Safety and feasibility of research lumbar puncture in Huntington's disease: the HDClarity cohort and bioresource

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STRUCTURED ABSTRACT

Background

Biomarkers are needed to monitor disease progression, target engagement and efficacy in Huntington's disease (HD). Cerebrospinal fluid (CSF) is an ideal medium to research such biomarkers due to its proximity to the brain.

Objectives

To investigate the safety and feasibility of research lumbar punctures (LP) in HD.

Methods

HDClarity is an ongoing international biofluid collection initiative built on the Enroll-HD platform, where clinical assessments are recorded. It aims to recruit 1,200 participants. Biosamples are collected following an overnight fast: blood via venipuncture and CSF via LP. Participants are healthy controls and HD gene expansion carriers across the disease spectrum. We report on monitored data from February 2016 to September 2019.

Results

Of 448 participants screened, 398 underwent at least 1 sampling visit, of which 98.24% were successful (i.e. CSF was collected), amounting to 10,610mL of CSF and 8,200mL of plasma. In the total 572 sampling visits, adverse events were reported in 24.13%, and headaches of any kind and post-LP headaches in 14.86% and 12.24%, respectively. Frequencies were less in manifest HD; gender, age, body mass index and disease burden score were not associated with the occurrence of the events in gene expansion carriers. Headaches and back pain were the most frequent adverse events.

Conclusions

HDClarity is the largest CSF collection initiative to support scientific research into HD and is now established as a leading resource for HD research. Our data confirm that research LP in HD are feasible and acceptable to the community, and have a manageable safety profile.

Keywords:

Huntington Disease, Spinal Puncture, Biomarkers, Cohort Studies, Cerebrospinal Fluid, Blood.

INTRODUCTION

Huntington's disease (HD) is a progressive autosomal dominant genetic disease, which typically manifests in adulthood with movement disorder, cognitive decline, and psychiatric changes(1). Its overall survival after clinical diagnosis is around 20 years(2). There are currently no disease-modifying interventions available (3), but several clinical trials are underway and planned in the next few years to explore novel therapeutic approaches to treating this disease(4-6). In preparation for such trials, biomarkers are needed – especially prognostic, pharmacodynamic, efficacy and safety biomarkers.

Cerebrospinal fluid (CSF) is a favourable biofluid compartment for assessing HD biomarkers, owing to its proximity to the brain and consequent enrichment of CNS-derived products. Blood is less CNS-enriched but more accessible, and may provide relevant hints to monitor disease progression and assess response to treatments(7, 8), whilst being useful to help interpret findings in CSF. The CSF is also the compartment into which the first targeted huntingtin-lowering experimental therapeutic was delivered and the fluid in which its successful target engagement was assessed(9, 10).

There have been numerous reports of potential biofluid biomarkers in HD(11-13); however, many were assessed in small-scale cross-sectional studies and remain unvalidated. Even findings from larger studies with longitudinal designs(7, 8, 14) need to be replicated in a well-powered and standardized new sample set. New samples are also invaluable for the discovery of novel biomarkers and validation of modern analytical methods.

While CSF is generally sampled through a minimally invasive procedure, the safety and feasibility profile of lumbar punctures (LP), has not been systematically investigated in HD. In the general population, this procedure has a low risk of serious adverse events, such as CNS infection and bleeding. Post-LP back pain and headache are common but transient, and either have spontaneous resolution or need simple measures and reassurance. LP has been widely studied for research and clinical purposes in other neurodegenerative conditions, notably Alzheimer's disease (AD), where older age and prominent generalised brain atrophy are associated with a lower risk of the most common low-pressure syndromes(15-19). The HD population has important characteristics that could

modify LP safety and feasibility profiles: relatively young age distribution compared to other dementias; degree of brain atrophy intermediate between healthy controls and AD patients; involuntary movements that could make LP more challenging; and a dysexecutive syndrome that could make recruitment, consent and toleration of procedure and adverse effects more difficult.

HDClarity (NCT02855476) commenced as a prospective nested CSF and blood collection initiative within Enroll-HD (https://enroll-hd.org)(20) with HD gene expansion carriers (HDGECs) and healthy control participants recruited from the main cohort to facilitate biomarker development in HD. Here we report the characteristics and experiences of the first 448 participants screened between February 2016 and September 2019. In addition, we examine the safety and feasibility of the study procedures in HDGEC and healthy controls, factors influencing complication risk, and quality control indicators of the collected CSF and plasma.

MATERIALS AND METHODS

Study protocol

The open-access HDClarity study protocol is available at http://hdclarity.net/study-information/(21). HDClarity was designed as a cross-sectional study with optional short-term resampling visits. It has since been extended to an annual collection for willing and eligible Enroll-HD participants at HDClarity sites to generate longitudinal samples and data.

Study aims

The primary objective of HDClarity is to generate a high-quality CSF collection for evaluation of biomarkers and pathways that will enable the development of novel treatments for HD. The secondary objectives are to generate a high-quality plasma sample collection matching the CSF collections, which will also be used to evaluate biomarkers and pathways of relevance to HD research and development.

Study design

HDClarity is a global longitudinal observational study, with the aim of enrolling 1,200 participants. All willing and eligible Enroll-HD participants at HDClarity sites are invited to participate in the HDClarity study.

The Enroll-HD study is a prospective longitudinal observational study that collects natural history data in HDGECs and healthy controls with core required assessments focused on neuropsychiatric, cognitive, motor and functional status conducted via a battery of validated and widely accepted measures. The Enroll-HD database includes clinical information on 24,391 participants (as of 21 Oct 2020) of which 17,734 are HDGECs, 2,406 are genotype unknown (but at risk of having inherited the expanded HD allele) and 4,251 are healthy controls. The mean age of HDGECs and healthy controls at enrolment into Enroll-HD was 49.1 years (range 11 – 92) and 48.8 years (range 18 to 91), respectively, with male to female ratio 1:1.16 and 1:1.51 respectively. Enroll-HD is conducted at 157 active sites in 19 countries across four continents.

Clinical and phenotypic data must be collected in the annual Enroll-HD visit within 2 months prior to the screening for HDClarity; otherwise the core assessments are repeated during HDClarity screening visit. Core Enroll-HD assessments include Unified Huntington's Disease Rating Scale (UHDRS) Total

Motor Score (TMS), Diagnostic Confidence Score (DCS), Total Functional Capacity (TFC), Independence Scale (IS), Problem Behaviours Assessment - short (PBA-S), Symbol Digit Modalities Test (SDMT), Stroop Word Reading (SWR), Stroop Color Naming (SCN), and Verbal Fluency Categorical (VFC)(22-26).

Participants who meet the HDClarity eligibility requirements at the screening visit, which includes testing platelet count and clotting function, return for a sampling visit within a month. Biosamples are collected between 8:00 and 10.30 am following an overnight fast: blood is obtained via venepuncture and CSF via LP. UHDRS TMS is repeated on the day of sampling. Participants are discharged following a period of observation and contacted within 72 hours to assess adverse events.

Adverse events were described by the local site investigators in terms of duration, severity (according to Common Terminology Criteria for Adverse Events v3.0), likely association with study procedures and compatibility with a low CSF pressure syndrome.

Approximately 20% of the HDClarity participants are invited to return for an optional short-term resampling visit approximately 4-8 weeks later. All participants are invited to return for annual visits.

Ethical considerations

HDClarity is performed in accordance with the principles of the Declaration of Helsinki, and the International Conference on Harmonization and the World Health Organization Good Clinical Practice standards. All participating sites sought appropriate ethical approval in accordance with each specific country legislation. All reported participants gave informed consent prior to undertaking study procedures.

Study population

Six participant groups are being recruited of which five are sub-categories of HDGEC (early pre-manifest, late pre-manifest, early manifest, moderate manifest and advanced manifest HD patients) in addition to healthy controls. Healthy controls have either no known family history of HD, or have had a negative genetic test for the HD CAG expansion (i.e. CAG < 36). All HDGEC participants need to

have been tested locally for the huntingtin gene glutamine codon (CAG) expansion and have a CAG ≥ 40 if premanifest or ≥ 36 if manifest. Further details of subgroup characteristics are provided in Table S3. Enrolment is anticipated to be similar in each group.

