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Zande, N.A. van de; Bulk, M.; Najac, C.; Weerd, L. van der; Bresser, J. de; Lewerenz, J.; ... ; Bot, S.T. de

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Study protocol of IMAGINE-HD: Imaging iron accumulation and neuroinflammation with 7T-MRI + CSF in Huntington's disease

Nadine Anniek van de Zande [a,1,*], Marjolein Bulk [b,1], Chloé Najac [b], Louise van der Weerd [c,d], Jeroen de Bresser [c], Jan Lewerenz [e], Itamar Ronen [f,2], Susanne Tamara de Bot [a,2]

[a] Department of Neurology, Leiden University Medical Centre, Leiden, The Netherlands
[b] C.J. Gorter MRI Center, Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands
[c] Department of Radiology, Leiden University Medical Centre, Leiden, The Netherlands
[d] Department of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands
[e] Department of Neurology, University of Ulm, Ulm, Baden-Württemberg, Germany
[f] Clinical Imaging Sciences Centre, Brighton and Sussex Medical School, Brighton, United Kingdom

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ABSTRACT

Introduction: Strong evidence suggests a significant role for iron accumulation in the brain in addition to the well-documented neurodegenerative aspects of Huntington's disease (HD). The putative mechanisms by which iron is linked to the HD pathogenesis are multiple, including oxidative stress, ferroptosis and neuroinflammation. However, no previous study in a neurodegenerative disease has linked the observed increase of brain iron accumulation as measured by MRI with well-established cerebrospinal fluid (CSF) and blood biomarkers for iron accumulation, or with associated processes such as neuroinflammation. This study is designed to link quantitative data from iron levels and neuroinflammation metabolites obtained from 7T MRI of HD patients, with specific and well-known clinical biofluid markers for iron accumulation, neurodegeneration and neuroinflammation. Biofluid markers will provide quantitative measures of overall iron accumulation, neurodegeneration and neuroinflammation, while MRI measurements on the other hand will provide quantitative spatial information on brain pathology, neuroinflammation and brain iron accumulation, which will be linked to clinical outcome measures.

Methods: This is an observational cross-sectional study, IMAGINE-HD, in HD gene expansion carriers and healthy controls. We include premanifest HD gene expansion carriers and patients with manifest HD in an early or moderate stage. The study includes a 7T MRI scan of the brain, clinical evaluation, motor, functional, and neuropsychological assessments, and sampling of CSF and blood for the detection of iron, neurodegenerative and inflammatory markers. Quantitative Susceptibility Maps will be reconstructed using T2* weighted images to quantify brain iron levels and Magnetic Resonance Spectroscopy will be used to obtain information about neuroinflammation by measuring cell-specific intracellular metabolites' level and diffusion. Age and sex matched healthy subjects are included as a control group.

Discussion: Results from this study will provide an important basis for the evaluation of brain iron levels and neuroinflammation metabolites as an imaging biomarker for disease stage in HD and their relationship with the salient pathomechanisms of the disease on the one hand, and with clinical outcome on the other.

1. Background

Huntington's Disease (HD) is a rare, autosomal dominant inherited, progressive, neurodegenerative disorder, caused by CAG repeat expansion of exon 1 in the HTT gene on chromosome 4 (Macdonald, 1993). The disease manifests at an age of onset between 30 and 50 years (Myers, 2004) and is characterized by a variety of motor disturbances (typically chorea and dystonia), cognitive impairment and behavioural

* Corresponding author.
E-mail address: n.a.van_de_zande@lumc.nl (N.A. van de Zande).
[1] Should both be considered as first authors.
[2] Should both be considered as last author.

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changes (Roos, 2010). The size of the CAG repeat expansion accounts for approximately 60–70% of variance in disease onset as the CAG repeat expansion length is inversely correlated with age of disease onset (Novak and Tabrizi, 2010; Sturrock and Leavitt, 2010).

Neurodegeneration in HD is a direct result of the CAG repeat expansion. The expansion results in intracellular aggregation of the mutant huntingtin protein, causing neuronal dysfunction and neuronal loss of the medium spiny neurons of the striatum. This eventually results in atrophy of the striatum and the external segment of the pallidum. As the disease progresses, white matter, thalamus, cerebral cortex, and other subcortical grey matter structures also exhibit marked atrophy, resulting in whole brain volume loss (Vonsattel et al., 2011).

In addition to the well-documented neurodegenerative aspect of HD, strong evidence suggests a significant role for iron accumulation in HD (Dexter et al., 1991; Domínguez D et al., 2016; Dumas et al., 2012; Muller and Leavitt, 2014; van Bergen et al., 2016; van den Bogaard et al., 2013; Ward et al., 2014; Zecca et al., 2004; Crotti and Glass, 2015; Möller, 2010; Silvestroni et al., 2009; Kumar and Ratan, 2016; Paul et al., 2014; Stack et al., 2008; Chen et al., 2019; Bulk et al., 2020). Iron dysregulation can cause cell toxicity via several mechanisms, including oxidative stress and ferroptosis, eventually followed by cell death (Ward et al., 2014; Zecca et al., 2004). Different MRI techniques have been used to measure brain iron levels in HD, all suggesting an increase in iron content in the putamen, caudate nucleus and pallidum (Domínguez D et al., 2016; Dumas et al., 2012; van den Bogaard et al., 2013; Chen et al., 2019; Bulk et al., 2020; Doan et al., 2014; Jurgens et al., 2010; Rosas et al., 2012). Interestingly, brain levels were found to be already increased in premanifest patients and further increase when the disease progresses (Domínguez D et al., 2016; van Bergen et al., 2016; Johnson et al., 2021).

