Huntington's Disease Young Adult Study 2.0

5th December 2023

HD-YAS 2.0 SAP 5th December 2023

Overview:

The Huntington's Disease Young Adult Study (HD-YAS) is part of the Wellcome Trust funded TREAT-HD programme, which aims to deliver therapies to prevent neurodegeneration in HD. In parallel to exploring novel therapeutic strategies in HD, HD-YAS aims to identify the optimum time window to deliver preventative treatments. Specifically, it seeks to determine early systems-level pathology in very early premanifest HD gene carriers, and to assess the impact of this degeneration on symptomatology. From 2017 to 2019 a cohort of 64 premanifest HD gene carriers approximately 24 years from expected symptom onset and 67 matched controls underwent clinical, cognitive, neuropsychiatric, neuroimaging and biofluid assessments (Scahill *et al*, 2020). There was no evidence of disease-related functional impairment. No significant differences in brain structure and function were evident between gene carriers and controls with the exception of smaller striatal volumes. Neurofilament light (NfL), a marker of neuronal damage, was elevated in gene carriers, with higher levels in those closest to expected symptom onset. HD-YAS 2.0 provides follow up approximately 4.5 years after baseline with the aim of determining whether ongoing longitudinal degeneration is evident and whether this impacts function. We will determine this by addressing the following hypotheses:

Hypothesis 1: There is evidence of a disease-related measurable phenotype ~20 years from expected symptom onset in:

a) brain imaging;

b) cognition;

c) neuropsychiatric function; and

d) biofluid measures.

Hypothesis 2: There is evidence of disease-related *longitudinal* change ~20 years from expected symptom onset in:

a) brain imaging;

b) cognition;

c) neuropsychiatric function; and

d) biofluid measures.

Hypothesis 3: Early pathological events lead to impairment in isolated functional domains such as cognitive flexibility.

Hypothesis 4: Individuals with higher levels of somatic CAG-repeat expansion are more vulnerable to early striatal atrophy, breakdown of corticostriatal connections and cortical pathology.

Hypothesis 5: Measures identified as showing disease sensitivity in hypotheses 1 and 2 have utility for future trials of disease-modifying therapy in premanifest HD gene carriers.

The Cohort

Approximately 4.5 years after baseline 57 premanifest HD gene carriers (89% retention) and 45 healthy controls (67% retention) returned for a follow-up visit (HD-YAS 2.0). An additional 23 new participants were recruited: 9 premanifest HD gene carriers and 14 controls. The full cohort consists of 66 premanifest HD gene carriers and 59 healthy controls, matched for age, gender and education. All participants completed the clinical and cognitive assessments, 91% had MRI and 86% underwent CSF collection. The cohort will be characterised in terms of the new Huntington's Disease Integrated Staging System (HD-ISS) (Tabrizi *et al*, 2022), which incorporates disease pathology and biomarkers within staging classification, In addition, predicted years to clinical onset will be linked to standardisation of the CAG-Age-Product (CAP) score via previously described statistically modelling of CAP=100 at the expected age of diagnosis (Warner *et al*, 2022). These measures will be important for disease staging in future clinical trials.

General Methods

Measures

Approximately 70 individual variables from various measurement domains will be

analysed as outcome variables that may differ between the premanifest HD gene carriers and controls. The majority of measures are identical to baseline, with a few exceptions:

- One cognitive task, the breakpoint assessment, was removed from the battery due to feedback from participants. A novel task, The Goals Prior Assay, was introduced to capture the motivational domain.
- Neurogranin, a marker of synaptic function, was not assessed at this timepoint. There was no significant difference in CSF neurogranin concentration between the premanifest HD gene carrier and control groups at baseline in HD-YAS.
- Total huntingtin (tHTT) was not assessed at this timepoint. At baseline, the presence of the mutant HTT gene did not have any effect on tHTT concentrations; tHTT concentrations in CSF were unchanged in premanifest HD gene carriers.
- Volumetric neuroimaging measures, in addition to cross-sectional measures at this timepoint, include a direct measure of change, the boundary shift integral (BSI). This method has shown greater sensitivity to subtle longitudinal volumetric change than independent volume measures at each timepoint (Freeborough and Fox).
- We have included a measure of CAG-repeat expansion derived from blood DNA. This will be assessed using both the HD-YAS 1.0 and HD-YAS 2.0 samples.

The complete set of outcome measures are listed by domain in Table 1.

Descriptive statistics: All measures will be summarized by descriptive statistical tables and plots. These will include tables of means, medians, standard deviations, and ranges for the total values and the longitudinal change in values (where applicable). These will be accompanied by box plots or scatter plots as applicable. Tables and plots will divide the data in several ways, including CAG-expanded versus controls, and (within the CAG-expanded) HD ISS stage 1 versus 0, CAP100 score, and CAP100 score >=100 versus < 100.