Eligible participants are aged between 21 and 75 years, inclusive, and of both genders. They need to be able to provide informed consent or have a legal representative authorized to give consent on their behalf. Compliance with study procedures, including fasting, blood sampling and LP is required, as is participation in the Enroll-HD study(20).

The main exclusion criteria are: participation in a clinical drug trial within 30 days prior to the sampling visit or use of an investigational drug; significant medical, neurological or psychiatric co-morbidity likely to impair participant's ability to complete study procedures, or likely to reduce the utility of the samples and data for studies of HD; clinical or laboratory bleeding and inflammatory abnormalities. For a detailed description please refer to the study protocol.

Biosample collection, processing, and storage

To minimize inter-site and inter-sample variability, biosamples (i.e. CSF and blood) are collected and processed according to a standardized and pre-piloted protocol (see Supplementary appendix 1).

Briefly, local sites are provided with centrally sourced kits for CSF and blood collection and processing. A LP is performed using a 22G Whitacre atraumatic BD spinal needle. Up to 20 mL of CSF are collected into a 50 mL pre-cooled polypropylene collection tube on wet ice. Biosamples are transported to the laboratory on wet ice, with the exception of serum which is kept at room temperature, and sample processing starts within 15 minutes of collection.

Red and white cell counts are carried out in up to 200 µL of CSF onsite for safety and quality control purposes, and the remaining CSF is prepared and aliquoted into 300 µL cryovials. Plasma is prepared and aliquoted into 300 µL cryovials and serum into 1,500 µL cryovials. All biosamples are frozen at -

80ºC and then shipped to a central biorepository using centrally sourced boxes with dry ice and temperature probes.

Site recruitment

Larger Enroll-HD sites were prioritised for HDClarity study to facilitate recruitment. Subsequent sites were added after assessment for suitability based on experience, expertise and facilities, local ethical approval, legal approval, translation of materials and site training. The number of sites opened annually from 2016 to 2019 were 2, 7, 5 and 1, for a total of 15 sites.

Data managing and statistical analysis

This analysis reports on fully monitored data from participants recruited from February 2016 to September 2019. All data were recorded on the Enroll-HD electronic data capture system. Data were remotely monitored by HDClarity central coordination team, and on-site by trained Enroll-HD data monitors. The final analysis dataset was queried for implausibilities, which were removed from the dataset and assumed as missing. No imputation procedures were used.

To describe study visits, the unit of analysis was the study visit irrespective of the fact that a proportion of participants who had annual sampling visits had more than 1 screening (n= 71; 15.85%) and/or sampling visits (n= 68; 17.09%). Participants with at least one successful sampling visit (i.e., where dura was pierced and CSF was collected, irrespective of amount of CSF) are characterized using data from the initial sampling visit. Adverse event data are analyzed for successful and unsuccessful (i.e., where a LP was attempted, but no CSF was collected) sampling visits. Headaches were defined as any kind of head pain, and post-lumbar puncture headaches (PLPH) as a headache secondary to a low CSF pressure syndrome as judged by the local site investigator.

Continuous variables were reported as mean ± standard deviations (SD); counts as median ± interquartile range (IQR), minimum and maximum; and categorical variables as absolute (n) and relative frequencies (%). Intergroup differences in continuous variables were tested with linear regression, and intergroup independence across categorical variable was examined with logistic regression. 2-sided Fisher exact tests were used for categories with zero events. We reported unadjusted p-values for the omnibus group membership main effect test and relevant contrasts.

To identify possible factors associated with LP success and post-LP complications, we examined the associations between the event of interest (i.e., LP success, adverse events, headache, PLPH) and exposures of interest using univariable mixed effects logistic regression, with the event as the dependent variable and participant as a random intercept. A multivariable model including study group, age, gender, body mass index (BMI; $kg \cdot m^{-2}$), and Disease Burden Score (DBS; [CAG-35.5]·age)(27) as fixed independent variables was used to report the adverse event frequency within each study group. We reported unadjusted p-values for the omnibus group membership main effect test and relevant contrasts. To study the impact of age, gender, BMI and DBS in HDGEC only these four variables were included in the model as fixed variables. DBS is a measure of cumulative exposure to HD pathology as a function of CAG repeat length and time exposed to the effects of the expansion. The output was reported as odds ratio (OR) and 95% confidence interval (95% CI). P-values were not adjusted for multiple comparisons, and data analysis was performed with the statistical package StataMP 16 (StataCorp, Texas, USA).

RESULTS

Participant and visit characteristics

In the first 43 months of the study, 448 participants were screened, of whom 398 went on to have one or more sampling visits. 459 successful sampling visits were completed at 15 study sites, followed by 101 successful short-term repeat sampling visits. Overall, 560/572 (97.9%) sampling visits were deemed successful (Figure 1 and **Figure S1**). Characteristics of those who underwent successful sampling are shown in

Table 1 and a comparison between participants' characteristics at successful and unsuccessful visits in **Table S4**.

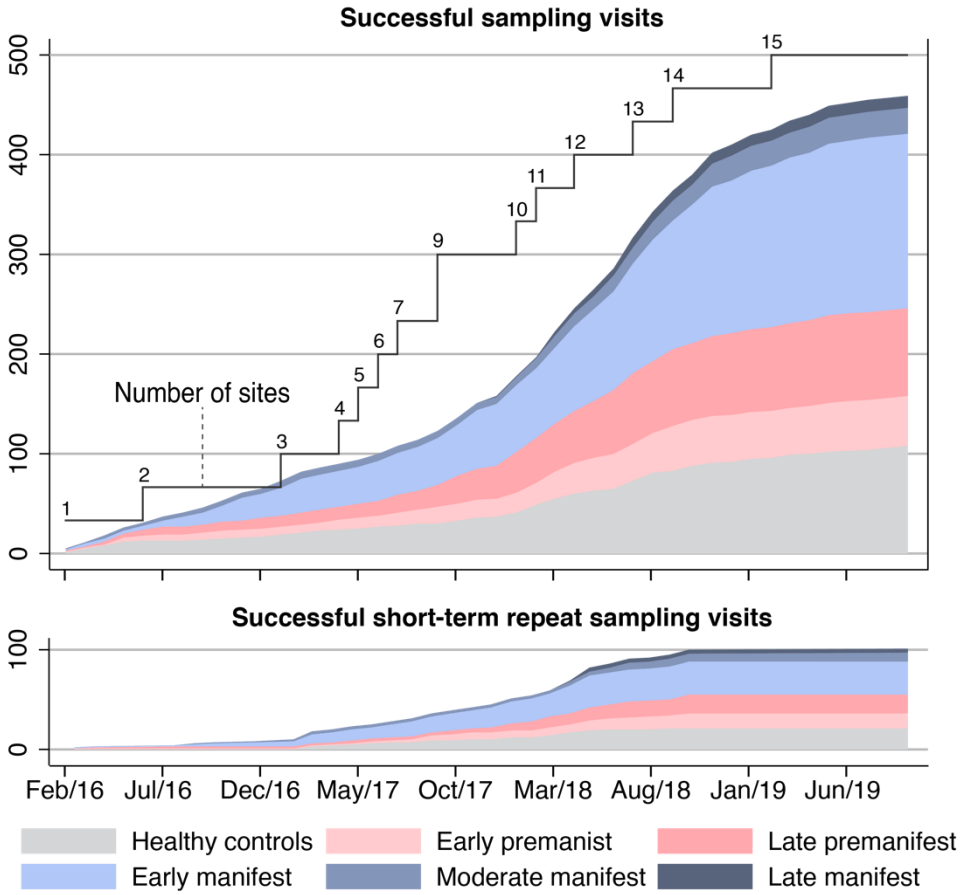

Figure 1. ***Successful sampling and short-term repeat sampling visits over time.*** *Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. Short-term repeat sampling visits were paused across most participant groups in early 2019 when the initial target numbers were reached; hence no fully monitored visits of this kind were captured in the dataset from January to September 2019.*

