useful to assess changes in specific neurotransmitters. For the latter, positron emission tomography (PET) may be deployed, aiding in understanding the progression and complexity of HD. [7] Especially in relation to PET markers, iMarkHD will provide innovative longitudinal assessments of HD pathology, complemented by MRI imaging.

Methods

Protocol Title

Longitudinal Adaptive Study of Molecular Pathology and Neuronal Networks in People with Huntington's disease (PwHD) and Healthy Controls using Positron Emission Tomography (PET) and Multi-modal Magnetic Resonance Imaging (MRI). The short title for the project is: iMarkHD In Vivo Longitudinal Imaging of HD Pathology.

Study aims and objectives

The primary objectives of the iMarkHD study are to investigate the change in binding profile of four discrete molecular PET tracers across different disease stages in HD and age- and sex-matched healthy volunteers, measured over two years, to determine whether these PET imaging methods could be used as markers of HD progression and future treatment response in therapeutic trials. The PET tracers used in the study are: 1) [^{11}C]MDL100907 for serotonin receptor 2A (5HT2A); [8] 2) [^{11}C]MK8278 for histamine 3 receptor (H3R); [9] 3) [^{11}C]MePPeP for cannabinoid 1 receptor (CB1R); [10] and 4) [^{11}C]IMA107 for phosphodiesterase 10A (PDE10A). [11]

Secondary objectives include:

1) Quantitative cross-sectional and longitudinal assessment of brain structure, function, blood flow and iron level changes measured through MRI including arterial spin labelling (ASL), quantitative susceptibility mapping (QSM), quantitative relaxation times, diffusion tensor imaging (DTI) and resting-state functional MRI (rs-fMRI).
2) To explore the cross-sectional and longitudinal relationships between PET imaging measures and MRI measures with:
    a. Number of *HTT* amino acid glutamine (CAG) repeats.
    b. CAG-Age Product (CAP) score (=age x (CAG-30)/6.49). [12]
    c. Clinical rating scales, including Unified Huntington's Disease Rating Scale (UHDRS), UHDRS Total Motor Score (UHDRS-TMS), UHDRS Total Functional Capacity (UHDRS-TFC) scores, and psychiatric and cognitive assessments.
    d. Disease duration as assessed by time at which first motor clinical manifestation was noticeable.
3) To develop and evaluate the application of combined multi-modal MRI and PET in assessing cross-sectional and longitudinal structural, functional, and molecular changes in the brain of PwHD compared to healthy controls.
4) To develop probabilistic tractography methodology fusing combined PET/DTI for assessing pathway-based functional molecular changes and associations in line with known brain circuits and primary and secondary objectives in PwHD and healthy controls.
5) To determine whether the [11C]IMA107 PET marker can be a prognostic marker of HD phenoconversion (clinical motor diagnosis) and how it compares to the prognostic accuracy of the other three PET markers included in the study.

Rationale for positron emission tomography targets

PDE10A activity appears to be one of the most promising markers for disease progression as PET imaging data in PwHD have shown that changes in PDE10A activity in the striatum may be one of the first detectable neuroimaging changes in premanifest PwHD. [13] Similarly, striatal PDE10A mRNA and protein levels are reduced in HD transgenic mice prior to the onset of motor symptoms. [14,15] Due to these early changes occurring in premanifest PwHD, [16,17] it has been suggested that PDE10A activity could serve as a marker to predict the occurrence of manifest motor symptoms and could be more sensitive than the dopamine-receptor and volumetric methods currently used. [17]

CB1R has been implicated as neuroprotective through an effect on glutamatergic synapses. In animal models of striatal damage, activation of CB1R on cortico-striatal projections selectively protect medium spiny neuron populations. [18] Interestingly, genetic deficiency of CB1R has been shown to worsen disease signs in the N171-82Q transgenic HD mice, [19] and a delayed onset of HD-like symptoms in other rodent HD models were associated with less pronounced loss of this receptor. [20] Similar findings were obtained in the R6/2 HD mice. [14] In PwHD, small-scale PET studies have shown profound early and widespread reduction of CB1R availability. [21]

Serotonergic neurotransmission is an area of interest across several neurodegenerative conditions, including HD. Decreased serotonergic neurotransmission has been linked to depressive symptoms, at least in the R6/1 HD mouse model. [22] Moreover, specifically 5HT2A shows an age-dependent decrease in the striatum, caudate, and hippocampus of zQ175 HD mice, [8] in line with findings from the R6/1 HD mice. [23] In the zQ175 HD mouse model, a PET study has shown that at six months of age, striatal binding ratios for [^{11}C]-MDL 100907, as a marker for 5HT2A, was decreased by 11% compared to wild-type mice. In addition, an overall reduction in hippocampal and cortical 5HT2A was observed with further reductions of striatal binding at nine months of age, whereas PDE10A

reached a plateau. [8] This suggest that 5HT2A might be a useful biomarker in more advanced stages of HD.