Previous studies, focusing on iron accumulation in neurodegenerative diseases such as Alzheimer's disease as well as HD, have shown that iron appears to be absorbed by activated microglia, the resident macrophages of the brain (Meadowcroft et al., 2015; Thomsen et al., 2015; Zeineh et al., 2015; Pavese et al., 2006; Sapp et al., 2001). It is therefore thought that cerebral iron accumulation in humans is at least in part explained by iron-accumulating microglia in affected brain regions, linking iron to inflammation, another key pathological mechanism in neurodegenerative diseases (Crotti and Glass, 2015; Möller, 2010; Thomsen et al., 2015; Edison et al., 2018; Heneka et al., 2015; Urrutia et al., 2014). Neuroinflammation is also increasingly recognized as a strong component of HD pathogenesis that causes disease progression, via the cell-autonomous proinflammatory activation of microglia due to the expression of mutant HTT (Pavese et al., 2006; Sapp et al., 2001; Crotti and Glass, 2015; Möller, 2010; Silvestroni et al., 2009; Simmons et al., 2007; Tai et al., 2007; Tai et al., 2007). Therefore, iron neuroimaging seems a good candidate to gain neuroimaging biomarkers for disease progression in HD, reflecting neuroinflammation in HD. So far, no previous study in a neurodegenerative disease has linked the observed increase of iron accumulation as measured by MRI with direct, well-established CSF markers for iron accumulation and neuroinflammation. Previous studies have already shown the clinical utility of CSF biomarkers for iron accumulation and neuroinflammation: for example, neuroinflammatory markers YKL-40 and IL-6 were found to be elevated in HD patients, and CSF markers for iron accumulation were shown to predict a more rapid cognitive decline in AD (Ayton et al., 2015; Björkqvist et al., 2008; Vinther-Jensen et al., 2016).

Most of the studies investigating iron accumulation in HD-brains with MRI have been qualitative, or semi-quantitative at most, and thus their sensitivity to assess the association between striatal and cortical iron content with clinical hallmarks of the disease is limited (Haacke et al., 2005; Ropele and Langkammer, 2017). Quantitative susceptibility mapping (QSM) is an MRI method that allows accurate iron quantification in the brain, based on the quantitative link between iron concentration in tissue and the local phase of the MRI signal. QSM provides a direct quantitative measure of tissue magnetic susceptibility, and thus of iron content (Deistung et al., 2017). QSM has been shown to correlate linearly with tissue iron content in grey matter, with high sensitivity and specificity to tissue iron changes, as demonstrated by post-mortem studies on brain tissue from healthy subjects and patients with neurodegenerative diseases (Deistung et al., 2013; Langkammer et al., 2012; Langkammer et al., 2018; Wang et al., 2017). QSM at ultrahigh field (7T) is particularly advantageous, since MRI at higher magnetic field strength is more sensitive to tissue-related magnetic field disturbances caused by presence of iron and allows scanning at higher resolution due to the increased signal to noise ratio (Ladd et al., 2018).

In addition to the neuroinflammation markers in CSF, we will also measure the level of brain metabolites using proton Magnetic Resonance Spectroscopy (^{1}H MRS). ^{1}H MRS provides information on intracellular processes, as metabolites of interest are primarily located in the intracellular space of neurons and glial cells. While glutamate (Glu) and total N-acetyl-aspartate (tNAA) are primarily found in neurons, myo-inositol (mI) and choline (Cho) are mostly concentrated in glia (Chang et al., 2013; Urenjak et al., 1993). When combined with diffusion-weighting spectroscopy (DWS), cytomorphological changes in the intracellular space can be evaluated (De Marco et al., 2022; Ronen et al., 2015). Previous studies using DWS have shown changes in glial cells comparable to earlier histological studies (De Marco et al., 2022). ^{1}H MRS and DWS therefore offer us the possibility to evaluate cell-specific markers of neuroinflammation. This is a highly innovative field within Huntington's Disease, as changes in neuroinflammation metabolites are seen (Sturrock et al., 2010), but until now there is no pathognomic alteration of any metabolite for HD (Reynolds et al., 2005; Adanyeguh et al., 2018). However, in other diseases, these imaging techniques have been used more widely. DWS has shown to be a useful technique, sensitive to glial cytomorphological changes in neuroinflammatory disease like MS, neuropsychiatric Systemic Lupus Erythematosus and LPS-induced inflammation (De Marco et al., 2022; Wood et al., 2017; Ercan et al., 2016). Previous MRS studies assessing patients with various neuroinflammatory and neurodegenerative disorders, including Alzheimer's disease, show elevated glial metabolites, such as mI and Cho in the affected brain regions (Chang et al., 2013).