Table 1 – ***Characteristics at first screening visit of participants who underwent at least 1 successful sampling visit.*** *Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. Continuous variables are reported as mean ± standard deviations. Categorical variables are reported as absolute and relative frequencies. BMI, body mass index; CAG, CAG repeat count; DBS, Disease Burden Score; UHDRS, Unified Huntington's Disease Rating Scale; TMS, UHDRS Total Motor Score; TFC, UHDRS Total Functional Capacity; IS, UHDRS Independence Score; FA, UHDRS Functional Assessment; SWR, Stroop Word Reading test; SCN, Stroop Color Naming test; SDMT, Symbol Digits Modality Test; VFC, Verbal Fluency Categorical; n/a, not applicable.*

Adverse events

In 572 sampling and repeat sampling visits, one or more adverse events were reported in 138 visits (24.13%, Table 2, Table S5); in 86 (62.32%) visits they were mild, in 51 (36.96%) visits moderate and in 1 (0.72%) visit was severe. The median duration was 4 days (IQR 5, max 28, min 1).

Overall, there were 189 reported adverse events (Table S4): 118 (62.43%) were mild, 70 (37.04%) were moderate and 1 (0.53%) was severe; and 152 (80.42%) were deemed "probably" related to the study procedure, 25 (13.23%) were "possibly" related, and 12 (6.35%) were unrelated. The most frequent side effect was headache, followed by back pain (**Error! Reference source not found.**). Headaches of any kind were reported after 85 visits (14.86%, Table 2). Of these, 50 (58.82%) were mild, 34 (40.00%) were moderate and 1 (1.18%) was severe. The median duration was 4 days (IQR 4, max 18, min 1). PLPH, defined by the local site investigator as headache secondary to a low CSF pressure syndrome, were reported after 70 visits (12.24%, Table 2, Table S3); 36 (51.43%) were mild, 33 (47.14%) were moderate and 1 (1.43%) was severe. The median duration was 5 days (IQR 4, max 18, min 1).

There was a single (0.17%) serious adverse event, where one female participant had to be admitted for a blood patch due to a prolonged, moderate intensity PLPH. A second blood patch was recorded following a prolonged non-serious PLPH of moderate intensity in female participant. In total there were 2 (0.35%) blood patches, both effective in relief of PLPH.

Participants with manifest HD had numerically fewer adverse events, headaches and PLPH than healthy controls; premanifest HD participants were not different from healthy controls, but given the low numbers of adverse events overall, these findings should be interpreted with caution (Table 2).

*Table 2 – **Frequency of visits with adverse events.** Categorical variables are reported as absolute and relative frequencies. The unit of analysis is the visit. PLPH, post-lumbar puncture headache; HD, Huntington's disease.*

We assessed whether age, gender, BMI and disease burden score – a measure combining age and CAG repeat length that is associated with clinical severity and degree of brain atrophy in HD(28, 29) – were predictors of PLPH. None of these variables were consistently associated with the occurrence of adverse events in HD mutation carriers in our dataset (Figure 2). The only predictor whose odds ratio deviated from 1.0 was gender: there was a somewhat higher odds of adverse events, headaches and PLPH in female gene expansion carriers (OR 1.57 for adverse events, OR 1.34 for headache and 1.12 for PLPH), but the 95% confidence intervals included 1.0 (0.64-3.89, 0.53-3.39 and 0.38-3.33 respectively).

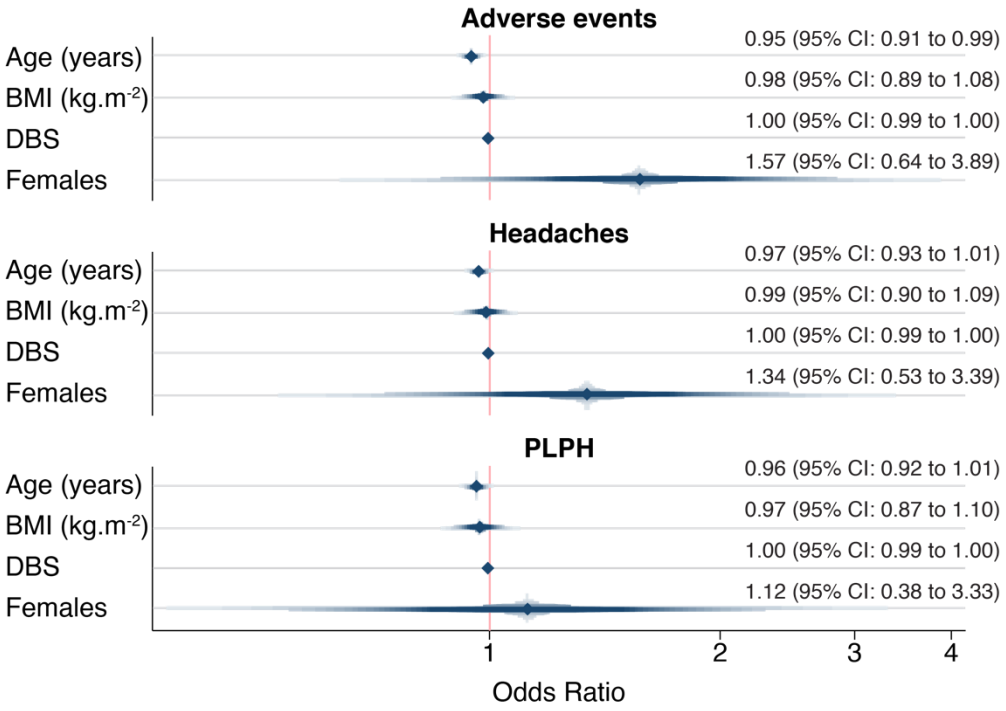

*Figure 2 – **Adverse events' association with age, gender, BMI and DBS in gene expansion carriers.** PLPH, post-lumbar puncture headache; BMI, body mass index; DBS, Disease Burden Score.*

CSF quality

10,610 mL of CSF was collected, of which 10,430.5 mL (98.31%) were deemed usable, amounting to 32,808 300µL-cryovials of CSF (Figure 3). Each LP produced a median of 20mL of CSF (IQR 0, minimum 2, maximum 24), of which a median of 20mL were deemed usable (IQR 1, minimum 2, maximum 24), generating a median of 63 cryovials of CSF (IQR 11, minimum 1, maximum 86). CSF sample processing began a median of 6 min from the end of collection (IQR 7.5, minimum 1, maximum 51); the processing itself took a median of 27 min (IQR 11, minimum 10, maximum 132), and samples were stored in the freezer in a further 1 min (IQR 2, minimum 0, maximum 242). Overall the median time from collection to storage was 36 minutes (IQR 15, minimum 12, maximum 292).

Samples had a median of 0 white blood cells per µL (IQR 1, minimum 0, maximum 24) and 1 red blood cell per µL (IQR 6, minimum 0, maximum 2,645). Haemoglobin was measured in 245 samples and the median concentration was 0.322 µg/ml (IQR 0.731, minimum 0, maximum 17.080). 224 (91.43%) were below 2 µg/ml, defined as the maximum acceptable amount of blood contamination for accurate quantification of mutant huntingtin (one critical measurement in HD research) by the most commonly used assay(30).