Finally, the central histaminergic neuropeptidergic system also shows promise as a biomarker for HD progression. Histamine has been suggested to play a role in the neuronal and oxidative damages observed in HD mouse models, focusing on striatal changes. [24] Evidence from PwHD shows not only an increase and a diurnal phase shift in L-histidine expression as a measure for histamine production in the hypothalamic tuberomammillary nucleus, but also a selective increase in H1, H2, and H3 histamine receptor expression in cortical areas, [25,26] indicating hyperactivity of the neuronal histaminergic system. This is likely to be of relevance given the effect of histamine on neuronal and oxidative damage which has been observed in quinolinic acid induced HD mouse models. [25] Another animal study has demonstrated that overactivation of dopamine D1 receptors leads to an imbalance in dopaminergic neurotransmission and cell death, which could be mitigated by targeting dopamine D1 and H3 receptor complexes through an H3R antagonist. Moreover, receptor complex levels were associated with disease duration suggesting a possible role as biomarker for H3R. [27]

Rationale for multimodal magnetic resonance imaging

Conventional T1-weighted structural MRI, at a spatial resolution in all three dimensions of 1 mm, allows the quantification of total white and grey matter as well as that of relevant anatomical regions of interest such as the caudate and striatum relevant to HD pathology, [28] in addition to these data being used for co-registration with PET imaging data to allow anatomical delineation of the receptor distribution. ASL will provide quantitative maps of cerebral perfusion and blood flow, which have been previously shown to be locally reduced in PwHD not solely explained by tissue atrophy. [29] The rs-fMRI will be used to evaluate functional connectivity through measures of blood-oxygen- level

dependent (BOLD) signal as there is some evidence to suggest that functional connectivity may be profoundly altered in PwHD. [30] Emerging evidence suggests that dynamic functional connectivity metrics may indicate changes in macroscopic neural activity patterns underlying critical aspects of cognition and behaviour. [31] DTI, used to probe the integrity of structural white matter connections, has previously been used to show that diffusion metrics were significantly altered in premanifest and early manifest PwHD. [32,33] Finally, QSM, sensitive to cerebral iron accumulation, has also been previously shown to be abnormal in PwHD. For example, iron deposition in subcortical areas is increased in premanifest and manifest PwHD. [34] The iMarkHD study will evaluate which MRI measure or combination thereof is most predictive of disease progression.

Ethical approval

The study received ethical approval from London - Bromley Research Ethics Committee (reference 19/LO/0339; Integrated Research Application System reference 242859). This study will be conducted in accordance with the principles of the Declaration of Helsinki and General Data Protection Regulation. Written informed consent will be obtained from all participants prior to study-related activities.

Study design

The iMarkHD study is a UK-based, longitudinal, adaptive, observational, multi-target PET molecular and multi-modal MR imaging study designed to identify candidate disease progression and treatment response biomarkers to increase knowledge of HD-related mechanisms. The study is registered on clinicaltrials.gov (https://classic.clinicaltrials.gov/ct2/show/NCT03434548). The recruitment target for the study is 113 participants across two cohorts. Cohort 1 consists of five healthy participants

undergoing MR scanning to optimise data acquisition and MR sequences for Cohort 2. In Cohort 2, 72 PwHD and 36 healthy volunteers across four different groups (Table 1) will be recruited:

- Group B: PwHD without symptoms (approximately HD-ISS stage 0/1; [35] CAP score >70) (n=24)
- Group C: PwHD with early disease (approximately HD-ISS stage 2) [35] (n=24)
- Group D: PwHD with later disease (approximately HD-ISS stage 3) [35] (n=24)
- Group E: Healthy volunteers (n=36)

Table 1 describes the eligibility criteria for the study and for each of the groups. We have chosen to allow participants with a TFC score of 12 to participate in Groups A (see below) and B and not consider a score of 12, in the absence of other major symptoms, indicative of more advanced stages of HD. An example where a participant in these groups scores 12 on the TFC is unemployment which can occur without clinical signs or symptoms. This is in line with previous neuroimaging studies in PwHD and has also been taken into account in the HD-ISS classification. [38]

Pending interim analysis taking place at 25-50% completion of baseline assessments of groups B and E, a decision will be taken whether or not to introduce a Group A consisting of PwHD without symptoms and expected far to onset of motor symptoms (CAP score ≤70; approximately HD-ISS stage 0) [35] (n=24), as well as 12 additional healthy volunteers. The decision will be based on whether a significant marker difference between PwHD in Group B and healthy controls is present, supporting the rationale for investigating the earliest change in marker profile in further to expected motor onset PwHD (Group A). Phenoconversion in the iMarkHD study, separating participants in Group C from those in Group B, is defined by the occurrence of motor symptoms (TMS scores ≥7) and/or decline in total functional capacity (TFC scores 11 and 12). In those Group C participants in whom higher motor scores may be present (TMS 24-33) a further requirement is a score of >50 on

the symbol digit modalities test (SDMT) which has been suggested as a useful early neuropsychological sign to indicate disease process status prior to reaching motor diagnosis criteria.[36]

In cohort 1, the five healthy volunteers will undergo a screening visit to determine eligibility, followed by two visits for MR and MR retest scanning with no further visits. For Cohort 2, following a screening visit to determine eligibility, participants will undergo clinical, PET and MR imaging assessments at baseline, and 1- and 2-year follow-up (Figure 1). Study procedures for cohort 2 are outlined below.