In IMAGINE-HD, we will link the brain iron levels obtained from QSM of pre-manifest, early and moderate manifest HD patients with specific and well-known clinical CSF markers for iron accumulation, neurodegeneration and neuroinflammation. CSF markers will provide quantitative measures of overall iron accumulation, neurodegeneration and neuroinflammation, while MRI measurements provide quantitative information on the spatial distribution of brain pathology. This study combines neuroimaging, fluid biomarkers and clinical characteristics (Fig. 1) in a clinically and genetically well-established HD cohort. This will provide an important basis for the evaluation of brain iron levels as a possible imaging biomarker for disease stage and ultimately disease progression in HD and their relationship with the salient pathophysiological mechanisms of the disease on the one hand, and with clinical outcome on the other. Besides that, we will use ^{1}H MRS and DWS to evaluate changes in metabolism and microstructure associated with neuroinflammation. We will link this data with the iron measurements and biofluid data to extend our knowledge of the pathophysiology and to evaluate whether neuroimaging of neuroinflammation could also be a useful method to obtain markers for disease stage. Although HD is a monogenic disease, it remains unknown what mechanisms cause the considerable variation in clinical characteristics, as some patients have more psychiatric or cognitive disturbances and others show more motor symptoms. What determines the difference in clinical phenotype, is still unclear. Neuropsychiatric, neuropsychological and neurological outcome measures can be linked to measurements of neuroimaging and CSF biomarkers to fill this gap of knowledge.

Specific objectives include the following:

(1) To quantify brain iron accumulation in patients with HD (at different disease stages) using quantitative susceptibility

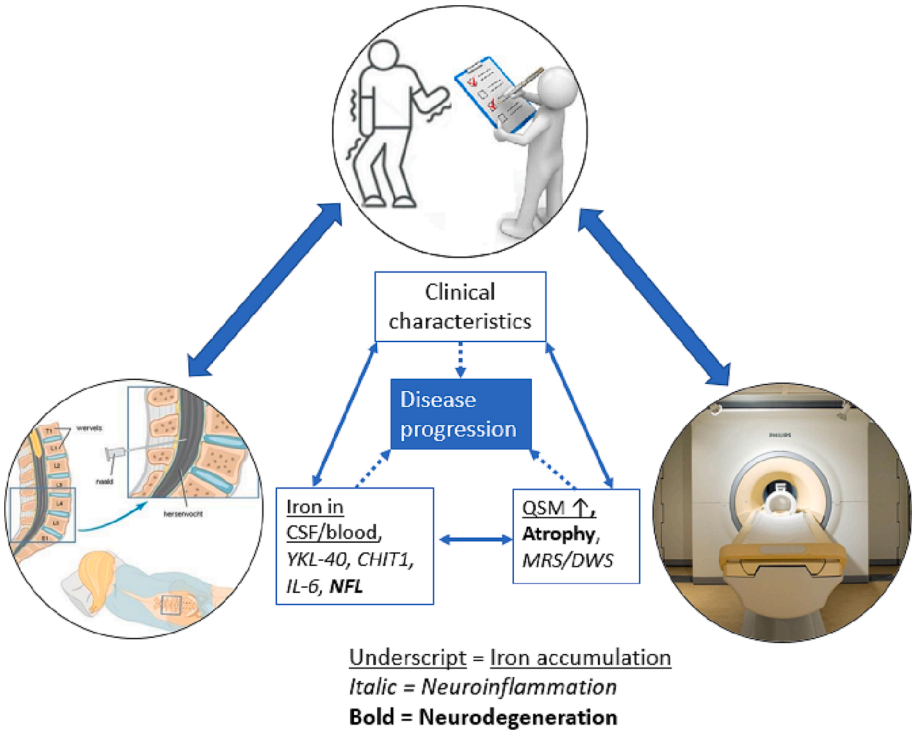

Fig. 1. Three modalities covered by the study: neurological and neuropsychological assessments, CSF and 7T-MRI, showing the pathophysiological mechanisms and associated markers that will be studied. *Abbreviations: CHIT-1 = Chitinase 1, CSF = Cerebrospinal fluid, DWS = Diffusion Weighted Spectroscopy, IL = Interleukin, MRS = Magnetic Resonance Spectroscopy, NFL = Neurofilament light.*

mapping (QSM) at ultrahigh field (7T) MRI and compare this with healthy controls;

(2) To link QSM results with specific and well-known biofluid markers for iron, neurodegeneration, and neuroinflammation;

(3) To quantify levels of metabolites (e.g. Cho, mI), using ^{1}H MRS, and to measure their diffusion rate by looking at the Apparent Diffusion Coefficient (ADC), using DWS. We will compare the results between the different subgroups.

(4) To link spectroscopy data with QSM results and with well-known biofluid markers for iron, neurodegeneration and neuroinflammation.

(5) To investigate the relationship between brain iron accumulation as detected by QSM, and neuroinflammation as detected by spectroscopy, with clinical signs of HD (cognitive, psychiatric, motor impairments) encoded for their symptoms and severity by well-established clinical scales.

(6) To follow-up these patients clinically to evaluate clinical disease progression after one year.

The first and primary objective is to quantify brain iron accumulation in patients with HD using quantitative susceptibility mapping (QSM) at ultrahigh field (7T) MRI and compare this with healthy controls. We hypothesize that there is an increased brain iron accumulation in patients with HD compared to healthy controls, as measured on QSM-maps (Chen et al., 2019; Domínguez D et al., 2016; van Bergen et al., 2016). Another hypothesis is that there is an increasing brain iron accumulation during disease progression, as measured by clinical assessments and scales (Domínguez D et al., 2016; van Bergen et al., 2016; Chen et al., 2019).