Blood product quality

So far 27,343 300µL-cryovials of 300µL plasma (Figure 3) and 1,142 1500µL-cryovials of serum have been collected. Each participant donated a median of 51 cryovials of plasma (IQR 17, minimum 2, maximum 75) and 2 cryovials of serum (IQR 0, minimum 1, maximum 3). Overall the median time from blood collection to storage was 47 minutes (IQR 19, minimum 14 maximum 288). Each plasma sample took a median of 15 min from the end of collection to start processing (IQR 12, minimum 1, maximum 150), the processing took a median of 26 min (IQR 13, minimum 10, maximum 93), and samples were stored in the freezer in 1 min (IQR 5, minimum 0, maximum 217). Each serum sample took a median of 17 min from the end of collection to start processing (IQR 20, minimum 1, maximum 169), the processing took a median of 14 min (IQR 7.5, minimum 10, maximum 66), and samples were stored in the freezer in 8 min (IQR 17, minimum 0, maximum 189)

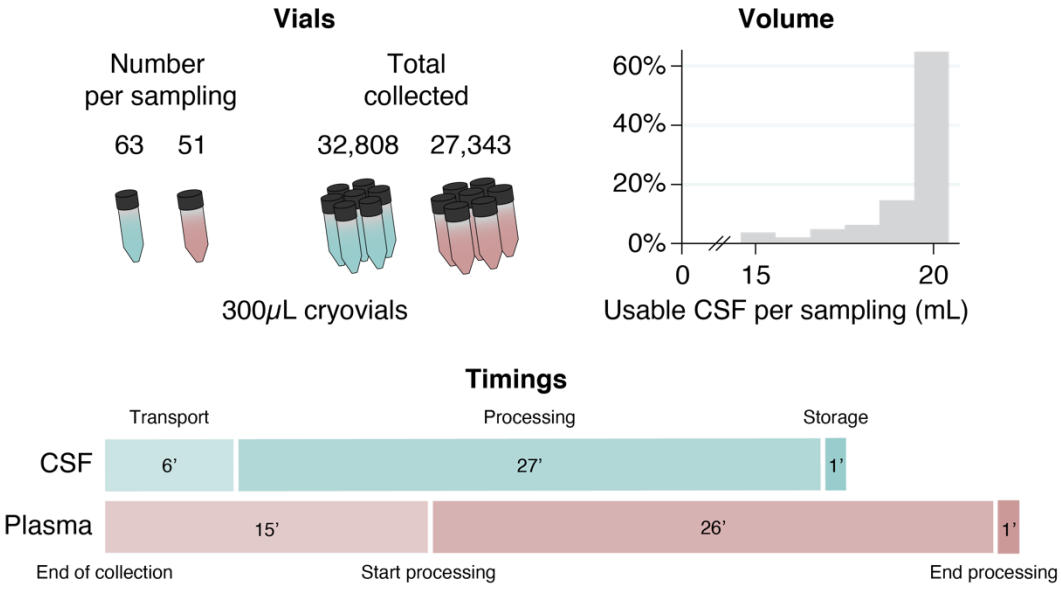

Figure 3 – ***Number of vials collected, volume of usable CSF per sampling visit, and median biosample processing times in minutes.***

Sample distribution

There have been 14 sample requests from qualified investigators (6 from industry and 8 from academia or non-profit organizations), of which 11 have been approved by an independent scientific review committee, and 3 are currently being evaluated. In total 3,251 CSF cryovials, 2,662 plasma cryovials and 238 serum cryovials have been shipped to collaborators.

DISCUSSION

The HDClarity study is the largest CSF collection initiative in HD, with the added advantage of matched phenotyping and plasma/serum samples in both, HDGECs and healthy controls. The experience reported here of the first 572 sampling visits over 43 months, with low rates of screen failure (2%) and adverse events (24.13%) suggests that LPs in HD have a safety profile akin to what is known in the healthy population. Large-scale, international, multisite biosample collection initiatives including CSF collection are viable in the HD population across all disease stages(31).

Having phenotypic data matched for all CSF samples will facilitate validation of future biomarkers while blood will grant the opportunity to explore correlations between blood and CSF levels of biomarkers. Over 100 repeat sampling visits, conducted 4 to 8 weeks after initial sampling visit permit the study of the short-term stability of potential biomarkers – crucial for the generation of sample size calculations and longitudinal clinical study designs(8).

Adverse event rates in our study are aligned with what has been reported in literature for HD(10), Parkinson's(32) and Alzheimer's disease(33-35), and in studies investigating the use of atraumatic needles for diagnostic LPs (i.e., where CSF is collected)(36). In a multivariable analysis, we did not find any factors such as gender, age and BMI to influence the frequency of adverse events, including PLPH. DBS, as a continuous surrogate of disease state, did not seem to associate with the frequency of headaches, although participants with manifest HD had fewer events than healthy controls. Lower frequencies could be explained by increased CSF and lower brain volumes or by the decreased levels of awareness or insight the characterise HD. In the only serious adverse event, where the subject underwent a blood patch for relief of PLPH, the procedure had been performed using a larger-bore, LP needle with a cutting tip, provided locally at the site rather than from the HDClarity kit. Causation between use of a different needle and this PLPH cannot be established. The study uses 22G pencil-point (Whitacre) needles and will shortly switch to 24G needles which may be helpful in reducing adverse events of back pain and headache. Lack of standardisation in methodology, which has been a frequent limitation of biomarker research in HD(13) ought to be much less of an issue, especially since the HDClarity protocol is open-access.

The data from HDClarity show that LP(s) is generally safe and tolerable in HDGECs. This will allow wide scale exploration of future biomarker avenues. Currently proposed biomarker candidates of HD disease progression, such as neurofilament light (NFL) and mutant huntingtin (mHTT), need to be validated in larger longitudinal cohorts(8, 14). The combination of HDClarity samples and Enroll-HD data provide the tool for testing and validating biomarkers in HD research and drug development, while the Enroll-HD platform has greatly assisted in recruitment of sites and participants. Studies proposed using the HDClarity biorepository aim at studying the utility of candidate biomarkers (e.g. mHTT, total HTT, NFL, YKL-40, total Tau, phospho-Tau, IL6) in predicting progression through different stages of HD with an emphasis on premanifest and early HD. Cross sectional studies will be used to identify most promising candidates, other than mHTT and NFL, followed by longitudinal validation studies. The current recruitment goal of 1,200 participants balanced across study groups will enable such analyses incorporating sophisticated modelling of data accounting for confounding factors. Eventually, a high-quality representative data set will be generated depicting the profile of each biochemical biomarker that can be utilized by all stakeholders for research and drug development. ImageClarity, another prospective nested study, will be launched in the near future where HDClarity participants who are willing and eligible will undergo a multisequence brain MRI that incorporates structural and functional modalities- offering a valuable opportunity to unite clinical, biofluid and imaging data for each participant. Availability of longitudinal phenotypic, biosample and imaging data on HDGECS at all stages and healthy controls will be a powerful asset in biomarker development for HD. Interested investigators should visit http://hdclarity.net for more information.

Acknowledgements

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Data used in this work were generously provided by the participants in the Enroll-HD study and made available by CHDI Foundation, Inc. Enroll-HD is a global clinical research platform intended to accelerate progress towards therapeutics for Huntington's disease; core datasets are collected annually on all research participants as part of this multi-center longitudinal observational study. Enroll-HD is sponsored by CHDI Foundation, Inc., a nonprofit biomedical research organization exclusively dedicated to developing therapeutics for Huntington's disease. Enroll-HD would not be possible without the vital contribution of the research participants and their families.

Samples and data used in this work would not be possible without the vital contribution of the research participants and their families in the HDClarity and HD-CSF studies. HDClarity and HD-CSF are cerebrospinal fluid collection initiatives designed to facilitate therapeutic development for Huntington's disease. HDClarity and HD-CSF are led by Dr. Edward Wild and sponsored by University College London. HDClarity is funded by CHDI Foundation, Inc., a nonprofit biomedical research organization exclusively dedicated to developing therapeutics that will substantially improve the lives of those affected by Huntington's disease. The Medical Research Council UK (MR/M008592/1) funded HD-CSF.

The HDClarity Investigators are Edward J Wild, Mark Guttman, Blair Roland Leavitt, Ralf Reilmann, Carsten Saft, Jürgen Winkler, Zacharias Kohl, Jan Lewerenz, Hugh Rickards, Stuart Ritchie, Jeremy Cosgrove, Nayana Lahiri, Roger Barker, Jee Bang, Francis Walker, Erin Furr-Stimming (more details in Supplementary Appendix 2).