Study sample and recruitment

Participants for this study will be recruited through King's College London and King's College Hospital, London, United Kingdom. In addition to local recruitment, PwHD will be referred by centres specialised in HD, currently consisting of 11 participant identification centres (PICs) from academic healthcare and National Health Service (NHS) organisations across the United Kingdom. The referring PICs and the clinical staff at King's College London will identify potentially eligible participants and their willingness to participate in the study. Recruitment will be supported through a public advertisement approved by the Health Research Authority (HRA). All participants will receive travel reimbursement as well as HRA-approved stipends for each attended study visit. Further reimbursement will be provided for overnight stay, where applicable, for participants travelling from outside London.

Sample size

The sample size of each group in the iMarkHD study has been based on previously reported differences in cross-sectional studies showing standardised differences (Cohen's d) between PwHD and healthy volunteers in PET markers to range between 1.8 ([^{11}C]MePPEP PET in the striatum) and 2.55 ([^{11}C]MePPEP PET in cortex) (unpublished data), in addition to differences between 0.5 ([^{11}C]IMA107 in substantia nigra), and 2.56 ([^{11}C]IMA107 in the putamen). [37] Based on this, 20 participants per group (100 in total), will provide 80% power to detect baseline differences between PwHD subgroups and healthy volunteers corresponding to a Cohen's d of 0.78 or larger with a one-sided test, 5% type I error rate. In order to ensure that 20 participants reach 2-year follow up, 24 participants will be recruited per group, to account for the expected approximate 20% dropout rate (10% attrition per year). Differences across groups will only be tested when differences versus healthy volunteers are found.

Power to detect longitudinal differences has been estimated for a mixed-model with repeated measures; one baseline and two follow up measurements, with a general correlation structure (assuming 0.5 between baseline and 2-years, and 0.8 between consecutive measurements). Based on 20 participants with complete follow up this will provide 80% power to detect standardized differences of 0.91 or higher, with a 2-sided 5% type I error rate, between PwHD participants and healthy volunteers. A previous study based on a small number of participants (n=12) has indeed shown that correlations between PDE-10A expression and probability for symptomatic conversion after 2 years, in far from motor onset PwHD, range from 0.59 in the higher motor-thalamic-nuclei/striatal, to 0.85 in the higher motor-thalamic-nuclei/striatopallidal. [37] It is expected that PET markers under investigation in this study will be more sensitive and detect larger changes earlier than previously established clinical and MRI markers. [2-4,6,32,33]

A sample size of 36 healthy volunteers has been chosen to adequately age match against three PwHD groups based on previous data in similar groups in the TRACK-HD study at visit 1. [2-4]

Study procedures

For all participants in the study a screening visit will be performed to assess eligibility and to enable grouping of participants based on the measures outlined in Table 1. During the screening visit participants will be consented to the study and demographics, such as age and sex, will be collected. In addition, past medical history will be obtained along with current medication use, and a medical and neurological examination performed, including Snellen chart and Rinne and Weber testing. Also, vital signs will be recorded and an electrocardiogram performed. To check for exclusion criteria, blood and urine samples will be obtained along with analysis of *HTT* CAG-repeat length that will be anonymously linked to the Enroll-HD database, [38] as well as performing the Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV). Finally, to group participants into one of the iMarkHD groups, the Unified Huntington's Disease Rating Scale (UHDRS) [39] Total Motor Score (UHDRS-TMS), Diagnostic Confidence Level (UHDRS-DCL), and Total Functional Capacity (UHDRS-TFC) will be assessed, along with Symbol Digit Modalities Test (SDMT), [40] Hospital Anxiety and Depression Scale (HADS), [41] and Mini Mental State Examination (MMSE) scores. [42]

As mentioned above, for Cohort 1 the five healthy volunteers will undergo a screening visit, followed by a MR visit and a MR retest visit. These two latter visits will consist of MR scanning taking up to two hours per visit. MRI scanning will be performed on a GE MR750 3Tesla MRI scanner (for Cohort 1 and 2). In Cohort 2, all participants will complete a total of 10 study visits, consisting of a screening visit (visit 1), followed by a combined clinical and MR visit (visit 2) and two PET visits (visits 3 and 4; during each PET visit participants will undergo two PET scans), at baseline, year 1

(visits 5, 6 and 7), and year 2 (visits 8, 9, and 10). An overview of study visits is provided in Figure 1 and clinical outcomes are summarised in Table 2.

PET targets and MR sequences that will be performed are summarised in Figures 2 and 3 and clinical assessments are summarised in Table 2. For Visits 2, 5 and 8, an effort will be made to perform clinical assessments and the MR sessions in the same visit, but based on availability it is possible that these visits may be performed over two separate days; for Visits 3, 4, 6, 7, 9 and 10, an effort will be made to perform two PET scans on the same day, but based on availability it is possible that these visits may be performed over two separate days. The overall study design is summarised in figure 1.