Our secondary objective is to quantify levels of neuroinflammation metabolites in a specific region of interest. We hypothesize that there is an increased level of neuroinflammation metabolites in patients with HD compared to controls, due to neuroinflammation (Chang et al., 2013). DW-MRS has not been used in a HD-study population, nevertheless we hypothesize that there is an increased ADC in patients with HD, due to the glial activation, as has been seen in other diseases with neuroinflammation (Palombo et al., 2018; Verkhratsky et al., 2014).

There has been no study so far linking QSM results with spectroscopy data, and with CSF biomarkers, in a neurodegenerative disease. These analysis are seen as exploratory analysis. Analyzing differences in clinical signs (cognitive, psychiatric and motor) and MRI outcome measurements are also seen as an exploratory analysis.

2. Methods

2.1. Study design

IMAGINE-HD is an observational cross-sectional cohort study with a multimodal design and a clinical one-year follow-up. The study was approved by the Medical Ethics Committee Leiden Den Haag Delft. All participants will be asked for written informed consent before inclusion. Furthermore, this study is registered on ClinicalTrials.gov with the ID-number: NCT05534139. This study will involve a two day visit for each participant and will include the following procedures: motor, functional and neuropsychological assessments of 45–60 min, a 7T MRI scan of 60 min, a lumbar puncture and blood sampling. See Fig. 2 for a flowchart of the study design. After one year, the clinical study assessments will be repeated, to assess disease progression. The assessments in the follow-up consist of the motor, functional and neuropsychological assessments and changes in demographics and HD-stage will be checked.

2.2. Population

The study population will include 65 HD gene expansion carriers (HDGEC) and 25 healthy control subjects consisting of males and females of 21 years of age and older. The two groups will be age and sex matched as much as possible, given the limitation of the rarity of the disease and the difficulty in recruiting healthy controls to a study that includes a lumbar puncture. The participants will be recruited via the HD outpatient expertise centre of the Leiden University Medical Centre