Conflict of Interest

FBR, GO, KS and EJW are University College London employees. FBR has provided consultancy services to GLG and F. Hoffmann-La Roche Ltd. EJW reports grants from Medical Research Council, CHDI Foundation, and F. Hoffmann-La Roche Ltd during the conduct of the study; personal fees from Hoffman La Roche Ltd, Triplet Therapeutics, PTC Therapeutics, Shire Therapeutics, Wave Life Sciences, Mitoconix, Takeda, Loqus23. All honoraria for these consultancies were paid through the offices of UCL Consultants Ltd., a wholly owned subsidiary of University College London. University College London Hospitals NHS Foundation Trust, has received funds as compensation for conducting clinical trials for Ionis Pharmaceuticals, Pfizer and Teva Pharmaceuticals. SS, EP, AGE, DK, SL, JLev and CS derive their compensation from CHDI Management/CHDI Foundation. JT is employed by the Enroll-HD platform. BRL reports related research grant funding from Canadian Institutes of Health Research, CHDI Foundation, Weston Foundation, the Huntington Society of Canada, uniQure, Teva, and Roche during the conduct of the study; Paid scientific consultancies from Ionis Pharmaceuticals, Roche, Triplet Therapeutics, PTC Therapeutics, Novartis, Teva, Mitoconix, Takeda, and uniQure; and the Centre for HD at UBC Hospital has received funding to conduct clinical trials in HD from CHDI, Ionis Pharmaceuticals, Roche, Vaccinex, and Teva Pharmaceuticals via the HSG. MG has provided consultancy services to F. Hoffmann-La Roche Ltd, Novartis, PTC Therapeutics and CHDI Foundation. Research support from Hoffmann-La Roche, Ltd, Wave Life Sciences, Triplet Therapeutics, Neurocrine Biosciences and CHDI Foundation Inc. JB has served on the Scientific Advisory Board for WAVE and has provided consultancy services to F. Hoffman-La Roche Ltd. JLew is an employee of the Ulm University medical center. JLew received grants from the Bundesministerium for Bildung und Forschung (BMBF, German ministry for education and research) and the European Huntington's disease network (EHDN) during the conduct of the study. JLew is member of the extended board of the Deutsche Gesellschaft für Liquordiagnostik und klinische Neurochemie (German society for cerebrospinal fluid diagnostics and clinical neurochemistry), has received funds as compensation for conducting clinical trials for UCB Biosciences, Marinus Pharmaceutical and CHDI, received speakers honoraria by TEVA Pharmaceuticals, CHDI and the Movement Disorders Society. CS has received consultancy honoraria from from vTv Therapeutics, Pinteon Therapeutics, Kyowa Kirin, Pfizer, The Green Valley Pharmaceuticals, and Neuraly.

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Author's Roles

EJW and CS designed the study. FBR, BRL, MG, JB and Jlew were involved in participant recruitment, eligibility check, clinical examinations and sample collection. DK and EP managed sample storage. GO and KS monitored data. GO, EP, SL, JT, KS and Jlev managed the study. FBR developed and performed the statistical analysis; FBR and EJW interpreted the data and wrote the manuscript; and all authors contributed to reviewing the manuscript.

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Figure legends

Figure 1 – ***Successful sampling and short-term repeat sampling visits over time.*** *Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. Short-term repeat sampling visits were paused across most participant groups in early 2019 when the initial target numbers were reached; hence no fully monitored visits of this kind were captured in the dataset from January to September 2019.*

Figure 2 – ***Adverse events' association with age, gender, BMI and DBS in gene expansion carriers.*** *PLPH, post-lumbar puncture headache; BMI, body mass index; DBS, Disease Burden Score.*

Figure 3 – ***Number of vials collected, volume of usable CSF per sampling visit, and median biosample processing times in minutes.***

Tables

	All	Healthy controls (HC)	Premanifest HD (PM)	Manifest HD (M)	Group membership	HC vs PM	PM vs M
N	391	91 (23.27%)	124 (31.71%)	176 (45.01%)	n/a	n/a	n/a
Age	47.53 ± 12.59	48.67 ± 12.67	39.44 ± 10.84	52.63 ± 10.73	<0.001	<0.001	<0.001
Female	194 (49.62%)	52 (57.14%)	69 (55.65%)	73 (41.48%)	0.015	0.827	0.016
Caucasian	383 (97.95%)	91(100.00%)	119 (95.97%)	173 (98.30%)	0.144	0.074	0.282
Right-handed	341 (87.21%)	82 (90.11%)	107 (86.29%)	152 (86.36%)	0.642	0.398	0.986
BMI (kg/m^2)	26.17 ± 4.91	28.20 ± 5.69	25.52 ± 4.75	25.57 ± 4.28	<0.001	<0.001	0.925
CAG	n/a	n/a	42.93 ± 2.50	43.40 ± 2.77	n/a	n/a	0.124
DBS	n/a	n/a	278.13 ± 79.66	395.29 ± 98.16	n/a	n/a	<0.001
UHDRS TMS	15.78 ± 20.72	1.20 ± 2.14	2.63 ± 3.69	32.69 ± 20.64	<0.001	0.460	<0.001
UHDRS TFC	11.53 ± 2.63	12.98 ± 0.15	12.80 ± 0.73	9.90 ± 3.18	<0.001	0.550	<0.001
UHDRS IS	92.11 ± 13.91	100.00 ± 0.00	99.35 ± 2.55	82.93 ± 16.50	<0.001	0.676	<0.001
UHDRS FA	22.96 ± 4.45	24.98 ± 0.15	24.76 ± 0.92	20.65 ± 5.81	<0.001	0.686	<0.001
SWR	82.62 ± 27.14	101.85 ± 16.28	97.17 ± 19.08	62.20 ± 22.13	<0.001	0.090	<0.001
SCN	64.64 ± 21.36	79.43 ± 15.63	76.52 ± 12.74	48.43 ± 17.32	<0.001	0.178	<0.001
SDMT	43.26 ± 17.23	53.89 ± 12.08	53.69 ± 11.74	29.96 ± 13.36	<0.001	0.906	<0.001
VFC	19.23 ± 7.22	23.54 ± 5.11	22.73 ± 5.46	14.51 ± 6.40	<0.001	0.313	<0.001

*Table 3 – **Characteristics at first screening visit of participants who underwent at least 1 successful sampling visit.** Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. Continuous variables are reported as mean ± standard deviations. Categorical variables are reported as absolute and relative frequencies. BMI, body mass index; CAG, CAG repeat count; DBS, Disease Burden Score; UHDRS, Unified Huntington's Disease Rating Scale; TMS, UHDRS Total Motor Score; TFC, UHDRS Total Functional Capacity; IS, UHDRS Independence Score; FA, UHDRS Functional Assessment; SWR, Stroop Word Reading test; SCN, Stroop Color Naming test; SDMT, Symbol Digits Modality Test; VFC, Verbal Fluency Categorical; n/a, not applicable.*

	Absolute and relative frequencies				Group membership p-value	HC vs PM p-value	HC vs M p-value
	All	Healthy controls (HC)	Premanifest HD (PM)	Manifest HD (M)			
Adverse events	138 (24.13%)	34 (26.15%)	55 (31.07%)	49 (18.49%)	0.432	0.633	0.205
Headaches	85 (14.86%)	26 (20.00%)	32 (18.08%)	27 (10.19%)	0.065	0.305	0.020
PLPH	70 (12.24%)	22 (16.92%)	26 (14.69%)	22 (8.30%)	0.099	0.195	0.033

*Table 4 – **Frequency of visits with adverse events.** Categorical variables are reported as absolute and relative frequencies. The unit of analysis is the visit. PLPH, post-lumbar puncture headache; HD, Huntington's disease.*