For Cohort 1 the first MR visit will be performed within 30 days of screening, and the second visit will be performed within 14 days of the first MRI visit. For Cohort 2, visit V2 will be performed within 40 days of screening (V1), and the PET visit V3 will be performed within 30 days from V2 with V4 taking place within 14 days from V3. The same timelines (i.e. first PET visit within 30 days of MRI, and clinical visit and second PET visit within 14 days of first PET visit) will be repeated for year 1 and year 2 follow-up.

Data analysis

All PET and MRI outcome markers will be analysed using a generalised linear model, with the absolute values of each marker as dependent variable, and subgroup categorical variables as independent, in addition to sex and age as covariates in the model. Benjamini–Hochberg false discovery rate correction at 0.1 to correct for multiple testing will be used. Marginal means, and standardised effect sizes will be used to report differences across groups. In addition, partial correlations across different markers and clinical measurements, correcting for age and sex, will be

used. To analyse longitudinal differences across groups fit linear mixed models for repeated measures, with random intercept and slope, will be used. The presence of non-linear changes will be tested by adding a quadratic term for time. The outcome variable will consist of the absolute levels of the individual PET and MRI markers at baseline and the two annual follow up measurements, and categorical subgroups as the main independent variable.

Data from clinical assessments, including rating scales to measure disease progression, demographics, and other baseline characteristics, will be summarised using descriptive statistics and linked to PET and MRI markers using mixed linear models and correlations, where appropriate.

Data handling and storage

All data will be handled confidentially and processed, stored, and disposed of in accordance with the General Data Protection Regulation and all applicable legal and regulatory requirements in the UK. Data collection will be performed with the use of paper and secured electronic case report forms (CRF). The database will only be accessible for authorised personnel via a unique login and user ID. All participant data will be coded, and any identifiable data removed prior to uploading. All paper source documents will be stored in a secure location for the duration of the study and any time following this depending on legal and regulatory requirements.

PET neuroimaging data will initially be stored on local servers at the PET imaging acquisition centre and within one week after image acquisition securely transferred via Microsoft One Drive to the department of neuroimaging at King's College London. Here, together with the MRI neuroimaging data, the data will be securely stored on internal servers. At the end of the study, these data will also be transferred via secure environments to CHDI.

Benefits and risks assessment

As this is a non-interventional study, no excessive risks or ethical concerns are anticipated. Any adverse events will likely be restricted to blood sampling and MRI and PET procedures and will be recorded in the paper and electronic CRFs. In relation to PET imaging the main risk to participants consists of the risk due to ionising radiation exposure from injected radioligands and associated low dose computed tomography (CT). The target dose for the individual PET tracers is 200 MBq (between 0.76 and 1.08 mSv per scan) in addition to the effective radiation dose associated with CT scanning (0.36 mSv per scan). As such, it has been calculated that the total effective dose for each participant is 5.08 mSv per year and 15.20 mSv in total across all study visits. This compares to the average 2.3 mSv of annual background radiation for UK residents. The radiation dose has been chosen as providing acceptable image quality while minimising participant risk. While any additional ionising radiation exposure may increase the risk of future malignancy, this risk is mitigated by clinical safety monitoring and safety assessment procedures during study participation and inclusion/exclusion criteria and restrictions applied during study participation. As there are no alternative methods to PET targets in our study, we feel the added risk due to the additional radiation exposure is justified.

Serious adverse events will also be reported to the study sponsor. Planning of study visits will be done depending on participants' availability and wishes, insofar as possible. The frequency and duration of the scanning sessions might cause some distress to participants, but this will be specifically assessed as part of the eligibility criteria. All researchers involved in this study are experienced in neurological clinical research. Further potential untoward effects will be minimised by pilot testing of MRI sequences in Cohort 1, consisting of healthy volunteers.

Discussion

The aim of the iMarkHD study is to evaluate how specific molecular, functional, and structural changes may influence the development of symptoms in PwHD and how this might aid in the development of imaging biomarkers that can characterise and predict events over the course of the disease. Ultimately, the outcomes of this study may be used as outcome measures in future clinical trials and identification of novel treatment targets. PET molecular imaging is a powerful in vivo tool to investigate brain function such as metabolism, and receptor and enzyme distributions. As an analytical imaging method, PET has the potential to give both structural and kinetic information and, in comparison with other imaging techniques with high sensitivity and temporal resolution. [43] The PET assessment will be complemented by MRI to provide information on structural and functional organisation of the brain in PwHD, as well as brain volume, perfusion, iron levels, and structural and functional connectivity. The combination of these PET and MRI techniques have the potential to significantly contribute to our understanding of the complexity of HD. [7]

As with any study, some challenges are expected to arise during the implementation process of the iMarkHD study. These include participant recruitment in a single centre study, participant retention in relation to the relatively high number of imaging sessions, as well as factors related to eligibility criteria and the inclusion of PwHD without symptoms (HD-ISS stages 0-1). We aim to address these challenges by actively engaging with PICs to maximise our recruitment area and to engage to the largest extent possible with PwHD across the UK; currently, we are working with 11 PICs in the UK with a large geographical distribution and catchment area. This will enable us to screen a large population of PwHD, offsetting some of the stricter eligibility criteria in the study protocol. Moreover, as the HD community is generally very motivated with high willingness to participate in

research, we expect to overcome the challenges in relation to recruitment with accrual of sufficient participants for each group. Another possible challenge is the adequate classification of each participant into the relevant groups described in the iMarkHD study protocol. Rather than relying on what is considered as increasingly outdated, premanifest and manifest HD, [35] we have included a more dynamic classification system based on TFC and TMS, in addition to CAP scores, similar to designs used in other HD studies. [44] This system will allow for more accurate classification of participants and reduce subjective rater classification into premanifest and manifest. [35]