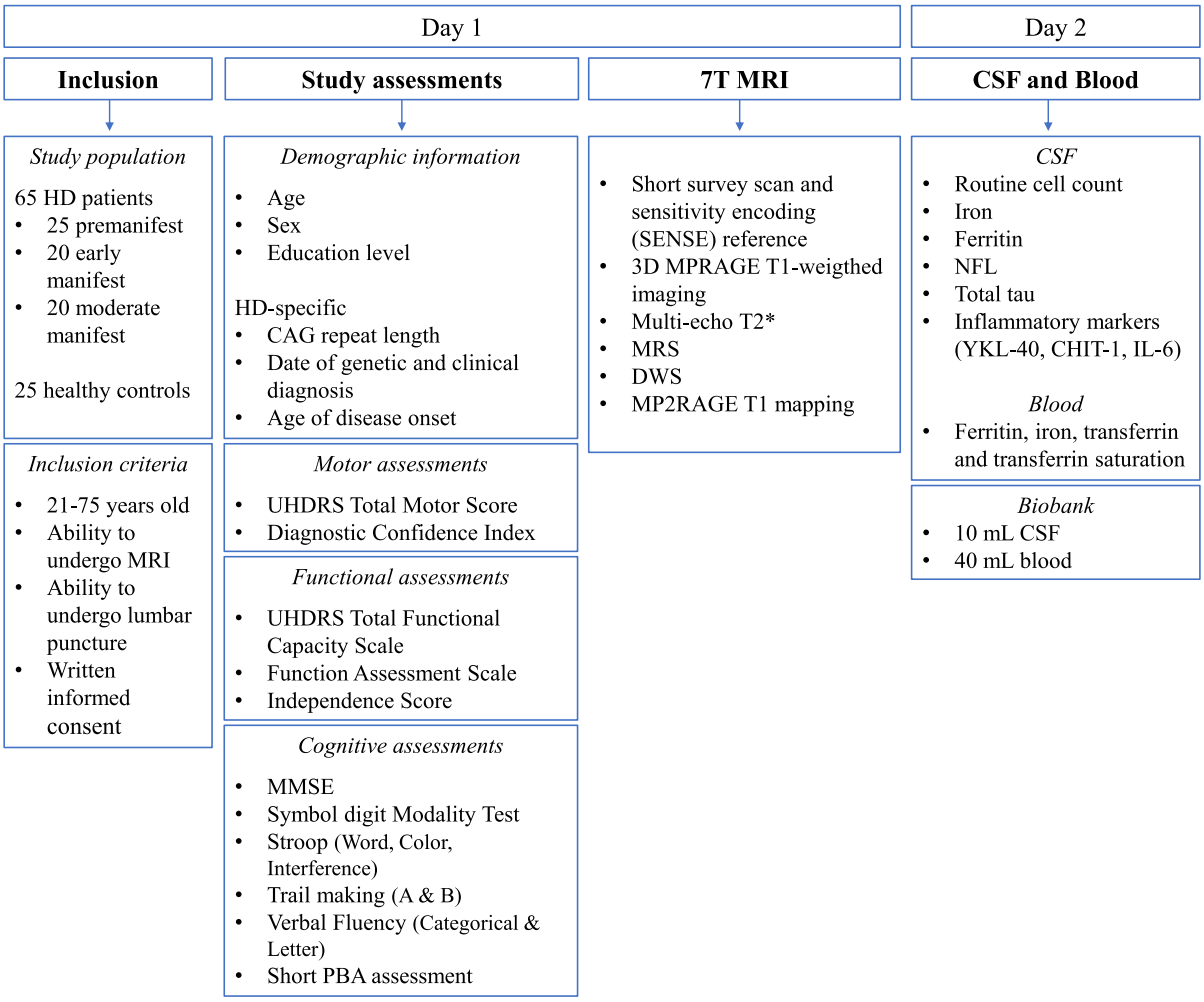

Fig. 2. Study design and flow chart of the Baseline visit. *Abbreviations: CHIT-1 = Chitinase 1, CSF = Cerebrospinal fluid, DWS = Diffusion Weighted Spectroscopy, HD = Huntington's Disease, IL = Interleukin, MMSE = Mini-Mental State Examination, MPRAGE = Magnetization-Prepared Rapid Acquisition Gradient-Echo, MP2RAGE = Magnetization-Prepared 2 Rapid Acquisition Gradient Echo, MRI = Magnetic Resonance Imaging, MRS = Magnetic Resonance Spectroscopy, NFL = Neurofilament light, PBA = Problem Behaviors Assessment, SENSE = SENSitivity Encoding, UHDRS = Unified Huntington's Disease Rating Scale.*

(LUMC), the Netherlands. We will include 25 pre-, 20 early-, and 20 moderate manifest HDGEC, resulting in variation in symptoms and disease severity, ensuring coverage of a broad spectrum of the disease. The study population is based on the sample size calculation and possible exclusion of data due to – for example – movement artefacts. We based the power calculation on quantitative QSM results from the previous studies using QSM in HD (Domínguez D et al., 2016; van Bergen et al., 2016; Chen et al., 2019). To test the sample size needed to identify differences in brain iron accumulation between HD patients and controls, the software G*Power is used (Faul et al., 2007). The aimed power is set on 0.80 and the α error probability on 0.05. Assuming two groups, HD patients and age-matched healthy controls, the caudate nucleus of healthy controls is estimated to have a susceptibility measurement and standard deviation of 0.043 ± 0.019 parts per million (ppm). HD manifest patients are estimated to have an increased susceptibility of 0.059 ± 0.016 ppm (Domínguez D et al., 2016). This results in an effect size of 0.9 and a required sample size of at least 17 subjects per group.

For individuals clinically diagnosed with HD, a positive genetic test with a CAG repeat expansion of ≥36 in the HTT gene is required to be enrolled in this study. For premanifest participants a positive genetic test with a CAG repeat expansion of ≥40 in the HTT gene is required, to avoid participants that will not get the signs and symptoms of HD, as repeat lengths between 36 and 40 are associated with reduced penetrance (Sturrock and Leavitt, 2010).

Premanifest HDGEC are defined by Unified Huntington's Disease Ratings Scale (UHDRS) Total Motor Score (TMS) ≤5, total functional capacity (TFC) = 13 (maximal score) and diagnostic confidence score <4. Both early- and moderate manifest HDGEC have a diagnostic confidence score equal to 4, which means they have clinical signs and symptoms that have a 99% certainty of being attributed to Huntington's disease. The manifest HDGEC subgroups are stratified based on the TFC score. For the TFC score, a lower score means a lower functional capacity. The TFC score is between 11 and 13 for early manifest HDGEC and 7 to 10 for moderate manifest HDGEC.

Age- and sex matched healthy individuals will be asked to participate as controls. Controls could be a partner or spouse of a patient, who is not at risk of HD; or a family member with genetic test results available that show a normal CAG repeat length for both HTT alleles (<36). Controls should have no other known major cognitive, neurological or psychiatric disorders.

Exclusion criteria include additional major comorbidities not related to HD, a history of severe head injury, use of investigational drug or participation in a clinical drug trial within the last 30 days, current intoxication and inability to understand information about the protocol. Participants that have severe physical restrictions or severe chorea are also excluded, as it makes undergoing an MRI-scan rather difficult. Participants with common contra-indications for MRI such as pregnancy, claustrophobia, or metal implants are also excluded. Before

lumbar puncture we will perform a screening blood test and participants with either results outside normal ranges (platelets, prothrombin time, activated partial thromboplastin time, haemoglobin, white cell count) or signs of increased intracranial pressure, local skin infections or use of anti-coagulant drugs will be excluded.

2.3. Methods – Clinical data

We will collect basic demographic information including age, sex and education level. For HDGEC, we will collect HD related information including CAG-repeat length, date of genetic and clinical diagnosis and age of disease onset according to the patient and family. Furthermore, we will calculate CAG Age Product (CAP)-score, to use as an index of disease progression. The CAP-score is widely used as a prediction tool for a variety of disease state variables in HD, such as age of onset and disease progression (Langbehn et al., 2010; Warner et al., 2022).