	Absolute and relative frequencies				Group membership p-value	HC vs PM p-value	HC vs M p-value
	All	Healthy controls (HC)	Premanifest HD (PM)	Manifest HD (M)			
Adverse events	138 (24.13%)	34 (26.15%)	55 (31.07%)	49 (18.49%)	0.432	0.633	0.205
Headaches	85 (14.86%)	26 (20.00%)	32 (18.08%)	27 (10.19%)	0.065	0.305	0.020
PLPH	70 (12.24%)	22 (16.92%)	26 (14.69%)	22 (8.30%)	0.099	0.195	0.033

Supplementary figure legends

*Figure S1- **Visit disposition from screening to sampling and short-term repeat sampling visit, by disease group.** Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. HD, Huntington's disease.*

Supplementary table legends

*Table S1 – **Participant cohorts.** *the current protocol (version 3 from 19 December 2018) requires manifest gene expansion carriers to have a CAG repeat count of 40 or more, but previous versions allowed participants with ≥ 36 repeats (version 1 from 6 October 2015, and 2 from 21 June 2016). CAG, CAG repeat count; DBS, Disease Burden Score ((CAG – 35.5) × age); DCS, Diagnostic Confidence Score; TFC, Total Functional Capacity; UHDRS, Unified Huntington's Disease Rating Scale.*

*Table S2 – **Comparison of participants characteristics at successful (i.e. when dura was pierced and CSF was collected, irrespective of amount of CSF) and unsuccessful visits (i.e. where an LP was attempted, but no CSF was collected).** Continuous variables are reported as mean ± standard deviations. Categorical variables are reported as absolute and relative frequencies. BMI, body mass index; HC, healthy controls; PM, premanifest HD; M, manifest HD; UHDRS, Unified Huntington's Disease Rating Scale; TMS, UHDRS Total Motor Score; TFC, UHDRS Total Functional Capacity; IS, UHDRS Independence Score; FA, UHDRS Functional Assessment; SWR, Stroop Word Reading test; SCN, Stroop Color Naming test; SDMT, Symbol Digits Modality Test; VFC, Verbal Fluency Categorical; n/a, not applicable.*

Supplementary material

Supplementary figures

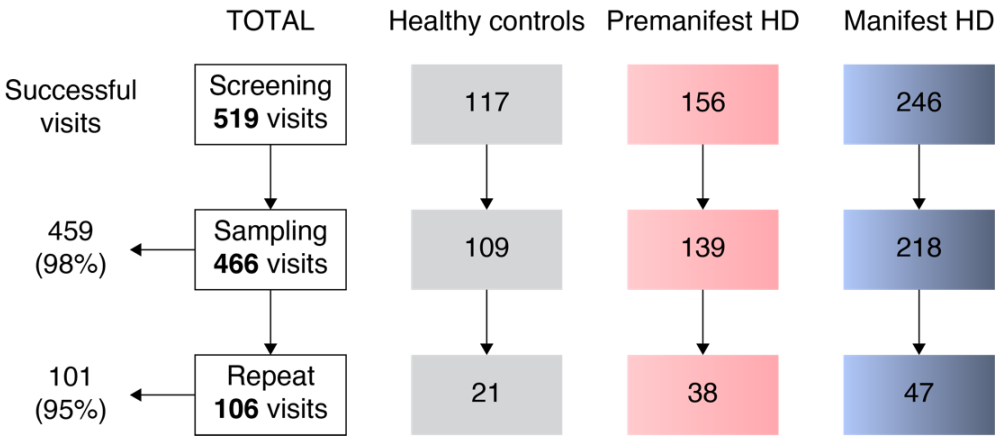


Figure S1- Visit disposition from screening to sampling and short-term repeat sampling visit, by disease group. *Successful visits were defined as when dura was pierced and CSF was collected, irrespective of amount of CSF. HD, Huntington's disease.*

Supplementary tables

Table S3 – Participant cohorts

Cohort		CAG	DBS	UHDRS DCS	UHDRS TFC
Healthy controls		<36 (or no known family history)	-	-	-
Gene expansion carriers	Early premanifest	≥ 40	< 250	< 4	-
	Late premanifest	≥ 40	≥ 250	< 4	-
	Early manifest	≥ 36*	-	4	7-13
	Moderate manifest	≥ 36*	-	4	4-6
	Late manifest	≥ 36*	-	4	0-2

**the current protocol (version 3 from 19 December 2018) requires manifest gene expansion carriers to have a CAG repeat count of 40 or more, but previous versions allowed participants with ≥ 36 repeats (version 1 from 6 October 2015, and 2 from 21 June 2016). CAG, CAG repeat count; DBS, Disease Burden Score ((CAG – 35.5) × age); DCS, Diagnostic Confidence Score; TFC, Total Functional Capacity; UHDRS, Unified Huntington's Disease Rating Scale.*

Table S4 – Comparison of participants characteristics at successful (i.e., when dura was pierced and CSF was collected, irrespective of amount of CSF) and unsuccessful visits (i.e. where an LP was attempted, but no CSF was collected).

	Successful visit	Unsuccessful visit	p-value
N	560 (97.90%)	12 (2.10%)	n/a
Age	48.35 ± 12.63	52.42 ± 9.97	0.272
Female	272 (48.57%)	7 (58.33%)	0.506
Caucasian	549 (98.04%)	12 (100.00%)	0.624
Right-handed	488 (87.14%)	10 (83.33%)	0.404
BMI (kg/m^2)	26.09 ± 5.00	29.94 ± 5.80	0.064
Study Cohort	**HC:** 129 (23.04%) **PM:** 259 (46.25%) **M**: 172 (30.71%)	**HC:** 1 (8.33%) **PM:** 5 (50.00%) **M**: 6 (41.67%)	0.487
UHDRS TMS	17.19 ± 22.30	24.67 ± 29.57	0.260
UHDRS TFC	11.33 ± 2.88	10.92 ± 3.45	0.624
UHDRS IS	90.83 ± 15.43	87.50 ± 20.17	0.465
UHDRS FA	22.55 ± 4.94	21.33 ± 6.85	0.406
SWR	81.88 ± 28.60	67.75 ± 25.36	0.095
SCN	64.32 ± 22.40	49.33 ± 15.50	0.108
SDMT	42.94 ± 17.93	32.75 ± 14.38	0.175
VFC	19.12 ± 7.36	16.25 ± 5.63	0.183

Continuous variables are reported as mean ± standard deviations. Categorical variables are reported as absolute and relative frequencies. BMI, body mass index; HC, healthy controls; PM, premanifest HD; M, manifest HD; UHDRS, Unified Huntington's Disease Rating Scale; TMS, UHDRS Total Motor Score; TFC, UHDRS Total Functional Capacity; IS, UHDRS Independence Score; FA, UHDRS Functional Assessment; SWR, Stroop Word Reading test; SCN, Stroop Color Naming test; SDMT, Symbol Digits Modality Test; VFC, Verbal Fluency Categorical; n/a, not applicable.