The unique aspect of the iMarkHD study is mainly related to its multimodal assessment of possible HD markers. Since it is likely that multiple pathophysiological changes influence disease progression over time, combining PET and multi-modal MRI assessments in this study will likely provide a comprehensive examination of the molecular, functional, and structural framework of HD progression in the brain. In addition, the inclusion of a group of healthy control volunteers to compare PET and MRI measurements at different disease stages will further contribute to our understanding of the development of symptoms and disease progression in HD. As such, the study will provide a solid base for the identification of treatment targets and could provide novel outcome measures for future clinical trials evaluating new targeted therapies. PET imaging offers molecular insights crucial for understanding disease mechanisms and progression, while complementing other imaging modalities such as MRI. In addition, developments over the years have resulted in safer radiotracer development utilising lower effective doses. Therefore, the use of PET in future clinical trials and other clinical research outweigh the risks when used responsibly.

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Conflict of interest

The authors declare that the research described is conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Rojas NG, Cesarini ME, Peker G, et al. Review of Huntington’s disease: from basics to advances in diagnosis and treatment. *J Neurol Res* 2022; 12: 93-113.

2. Tabrizi SJ, Scahill RI, Owen G, et al. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. *Lancet Neurol* 2013; 12: 637-649.

3. Tabrizi SJ, Scahill RI, Durr A, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. *Lancet Neurol* 2011; 10: 31-42.

4. Tabrizi SJ, Langbehn DR, Leavitt BR, et al. Biological and clinical manifestations of Huntington's disease in the longitudinal TRACK-HD study: cross-sectional analysis of baseline data. *Lancet Neurol* 2009; 8: 791-801.

5. Langbehn DR, Brinkman RR, Falush D, et al. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. *Clin Genet* 2004; 65: 267-77.

6. Tabrizi SJ, Reilmann R, Roos RA, et al. Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. *Lancet Neurol* 2012; 11: 42-53.

7. Niccolini F, Politis M. Neuroimaging in Huntington's disease. *World J Radiol* 2014; 6: 301-12.

8. Häggkvist J, Tóth M, Tari L, et al. Longitudinal small-animal PET imaging of the zQ175 mouse model of Huntington disease shows in vivo changes of molecular targets in the striatum and cerebral cortex. *J Nucl Med* 2017; 58: 617-22.

9. Van Laere KJ, Sanabria-Bohórquez SM, Mozley DP, et al. 11C-MK-8278 PET as a tool for pharmacodynamic brain occupancy of histamine 3 receptor inverse agonists. *J Nucl Med* 2014; 55: 65-72.

10. Terry GE, Liow J-S, Zoghbi SS, et al. Quantitation of cannabinoid CB1 receptors in healthy human brain using positron emission tomography and an inverse agonist radioligand. *Neuroimage* 2009; 48: 362-70.

11. Boscutti G, Rabiner EA, Plisson C. PET Radioligands for imaging of the PDE10A in human: current status. *Neurosci Lett* 2019; 691: 11-7.

12. Warner JH, Long JD, Mills JA, et al. Standardizing the CAP score in Huntington’s disease by predicting age-at-onset. *J Huntingtons Dis* 2022; 11(2): 153-71.

13. Wilson H, De Micco R, Niccolini F, et al. Molecular imaging markers to track Huntington's disease pathology. *Front Neurol* 2017; 8: 11.

14. Ooms M, Rietjens R, Rangarajan JR, et al. Early decrease of type 1 cannabinoid receptor binding and phosphodiesterase 10A activity in vivo in R6/2 Huntington mice. *Neurobiol Aging* 2014; 35: 2858-69.

15. Hebb A, Robertson H, Denovan-Wright E. Striatal phosphodiesterase mRNA and protein levels are reduced in Huntington′ s disease transgenic mice prior to the onset of motor symptoms. *Neuroscience* 2004; 123: 967-81.

16. Russell DS, Barret O, Jennings DL, et al. The phosphodiesterase 10 positron emission tomography tracer,[18F] MNI-659, as a novel biomarker for early Huntington disease. *JAMA Neurol* 2014; 71: 1520-8.

17. Fazio P, Fitzer-Attas CJ, Mrzljak L, et al. PET molecular imaging of phosphodiesterase 10A: An early biomarker of Huntington's disease progression. *Mov Disord* 2020; 35: 606-15.

18. Cristino L, Bisogno T, Di Marzo V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nat Rev Neurol* 2020; 16: 9-29.

19. Mievis S, Blum D, Ledent C. Worsening of Huntington disease phenotype in CB1 receptor knockout mice. *Neurobiol Dis* 2011; 42: 524-9.