In addition, motor, functional and neuropsychological assessments are performed. The motor assessments include the UHDRS total motor score (TMS) and the Diagnostic Confidence Index to assess the motor dysfunction characteristics of HD. The functional assessments include the UHDRS total functional capacity scale (TFC), function assessment scale and independence scale to assess general functioning. The neuropsychological assessments include the symbol digit modality test, Stroop word reading, Stroop colour naming, Stroop interference, Trail making A & B, categorical verbal fluency, letter verbal fluency, the Mini Mental State Examination (MMSE). Neuropsychiatric symptoms will be assessed with the short Problem Behaviour Assessment (PBA-s). Cognitive decline is an important hallmark of HD, which shows in different types of dysfunction, like executive dysfunction, attention problems and memory impairment (Papoutsi et al., 2014). These types of cognitive dysfunctions and the severity of it, as measured by the standard neuropsychological assessments mentioned before, will be linked with MRI and CSF outcome measurements, as will be done with the TMS results and the neuropsychiatric outcome measurements. This will be obtained to try to define a tool for prediction of phenotype. In addition, they can serve as another index for disease progression. Since these neuropsychological and neurological outcome measurements decline during disease progression, they form another index for disease progression besides the stages and CAP-score. These different outcome measurements have already been combined into a composite UHDRS (cUHDRS), an established formula using a composite of motor, cognitive, and global functional clinical outcome measurements (Schobel et al., 2017). It has been shown that this formula serves as a better index for clinical disease progression, compared to individual assessments or the widely used HD-stages (Schobel et al., 2017).

These assessments will be repeated after a one-year follow-up visit.

2.4. Methods – MRI

All scans are performed locally at the LUMC using a 7T Philips Achieva MRI scanner (Philips Healthcare). The standardized scan protocol consists of a short survey scan and a sensitivity encoding (SENSE) reference scan followed by 3D MPRAGE T_1-weighted scan for anatomical information. We also acquire a MP2RAGE for T1 mapping (Marques et al., 2010). Our central MRI tool for estimation of iron in the brain is QSM (Ropele and Langkammer, 2017). Therefore the protocol includes a multi-echo flow-compensated gradient echo scan. A QSM map will be made from the whole brain, with a specific Region of Interest (ROI) of the caudate nucleus (CN), putamen (PT) and globus pallidum (GP). For evaluating metabolites characteristic for inflammation and neurodegeneration, we acquire single-volume ^{1}H MR spectroscopy (MRS) (Wilson et al., 2019) and diffusion-weighted MRS (DWS) (Ronen et al., 2015). For the details of the standardized scan protocol, refer to Table 1. Spectra are acquired in a volume-of-interest (VOI), placed manually to include as much of the ROI: CN, PT and GP, without including CSF of the lateral ventricle.

Established pipelines for basic segmentation of anatomical images, co-registration and basic motion and intensity corrections are applied, using SPM (Friston et al., 1995), FSL (Smith et al., 2004) and/or MIPAV/JIST toolbox (Lucas et al., 2010; McAuliffe et al., 2001). We use the QSM processing pipeline tool SEPIA to generate QSM maps from multi-echo data (Chan and Marques, 2021). By using established pipelines we obtain voxel-wise susceptibility values indicative for iron content for predefined structures, such as the striatum. MRS and DWS data analysis is performed with in-house Matlab routine and LCModel (Provencher, 1993).

2.5. Methods – CSF

CSF will be drawn after overnight fasting by a lumbar puncture under standardised conditions and by experienced physicians. CSF for the proposed biomarker assays will be collected in polypropylene tubes, centrifuged, divided into 0.5 and 1 ml aliquots, and stored at −80 °C until analysis. In addition to routine cell count, which will be analysed locally within the LUMC laboratory, we selected the following markers for CSF analyses: iron and ferritin (iron storage protein) as markers for brain iron accumulation, neurofilament light protein (NFL) and total tau as reflecting neuronal damages, YKL-40, CHIT-1 and IL-6 as inflammatory markers. Analyses for CSF concentrations of these markers will be done using ELISA and will be performed in the Neurochemical Laboratory of Ulm University, Ulm, Germany. Measurements to determine CSF iron levels will be done in the Research Unit Analytical BioGeoChemistry Helmholtz Zentrum, Munich, Germany using inductively coupled plasma – mass spectrometry.

If participants give permission, by informed consent, for the LUMC Biobank Huntington (part of the LUMC Biobank Neurological Diseases), an additional 10 ml of CSF will be obtained. This CSF sample will be stored for future (yet unknown) biomarker analysis.

2.6. Methods – Blood

Immediately after the lumbar puncture, up to 10 ml of venous blood will be drawn according to clinical standards and procedures. Blood samples will be used for a local routine laboratory test for glucose, to confirm the overnight fasting, and full blood count. Blood samples will also be used to measure markers related to systemic iron (ferritin, iron, transferrin and transferrin saturation). These analyses will be performed in the Neurochemical Laboratory of Ulm University. If participants give permission, by informed consent, for the LUMC Biobank Huntington (part of the LUMC Biobank Neurological Diseases), an additional 40 ml blood will be drawn. These blood samples will be stored for future (yet unknown) biomarker analysis.