Table S5 – Comparison of participants characteristics at visits with and without adverse events, headaches, and post-lumbar puncture headache

	Adverse events			Headaches			Post-lumbar puncture headache		
	No	**Yes**	**p-value**	**No**	**Yes**	**p-value**	**No**	**Yes**	**p-value**
N	434 (75.87%)	138 (24.13%)	n/a	487 (85.14%)	85 (14.86%)	n/a	502 (87.76%)	70 (12.24%)	n/a
Age	49.84 ± 12.42	44.01 ± 12.09	>0.001	48.96 ± 12.60	45.43 ± 12.16	0.024	49.00 ± 12.53	44.42 ± 12.34	0.008
Female	200 (46.08 %)	79 (57.25%)	0.048	229 (47.02%)	50 (58.82%)	0.057	239 (47.61%)	40 (57.14%)	0.155
Caucasian	427 (98.39%)	134 (97.10%)	0.402	479 (98.36%)	82 (96.47423%)	0.313	492 (98.01%)	69 (98.57%)	0.793
Right-handed	375 (86.41%)	123 (89.13%)	0.297	423 (86.86%)	75 (88.24%)	0.428	435 (86.65%)	63 (90.00%)	0.267
BMI (kg/m^2)	26.36 ± 5.22	25.57 ± 4.41	0.113	26.28 ± 5.13	25.55 ± 4.46	0.233	26.27 ± 5.12	25.42 ± 4.36	0.213
Study Cohort	**HC:** 96 (22.12%) **PM:** 122 (28.11%) **M**: 216 (49.77%)	**HC:** 34 (24.64%) **PM:** 55 (39.86%) **M**: 49 (35.51%)	0.023	**HC:** 104 (21.36%) **PM:** 145 (29.77%) **M**: 238 (48.87%)	**HC:** 26 (30.59%) **PM:** 32 (37.65%) **M**: 27 (31.76%)	0.023	**HC:** 108 (21.51%) **PM:** 151 (30.08%) **M**: 243 (48.41%)	**HC:** 22 (31.43%) **PM:** 26 (37.14%) **M**: 22 (31.43%)	0.041
UHDRS TMS	19.68 ± 24.08	10.04 ± 14.19	>0.001	18.83 ± 23.52	8.88 ± 12.15	0.001	18.61 ± 23.31	8.34 ± 11.79	0.001
UHDRS TFC	11.08 ± 3.10	12.08 ± 1.91	0.002	11.17 ± 3.03	12.19 ± 1.60	0.006	11.20 ± 3.00	12.21 ± 1.61	0.011
UHDRS IS	89.48 ± 16.59	94.78 ± 10.65	0.002	89.89 ± 16.36	95.76 ± 7.73	0.003	90.10 ± 16.22	95.50 ± 7.72	0.012
UHDRS FA	22.10 ± 5.41	23.88 ± 2.92	0.002	22.25 ± 5.29	24.14 ± 1.85	0.004	22.30 ± 5.24	24.19 ± 1.76	0.010
SWR	79.35 ± 29.41	88.52 ± 24.71	0.004	79.99 ± 28.98	90.61 ± 24.50	0.004	80.08 ± 29.00	92.26 ± 22.97	0.003
SCN	62.39 ± 22.96	69.01 ± 19.70	0.007	62.84 ± 22.66	70.59 ± 19.55	0.006	62.88 ± 22.72	71.96 ± 17.94	0.004
SDMT	40.89 ± 18.03	48.35 ± 16.39	>0.001	41.57 ± 17.90	49.16 ± 16.66	0.001	41.68 ± 17.95	50.03 ± 16.00	0.001
VFC	18.44 ± 7.52	21.00 ± 6.39	0.002	18.75 ± 7.51	20.82 ± 6.02	0.025	18.78 ± 7.49	21.06 ± 5.84	0.025

Continuous variables are reported as mean ± standard deviations. Categorical variables are reported as absolute and relative frequencies. BMI, body mass index; HC, healthy controls; PM, premanifest HD; M, manifest HD; UHDRS, Unified Huntington's Disease Rating Scale; TMS, UHDRS Total Motor Score; TFC, UHDRS Total Functional Capacity; IS, UHDRS Independence Score; FA, UHDRS Functional Assessment; SWR, Stroop Word Reading test; SCN, Stroop Color Naming test; SDMT, Symbol Digits Modality Test; VFC, Verbal Fluency Categorical; n/a, not applicable.

Table S4 – *Overall* ***frequency of adverse events.*** *Categorical variables are reported as absolute and relative frequencies. The unit of analysis is the adverse event.*

Absolute and relative frequencies (n=189)		
Headache	90	47.62%
Back pain	54	28.57%
Vasovagal reactions	10	05.29%
Nausea & vomiting	7	03.70%
Bruising	7	03.70%
Paraesthesia	2	01.06%
Other	19	10.05%

Supplementary appendix 1 - Biosample collection and processing procedures

CSF collection

LUMBAR PUNCTURE

1. Identify L4/5 or L3/4 space using surface markings (i.e. the intercristal line)
2. Place subject into lateral decubitus position with pillow between knees
3. Disinfect skin using antiseptic applicator.
4. It is highly recommended to use adequate lidocaine to reduce the discomfort of this LP procedure. If, after noting allergies or sensitivities to lidocaine and discussing the risks and benefits of local anaesthesia, it is decided to forgo this step, it should be noted in the case report form. Inject up to 5ml of 2% lidocaine for local anaesthesia. Use the 25G 1" needle and inject lidocaine to raise a skin wheal. Then inject lidocaine more deeply using the 21G needle.
5. Obtain CSF using the supplied spinal needle. If the participant is thin, do not insert the deep infiltration needle all the way. Use only about 2/3 of its length (to prevent entering the subarachnoid space with anything other than the pencil-point spinal needle).
6. If CSF cannot be obtained, up to three needles may be used. An alternative design of spinal needle supplied by the site may be used if, after at least one attempt with the supplied needle, it is felt this will increase the chance of success.
7. If the CSF collection fails then there is no need to collect blood samples from the participant at this visit
8. An adjacent space may be used (with further lidocaine, max. total 10 ml, if needed).
9. If necessary, the CSF space may be located by sitting participant up, but once CSF is seen, it is recommended to have participant lie back in lateral decubitus position for 30 seconds before collection begins. Document positions of participant during puncture and collection in the eCRF
10. Document the space used for lumbar puncture, the number of needle passes (i.e. the number of times a needle is inserted and removed from the skin), the number of attempts (i.e. the number of times the lumbar space, the participant position, or the investigator conducting the LP change), the volume of lidocaine used, and the time CSF collection started and ended in the eCRF
11. Omit pressure measurement for all subjects (this is because polypropylene manometers are not available)
12. CSF is collected without suction in 50ml tubes placed on wet ice in the Styrofoam cup
13. Collect the first 1 ml of CSF into the supplied tube labelled ‘CSF’. If the first 1 ml (approx. 15 drops) is not macroscopically bloody, continue sampling CSF in the same tube up to 15-20 ml, as allowed locally, keeping the tube in the wet ice cup.

    If the first 1 ml is macroscopically bloody,

    - stop collecting CSF by reinserting the stylet partially
    - Discard the tube, and collect a second 1 ml in a new pre-cooled ‘CSF’ tube, and examine it visually for blood contamination
    - If it is free of blood, continue collecting CSF up to 14-19 ml (1ml less than the locally permitted maximum).
    - If the second separately collected ml of CSF is also macroscopically bloody, discard the tube, and continue to collect 13-18 ml of CSF in a third pre-cooled ‘CSF’ tube.
    - If the third tube is macroscopically bloody, stop collecting and abandon the procedure or attempt the LP in a different space, if there is reason to believe blood-free CSF can be obtained. You may need to open a new collection kit to provide sufficient tubes; if this

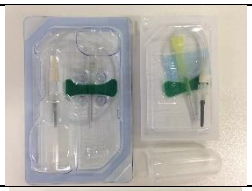
creates any discrepancies in the kit ID numbers, it must be noted carefully and explained in the eCRF.

- Stop collecting CSF when sampling time exceeds 20 minutes. Document these details in the eCRF.

14. Place cap on tube and leave on wet ice until further processing.
15. Reinsert the stylet before withdrawing the needle.
16. Cover the puncture site with sterile dressing.
17. Record time of CSF collection (time when CSF was first seen).
18. At the discretion of the Site Principal Investigator, participants may be instructed to lie flat for 1 hour.