20. Glass M, Van Dellen A, Blakemore C, et al. Delayed onset of Huntington′ s disease in mice in an enriched environment correlates with delayed loss of cannabinoid CB1 receptors. *Neuroscience* 2004; 123: 207-12.

21. Van Laere K, Casteels C, Dhollander I, et al. Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. *J Nucl Med* 2010; 51: 1413-7.

519 22. Pang TY, Du X, Zajac MS, et al. Altered serotonin receptor expression is associated with
520 depression-related behavior in the R6/1 transgenic mouse model of Huntington's disease. *Hum Mol*
521 *Genet* 2009; 18: 753-66.
522 23. Zajac MS, Renoir T, Perreau VM, et al. Short-term environmental stimulation
523 spatiotemporally modulates specific serotonin receptor gene expression and behavioral pharmacology
524 in a sexually dimorphic manner in Huntington's disease transgenic mice. *Front Mol Neurosci* 2018;
525 11: 433.
526 24. Martínez-Gopar PE, Pérez-Rodríguez MJ, Rodríguez-Manzo G, et al. Mast cells and
527 histamine are involved in the neuronal damage observed in a quinolinic acid-induced model of
528 Huntington's disease. *J Neurochem* 2022; 160: 256-70.
529 25. Shan L, Hofman MA, van Wamelen DJ, et al. Diurnal fluctuation in histidine decarboxylase
530 expression, the rate limiting enzyme for histamine production, and its disorder in neurodegenerative
531 diseases. *Sleep* 2012; 35: 713-5.
532 26. van Wamelen DJ, Shan L, Aziz NA, et al. Functional increase of brain histaminergic
533 signaling in Huntington's disease. *Brain Pathol* 2011; 21: 419-27.
534 27. Moreno-Delgado D, Puigdellívol M, Moreno E, et al. Modulation of dopamine D1 receptors
535 via histamine H3 receptors is a novel therapeutic target for Huntington's disease. *Elife* 2020; 9:
536 e51093.
537 28. Rosas H, Goodman J, Chen Y, et al. Striatal volume loss in HD as measured by MRI and the
538 influence of CAG repeat. *Neurology* 2001; 57: 1025-8.
539 29. Chen JJ, Salat DH, Rosas HD. Complex relationships between cerebral blood flow and brain
540 atrophy in early Huntington's disease. *Neuroimage* 2012; 59: 1043-51.
541 30. Werner CJ, Dogan I, Saß C, et al. Altered resting-state connectivity in Huntington's disease.
542 *Hum Brain Map* 2014; 35: 2582-93.

31. Hutchison RM, Womelsdorf T, Allen EA, et al. Dynamic functional connectivity: promise, issues, and interpretations. *Neuroimage* 2013; 80: 360-78.

32. Novak MJ, Seunarine KK, Gibbard CR, et al. White matter integrity in premanifest and early Huntington's disease is related to caudate loss and disease progression. *Cortex* 2014; 52: 98-112.

33. Novak MJ, Seunarine KK, Gibbard CR, et al. Basal ganglia-cortical structural connectivity in Huntington's disease. *Hum Brain Map* 2015; 36: 1728-40.

34. Ng AC, Poudel G, Stout JC, et al. Iron accumulation in the basal ganglia in Huntington's disease: cross-sectional data from the IMAGE-HD study. *J Neurol Neurosurg Psychiatry* 2016; 87: 545-9.

35. Tabrizi SJ, Schobel S, Gantman EC, et al. A biological classification of Huntington's disease: the Integrated Staging System. *Lancet Neurol* 2022; 21: 632-44.

36. Heim B, Peball M, Saft C, et al. Time will tell: Decision making in premanifest and manifest Huntington's disease. *Brain Behav* 2020; 10: e01843.

37. Niccolini F, Haider S, Reis Marques T, et al. Altered PDE10A expression detectable early before symptomatic onset in Huntington's disease. *Brain* 2015; 138: 3016-29.

38. Landwehrmeyer GB, Fitzer-Attas CJ, Giuliano JD, et al. Data analytics from Enroll-HD, a global clinical research platform for Huntington's disease. *Mov Disord Clin Pract* 2017; 4: 212-24.

39. Huntington Study Group. Unified Huntington's Disease Rating Scale: reliability and consistency. *Movement Disord* 1996; 11: 136-42.

40. Smith A. Symbol digit modalities test: Western psychological services Los Angeles; 1973.

41. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiat Scand* 1983; 67: 361-70.

42. Folstein MF, Robins LN, Helzer JE. The Mini-Mental State Examination. *Arch Gen Psychiatry* 1983; 40: 812.

43. Phelps ME. PET: the merging of biology and imaging into molecular imaging. *J Nucl Med* 2000; 41: 661-81.

44. Mühlbäck A, Frank W, Klempířová O, et al. Validation study of a German cognitive battery for Huntington's disease: relationship between cognitive performance, functional decline, and disease burden. *Arch Clin Neuropsychol* 2020; 36: 74-86.