2.7. Statistics

One-way ANOVA's will be used to assess baseline between group differences. Potentially confounding demographic variables (age, sex) will be examined and those found significant will be included as covariates for subsequent analyses. The distributions of the MRI and CSF markers will be tested for normality. If applicable, appropriate non-parametric test will be used and corrections for multiple comparisons will be performed. For all statistics the significance is set at $p < 0.05$.

The first and primary objective is to quantify brain iron accumulation in participants with HD using QSM at 7T and compare these values with healthy controls. Differences in QSM values between subgroups will be analysed using parametric (one-way ANOVA) or non-parametric tests when applicable.

The following objective is to evaluate changes in metabolites' level and diffusion specific for neuroinflammation in HDGEC, using ^{1}H MRS and DWS, compared to healthy controls. Differences in these values between subgroups will be analysed using parametric (one-way ANOVA) or non-parametric tests when applicable.

Table 1
Standardized scan protocol, on the 7T-MRI-scanner at the LUMC, to assess iron accumulation, neuroinflammation and neurodegeneration in the study population. *Abbreviations: CN = Caudate Nucleus, GP = Globus Pallidus, PT = Putamen.*

Sequence name	Purpose	Timing	Resolution	Field of View (FOV)	Acquisition time	Additional information
3D MPRAGE T1-weighted	Anatomical information	TR/TE = 5.0/2.2 ms	1 × 1 × 1 mm^3	246 × 246 × 174 mm^3	1 min, 54.7 s	flip angle = 7°
T2-weighted Multi-echo flow-compensated gradient echo scan*	Iron accumulation measurement using QSM	TR/TE1/ΔTE = 32/4.1/4.1 ms, 6 echos	0.70 × 0.70 × 0.70 mm^3 ROI = CN, PT, GP	224 × 196 × 156.8 mm^3	9 min, 6.1 s	flip angle = 15°
Short TE sLASER	Spectroscopy neuroinflammation metabolites	TR/TE = 7500 / 38 ms	18 × 23 × 17 mm^3 volume-of-interest (VOI) ROI = CN, PT, GP		7 min	48 signal averages Outer-volume suppression with saturation bands
DW-sLASER with and without water suppression	Cell-type specific intracellular microstructure	TR/TE = 9 cardiac cycle /110	18 × 23 × 17 mm^3 volume-of-interest (VOI) ROI = CN, PT, GP		~20–25 min	- Cardiac triggering – 4b-values: 19, 444, 2070, 3141 s/mm2 – 3 orthogonal directions – 12 signal averages per direction and b-value - Outer-volume suppression with saturation bands
3D MP2RAGE[33]	T1 mapping	TR/TE = 6.2/2.1 ms	1 × 1 × 1 mm^3	224 × 224 × 180 mm^3	6 min, 30 s	TI1/TI2 = 1/3.3 s Flip angle 1/flip angle 2 = 7/5°

To investigate the link between brain iron as detected with QSM and biofluid markers for iron, neurodegeneration and neuroinflammation, and the link between ^{1}H MRS/DWS and the biofluid markers, we will use linear or logistic regression analyses when applicable. The same statistical analyses will be used to investigate the link between brain iron as detected with QSM and clinical signs such as, for example, the UHDRS-TMS and neuropsychological test results. Furthermore regression analyses will also be used to investigate the link between QSM and ^{1}H MRS data.

Due to an expected enormous amount of data, machine learning strategies will be considered.

3. Discussion

Despite its monogenetic origin, the pathogenesis of HD is complex, resulting in multiple potential biomarkers ranging from markers reflecting the huntingtin protein to the devastating final pathway of neuronal death. Although a variety of clinical tools exist to assess disease progression in HD, the gold standard for disease severity of HD remains neuropathological confirmation upon post-mortem examination using the Vonsattel score (Vonsattel et al., 2011). This score, however, only gives information on the end stage of the disease, whereas a non-invasive tool to assess disease severity over the course of the disease in vivo is needed. Neuroimaging is appealing as a potential biomarker due to its ability to provide putative markers for several pathological mechanism in a non-ionizing and predominantly non-invasive manner. To date, several MRI studies in neurodegenerative diseases, including HD, showed the relevance of brain iron accumulation (Dexter et al., 1991; Domínguez D et al., 2016; van Bergen et al., 2016; Ward et al., 2014; Chen et al., 2019; Acosta-Cabronero et al., 2013). Specific for HD, increases of iron levels in the putamen, caudate nucleus and pallidum have been reported (Domínguez D et al., 2016; van Bergen et al., 2016; Chen et al., 2019; Bulk et al., 2020; Jurgens et al., 2010). Some reports showed elevated iron levels in premanifest patients compared to healthy controls and correlations with disease severity, pointing to MRI readouts of iron accumulation in the brain as a potential early biomarker (Domínguez et al., 2016; van Bergen et al., 2016). Nevertheless, before such biomarker can be used in the clinic, more research is needed to examine the clinical value and study its correlation with well-accepted biofluid markers. This study is especially designed to provide a basis for the evaluation of brain iron levels as an imaging biomarker for disease stage in HD.