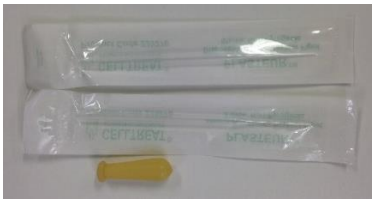
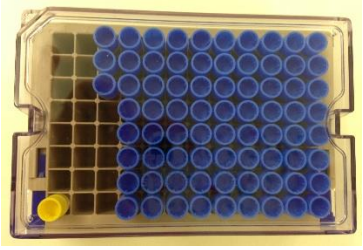
Transport CSF immediately to laboratory for processing, do not wait for the blood samples to be ready as this can cause delays

Blood collection

<table>
<tr><td colspan="2">! Please make sure that all caps are tightly secured.
! Check the expiration date on the tube- do not use expired tubes!
! Do not collect blood samples if CSF collection was not successful!</td></tr>
<tr><td>Specimens are best collected through venipuncture using a butterfly needle vacuumed directly into the required tube.</td><td>  </td></tr>
<tr><td>1. Fill 4 x 10 ml blood in lithium heparin tubes</td><td>  </td></tr>
<tr><td>2. Gently invert each lithium heparin tube 10 times immediately after collection, and place on wet ice</td><td></td></tr>
<tr><td>3. Fill 1 8.5ml serum tube</td><td>  </td></tr>
<tr><td>4. Immediately after collection transfer all blood samples to the lab for processing</td><td></td></tr>
</table>

CSF processing

Sample Collection	1. Lab to receive one 50ml CSF collection tube filled up to 20mls with CSF (collected from participant between 08:00 - 10:30 local time) 4 tubes are provided in case of blood contamination. All clean CSF sent to the lab should be in a single tube.	
	2. CSF sample is collected while the collection tube is in the styrofoam cup filled with wet ice. Sample is transported to the lab in wet ice (container to be supplied by site).	
	3. Samples transported immediately to laboratory for processing.	 **Processing must start within 15 minutes of sample collection**
	4. After CSF collection, details including the Kit ID are recorded in the **CSF** eCRF, 'CSF collection' box (Screen shot 2).	
Sample Processing	5. Note the CSF processing start time	
	6. Agitate the entire CSF sample for 10 seconds using a vortex mixer to homogenise CSF	
	7. Using a sterile individually wrapped polypropylene 1ml pipette tip, extract 200 µl of the CSF and use it to determine white blood cell count and erythrocyte count per µl in triplicate according to local GLP- approved laboratory practice as instructed at the	

	Site initiation visit and in the Manual CSF Cell Count SOP Cell counts should be recorded on the 'CSF Quality' eCRF in the 'Onsite CSF Sample Quality Control' box (Screen shot 3)	  **Triplicate cell count should be done within 60 minutes of sample collection.**
	8. Balance the centrifuge and before filling the balance tube with water please clearly mark the tube so that it can easily be identified as water (not CSF). 9. Centrifuge the 50ml tube containing residual CSF at **400 × g for 10 min at 4°C** to remove cells while preserving cell integrity for potential future use. Cell integrety in needed so that intracelular substances do not contaminate the non-cellular phase of the CSF	**Label your balance tube!**
	10. Using the polypropylene Pasteur pipette, transfer the supernatant into a single 30ml polypropylene tube labelled "CSF supernatant" and agitate for 10 seconds to homogenise CSF If the polypropylene Pasteur pipettes are damaged then it is acceptable to decant the supernatant into the tube. No pipettes should be used other than those supplied.	 
	11. Aliquot the CSF in 300 µl aliquots into the cryovials labelled "CSF", using a sterile individually wrapped polypropylene 1ml pipette tip Note the tube rack ID, tube ID (this must be the same for all aliquots) and the number of aliquots for later	

	recording on the eCRF. Please dispose of any unused aliquots CSF aliquots must have blue lids. Any samples that do not have the expected lid colour will be discarded by BioRep.	
	12. Re-suspend the CSF cell pellet in 300 µl of supplied RNA*later* solution, using gentle vortex agitation, and use another sterile pipette tip to transfer to a cryovial with yellow lid labelled “Cells from CSF” **Dispose of empty vials – Do not ship or re-use them**	 
Sample Storage and Shipment	13. Immediately after processing freeze CSF aliquots and the resuspended cells in your -80°C freezer.Ensure samples are stored upright and all lids are secure Plasma, Serum and CSF do not need to be stored in the freezer at the same time – if waiting for the blood to be ready will cause a delay, then store the CSF in the freezer first, rather than waiting. If there will be any delay in getting the samples into the freezer then they can be kept in dry ice for a short period of up to 5 minutes. Please document this on the worksheet or source notes to explain how the samples were stored if not transferred immediately to the freezer.	FREEZE AT -80°C AND SHIP AFTER A MINIMUM OF 3 MONTHS, AND WHEN YOU HAVE AT LEAST 5 SAMPLES
	Details of CSF processing are recorded on the **CSF** eCRF, ‘CSF processing’ box (Screen shot 2). Record the following parameters; Start time of CSF processing End time of CSF processing CSF tube rack ID CSF aliquot tube ID and number of cryovials Cells from CSF tube ID Date and time the samples are stored Any discrepancies in ID must be explained bearing in mind the ID is the only way to reconcile samples with participants	  

Blood processing

Sample Collection	1. Gently invert each tube 10 times immediately after collection, and place on wet ice	
	2. Samples transported immediately to laboratory for processing.	 Processing must start within 15 minutes of sample collection
	3. Lab to receive 4 x 10 ml blood in lithium heparin tubes	
Sample Processing	4. Note the following for later entry into the eCRF, or enter directly: Lithium heparin tube IDs Plasma aliquot tube ID Start time of plasma processing	 
	5. Spin lithium heparin tubes at **1300×g for 10 min at 4°C** immediately on arrival	
	6. Discard any tubes whose plasma is pink due to haemolysis. In the unlikely event that they are all pink then use all of the tubes but clearly label the sample as contaminated.	
	7. Combine the supernatant in one tube labelled “plasma” and mix by inverting 10 times. Place on wet ice.	
	8. Aliquot the plasma into 300 µl cryovials labelled ‘plasma’ using a sterile individually wrapped polypropylene 1ml pipette tip	Dispose of empty vials – do not ship! 

<table>
<tr><td></td><td>9. Plasma aliquots must have red lids. Any samples that do not have the expected colour lid will be discarded by BioRep.

Dispose of empty vials – Do not ship or re-use them</td><td></td></tr>
<tr><td rowspan="2">Sample Storage and Shipment</td><td>10. Freeze samples on dry ice and store at -80°C

Ensure samples are stored upright and all lids are secure</td><td rowspan="2">FREEZE AT -80°C
AND SHIP
AFTER A MINIMUM OF 3 MONTHS, AND WHEN YOU HAVE AT LEAST 5 SAMPLES</td></tr>
<tr><td>11. Record the following on the Blood Processing' tab in the eCRF (Screen Shot 4);

LiHep tube ID
Processing start time
Plasma aliquot tubes ID
Plasma aliquot tube count
Time plasma processing is completed
Time of frozen storage (if serum and plasma times of freezing are different then it is the time of freezing the plasma which is most important to record in the EDC)</td></tr>
</table>

Supplementary appendix 2 - HDClarity Investigators

Central Coordination

University College London: Edward J Wild (Chief Investigator), Gail Owen (Study Manager), Filipe B Rodrigues (Quality Control Officer), Katarzyna Schubert (Study Coordinator), Seema Maru (Study Coordinator), Alexander Lowe (Research Assistant), Stefanie Gosling (former Study Coordinator).

CHDI Foundation: Robi Blumenstein (President), Cristina Sampaio (Chief Clinical Officer), Eileen Neacy (Chief Operating Officer), Swati Sathe (Medical Director, Clinical Research), Anka G Ehrhardt (Director, Bio Fluid Clinical Research), Elena Pak (Clinical Research Program Manager and Study Lead), Shilpa Deshpande (Director, Clinical Operations), Sherry Lifer (Director, Contract Finance & Operations), Julia Keklak (Clinical Program Manager, Biorepository), Dipinder Kaur (former Clinical Biorepository Program Manager), Jamie Levey (Co-Director, Clinical Research Platform), Olivia Handley (Enroll-HD Global Project Manager), Jenny Townhill (Enroll-HD Trial Manager), Mette Gilling (Enroll-HD Scientific Project Manager).

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