Table 1. Eligibility criteria per group for the iMarkHD study. Abbreviations: PwHD: people with Huntington's disease; TMS: Unified Huntington's Disease Rating Scale (UHDRS) Total Motor Score; TFC: UHDRS Total Functional Capacity; CAP: CAG Age Product; SDMT: Symbol Digit Modalities Test; HD: Huntington's disease.

Inclusion criteria for the entire cohort 1. Age ≥ 21 to ≤ 75 years at the time of screening 2. Adequate visual (Snellen chart) and auditory (Rinne and Weber tests) acuity to complete the psychological testing as determined by the investigator 3. Capability of giving informed consent 4. Willing to comply with highly effective contraceptive measures 5. Vital signs (after three minutes resting in a supine position) which are within the following ranges at the screening evaluation: a. Systolic blood pressure 85-160 mmHg b. Diastolic blood pressure 50-100 mmHg c. Heart rate 45-110 bpm 6. Absence of clinically significant diseases (excluding disease under study) or clinically significant abnormal laboratory values 7. Ability to travel to imaging and clinical assessment centres in London
Group A (PwHD without symptoms far from expected motor onset) 1. PwHD with ≥40 CAG repeats in the *Htt* gene 2. TMS ≤ 6 AND TFC ≥ 12 AND CAP ≤ 70
Group B (PwHD without symptoms) 1. PwHD with ≥ 40 CAG repeats in the *Htt* gene 2. TMS ≤ 6 AND TFC ≥ 12 AND CAP > 70
Group C (PwHD with early disease) 1. PwHD with ≥ 40 CAG repeats in the *Htt* gene 2. If one of the following criteria is met a. TMS ≤ 6 AND TFC = 11 b. TMS is between 7 and 23 inclusive AND TFC is between 11 and 13 inclusive c. TMS is between 24 and 33 inclusive AND SDMT > 50 AND TFC is between 11 and 13 inclusive
Group D (PwHD with later disease) 1. PwHD with ≥ 40 CAG repeats in the *Htt* gene 2. If one of the following criteria is met: a. TMS ≤ 6 AND TFC is between 7 and 10 inclusive b. TMS is between 7 and 23 inclusive AND TFC is between 8 and 10 inclusive c. TMS is between 24 and 33 inclusive AND SDMT > 50 AND TFC is between 7 and 10 inclusive d. TMS is between 7 and 23 inclusive AND TFC = 7 e. TMS > 23 AND SDMT ≤ 50 AND TFC is between 7 and 13 inclusive f. TMS > 33 and SDMT > 50 AND TFC is between 7 and 13 inclusive
Group E (Healthy controls) 1. Age (±8 years) and sex matched with PwHD

2. No known family history of HD or have known family history of HD but have been tested for the huntingtin gene glutamine codon expansion and are not at genetic risk for HD (CAG < 36)
Exclusion criteria for the entire cohort 1. Presence or history of other neurological condition likely to interfere with imaging or abnormal neurologic examination findings suggestive of a central nervous system pathology (other than HD) 2. Presence of history of primary psychiatric disorders unrelated to HD 3. Participants using any medications with known actions on any of the PET targets 4. Pregnancy confirmed by a positive urine pregnancy test 5. Participants who are currently breastfeeding or intend to breastfeed during the study 6. Contraindication to MRI 7. History of alcoholism or substance abuse within three years prior to study entry 8. Failure of drug screen for substances of abuse such as amphetamines, barbiturates, benzodiazepines, methadone, opiates, cocaine, cannabinoids, phencyclidine, and creatine 9. History of cancer 10. Claustrophobia 11. Significant back pain that makes prolonged laying on the PET or MRI scanner intolerable 12. Contraindication for arterial cannulation as judged by the Allen test and the laboratory blood screening for coagulopathy 13. Inability to communicate or cooperate with the Principal Investigator/iMarkHD Team for any reason 14. Participants who are enrolled in or participated in clinical trials testing the efficacy of novel therapeutics with action on the specific PET targets being tested within 3 months of screening 15. Any concurrent conditions that could interfere with the safety and/or tolerability measurements.

Table 2. Clinical assessments in cohort 2 of the iMarkHD study. Abbreviations: UHDRS: Unified Huntington's Disease Rating Scale; TMS: Total Motor Score; DCL: Diagnostic Confidence Level; TFC: Total Functional Capacity; FAS: Functional Assessment Scale; IS: Independence Scale; HDQoL: Huntington's Disease Quality of Life; HD: Huntington's disease.

Motor assessments	**Cognitive assessments**
UHDRS-TMS UHDRS-DCL	Cognitive Assessment Battery: • Symbol Digit Modalities Test • Emotion Recognition • Trail Making Test • Hopkins Verbal Learning Test Revised • Paced Tapping Test • Tower-Z task Stroop Colour and Word Reading Test and Interference Test Categorical Verbal Fluency Test Mini-Mental State Examination
Functional assessments UHDRS-TFC UHDRS-FAS UHDRS-IS Physical Performance Test HDQoL	
Neuropsychiatric assessment Problem Behaviour Assessment Short Version Hospital Anxiety and Depression Scale Apathy Evaluation Scale HD Clinical Characteristics Assessment	

Figure 1. Overall study design of the iMarkHD study. Abbreviations: MRI: magnetic resonance imaging; PET: positron emission tomography; PwHD: people with Huntington's disease.