^{1}H MRS and DWS are neuroimaging methods to evaluate neuroinflammation by looking at intracellular cell-specific brain metabolites' level and diffusion. ^{1}H MRS has already shown differences in metabolites in neuroinflammatory diseases and neurodegenerative diseases, like AD (Chang et al., 2013). However in Huntington's disease, there is still a lack of sufficient and consistent evidence (Adanyeguh et al., 2018; Gómez-Ansón et al., 2007; Reynolds et al., 2005; Sturrock et al., 2010). DWS is a new innovative tool to evaluate glial activation in an even more detailed way, by looking at the cytomorphological changes (Ronen et al., 2015; Ronen et al., 2013), which has already been successfully applied in other neurodegenerative (Hübers et al., 2021) and neuroinflammatory diseases (De Marco et al., 2022; Wood et al., 2017; Ercan et al., 2016). Since both MRS and DWS are non-invasive, they could be used as a monitoring marker for disease progression in clinical trials. However, before this can be used as a neuroimaging biomarker, more research is needed to examine the clinical value and study its correlation with well-accepted neuroinflammation biofluid markers.

Even though biofluid markers for disease progression are currently not used in clinical HD setting, CSF can provide a set of objective markers for the interpretation of MRI findings. Studies on HD strongly suggest that CSF neurofilament light protein (NFL), a marker for axonal damage, and total tau (t-Tau), reflecting damage of neurons, are promising biomarkers for overall neuronal damage in HD (Constantinescu et al., 2009; Constantinescu et al., 2011; Rodrigues et al., 2016). Especially for NFL, a close correlation with disease progression and disease onset has been described (Byrne et al., 2017; Byrne et al., 2018). NFL has also been identified in blood, with the ability to predict atrophy in HD brains (Johnson et al., 2018) and years to clinical onset (Parkin et al., 2022). Inflammatory markers have been less studied in HD, but CSF-markers YKL-40/chitinase-3-like-protein 1 (CHI3L1) secreted by astrocytes (Bonneh-Barkay et al., 2012; Bonneh-Barkay et al., 2010) and chitotriosidase-1 (CHIT-1) secreted by activated microglia (Rosén et al., 2014; Varghese et al., 2013) are increased in many inflammatory central nervous system disorders. Elevated levels of YKL-40 (Vinther-Jensen et al., 2016) as well as of the proinflammatory cytokine IL-6 have been found in the CSF of HD patients (Björkqvist et al., 2008; Rodrigues et al., 2016). In one study YKL-40 significantly correlated with disease stage, UHDRS total functional capacity score and UHDRS total motor score (Rodrigues et al., 2016). In the Huntington's Disease Young Adult Study (HD-YAS), where they studied premanifest

HDGEC, they also found elevated CSF YKL-40 compared to controls (Scahill et al., 2020). Previous studies on iron accumulation in several neurodegenerative diseases have also shown the clinical utility of CSF iron markers (Ayton et al., 2015; Genoud et al., 2020; Jouini et al., 2021). CSF-ferritin levels could serve as an index of brain iron load. In Alzheimer's disease (AD) it has been shown that CSF ferritin levels have similar clinical utility compared with more established AD CSF biomarkers in predicting various outcomes of AD (Ayton et al., 2015). More specifically, higher CSF ferritin predicted a more rapid cognitive decline in AD and is positively correlated with the rate of clinical disease progression (Ayton et al., 2015). There has been only one previous study that assessed iron, among other metals, in CSF and found an increased iron level in manifest HD patients (Pfalzer et al., 2022). Further analysis has yet to be performed in exploring the potential of these CSF markers to reveal new biomarkers in HD.

For pharmaceutical trials including HTT lowering therapies, biomarkers to measure treatment effect as well as to detect the earliest changes at the start of the disease are of utmost importance (Zeun et al., 2019). Ideally, in order to prevent neuronal damage, treating HDGEC starts as early as possible. Hopefully, the results from our study, which includes both premanifest and manifest HDGEC, will give more insight in potential biomarkers involved early in the disease. The information obtained in our study may potentially benefit the development of new treatments and, when treatments are available, these markers could guide the timing for treatment initiation and evaluation of effect.

One limitation of our study, although covering different disease stages, is the cross-sectional nature. Longitudinal data of brain iron accumulation within individuals over the years is needed to conclude, whether these neuroimaging biomarkers could be used to evaluate disease *progression*. This study would therefore ideally be extended into a longitudinal study and thus serves as a pilot for future studies. Also, standardization of protocols is important to allow comparison between different research sites. Therefore, the CSF and blood protocols follow the same guidelines as described for HD-Clarity. In addition, if given permission by the participant, additional CSF and blood samples are stored in a local biobank that can be used for future scientific studies on promising biomarkers related to HD.

In conclusion, our study IMAGINE-HD will provide an important basis for the evaluation of brain iron levels and neuroinflammation metabolites as potential imaging biomarkers at different disease stages in HD and their relationship with the salient pathophysiological mechanisms of the disease.

Author contribution

MB conceptualized and designed the protocol including methodology, with the help of IR, SdB, LvdW, and JL. Initial funding acquisition was obtained by MB and IR. NvdZ amended the protocol including methodology, with the help of CN. NvdZ is the current study-coordinator, responsible for project administration and organizing resources. Supervision overall is done by SdB. The technical imaging aspects are supervised by IR, CN, and LvdW. MB made the first draft of this article. NvdZ amended the first draft in according to current protocol and literature, and wrote the final draft. SdB, LvdW, JdB, CN and IR reviewed all drafts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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