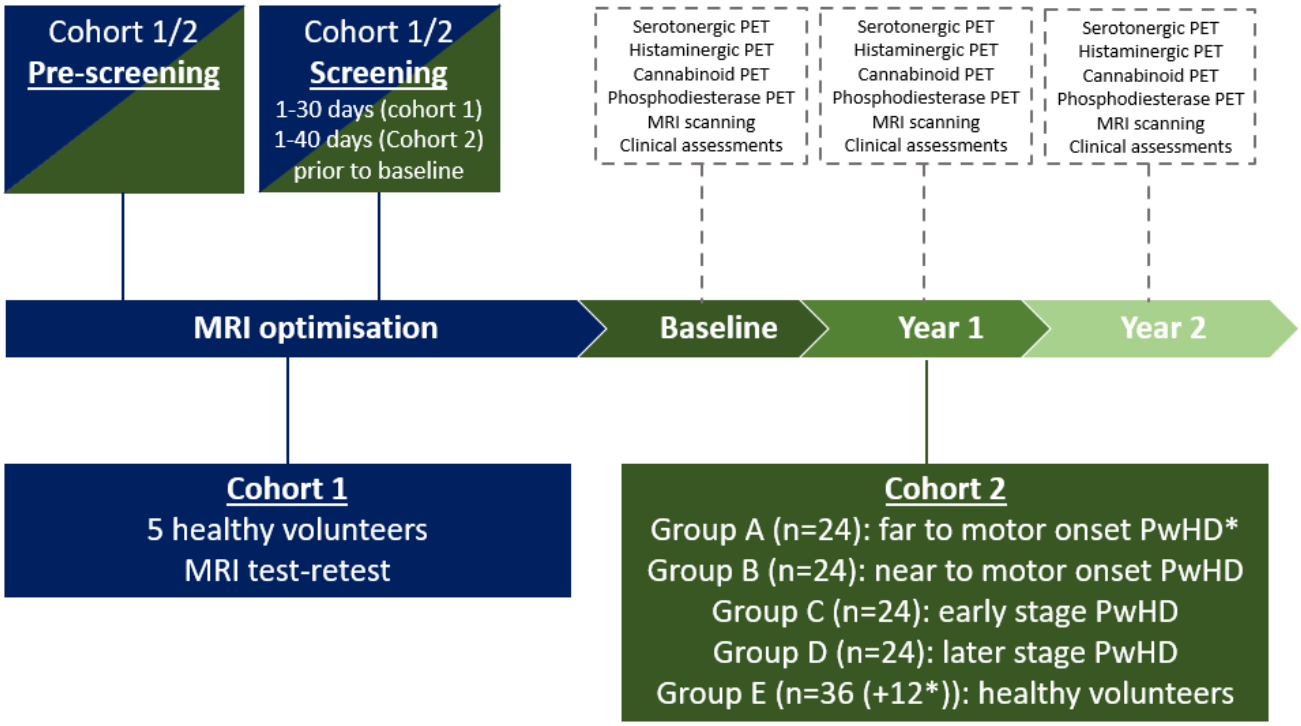

Figure 2. Positron Emission Tomography outcomes for the iMarkHD study. The figure shows receptors (centre panel) and PET tracers (bottom panel) with illustrative scan results. Abbreviations: 5HT2AR: Serotonin receptor 2A; H3R: Histamine 3 receptor; CB1R: Cannabinoid 1 receptor; PDE10A: phosphodiesterase 10A enzyme.

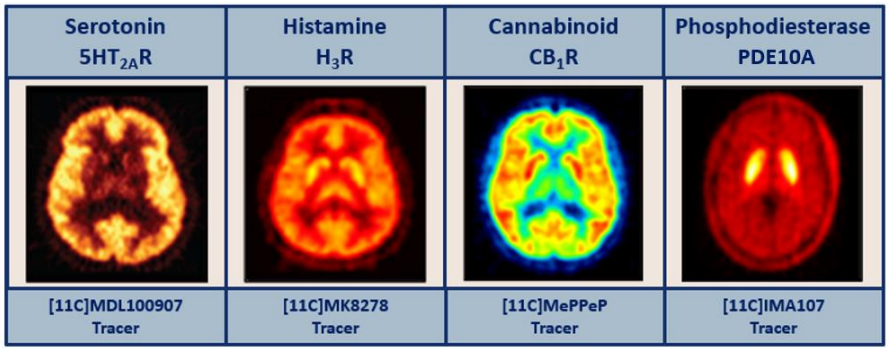

Figure 3. Magnetic resonance imaging outcomes for the iMarkHD study. The figure shows the different magnetic resonance imaging (MRI) outcomes (top panel) and what these outcomes represent (bottom panel) alongside illustrative scan results. Abbreviations: MP-RAGE: Magnetisation prepared rapid acquisition gradient echo; MPM: Multi-Parametric Mapping; DTI: Diffusion Tensor Imaging; fMRI: functional MRI; ASL: Arterial Spin Labelling; QSM: Quantitative Susceptibility Mapping.