Mutant huntingtin assay considerations:

By definition, mutant huntingtin (mHTT) levels should be 0 in bioassays collected from the non-CAG-expanded control group. Consequently, analyses to test mHTT levels associations will be restricted to the CAG-expanded group. To test possible artifacts in

measured mHTT levels, we will repeat this model with hemoglobin (Hb) levels included as an additional candidate covariate.

Somatic expansion assay:

The distribution of CAG lengths in DNA, as measured by polymerase chain reaction (PCR) will be available for the first time for HD-YAS 1.0 as well as HD-YAS 2.0. Somatic expansion lengths measured after PCR are subject to the artifact of PCR slippage, whereby true CAG lengths are occasionally shortened (and very rarely lengthened) due to errors in the chain reaction. This phenomenon is known as PCR slippage. Correction for PCR slippage has been an ongoing area of collaboration between Professor Darren Monckton's lab and Professor Douglas Langbehn. They have developed statistical methods to approximately correct for the slippage artifacts and generate what is believed to be a more accurate representation of the distribution of expanded CAG lengths. The HD-YAS somatic expansion analyses will be done using two different measures of net somatic expansion. The first of these will be the estimated mean expansion after slippage correction via the above-mentioned method. The second will be the "expansion ratio", which is the measure used in similar published research so far. The expansion ratio is the ratio (before slippage correction) of the proportion of CAG longer than the presumed baseline CAG length divided by the proportion of CAG at that baseline length.

Outliers

Outliers will be judged by both observed and residual values from the models. Extreme outliers with high model leverage will be queried with the data sources for accuracy. (The sources will not be given information about the type of influence such outliers exert on the analyses.) Outliers judged due to mistakes or poor-quality data will be excluded from analyses. Outliers confirmed to be accurate measurements will trigger a sensitivity analysis by refitting the relevant model with the outlier removed. Unless this reanalysis clearly demonstrates an absence of group or CAG effect, regardless of outlying data, we will perform nonparametric bootstrapping of the original analyses and report estimates and inference based on bias-corrected, accelerated confidence intervals.

Missing data

If more than 10% of data for any measure within an analysis (predictor or outcome) is missing, then we will use multiple imputations for parameter estimation and inference. We will also include a specific assessment of the plausibility of the missing at random (MAR) assumption that would justify this procedure. The imputation will be based on both the other variables being used in the analysis and also on other outcome variables that show a notable correlation with the variable in question when the values are not missing.

Analysis of drop-out effects

An exception to the use of multiple imputation will occur in the case of participant drop-out when longitudinal mixed effect models are fit, except if there is reason to suspect that the MAR assumption only holds conditionally after considering other available baseline measurements not used explicitly in the longitudinal model. Otherwise, correction for potential MAR dropouts will be done by including baseline values for those who dropped out within the modelling data. (This missing data strategy works only for random effect longitudinal models.) Similarly, the single measurements available from replacement participants will also be used in fitting the longitudinal models. This is consistent with the assumption that the original and replacement participants form random samples from the same underlying population of interest. It is known that approximately 30 of the original 131 participants dropped out. The majority of these were in the control group. Approximately the same number of new participants have been added to the study and will have a single, recent set of measurements available.

Violation of model distribution assumptions

For the default general linear models, the key standard assumptions are that residuals of the outcome variable will be approximately normally distributed, independent of the predicted values, and with uniform variance. The assumption is most commonly violated when the residuals show notable skewness. In such cases we will first evaluate whether the assumptions are reasonably satisfied by a square-root, log, polynomial, or inverse

transformation of the outcome, in which cases the analysis will proceed with the transformed variable. Such transformations are most commonly unsatisfactory due to floor or ceiling effects in the range of measurement of the outcome. In such cases we will consider the feasibility of using an ordinal logistic model or a censored model. If neither of these is practical and satisfactory, then the model will be subjected to bootstrapping as described above for outliers. If residual variance changes systematically by value of a covariate (e.g. age) then we will fit the models using iteratively reweighted least-squares, with weighting based on the relevant covariate.

Multiple testing

The issue of multiplicity in outcomes will be addressed by calculating estimated false discovery rates (FDR) for the covariate-adjusted difference between premanifest HD gene carriers and controls. We will perform the FDR analyses at the domain level. (Rigor is not diminished by performing these calculations at the domain level rather than in a single appraisal of the combined domains. Unlike correction for family-wise error rate, FDR calculations depend on the distribution but not on the number of hypothesis test p values considered simultaneously.) One cognitive measure, the CANTAB *inter-extra dimensional shift* task (IED), will be treated as a confirmatory rather than exploratory analysis, based on previous data showing HE differences far from onset. Significance tests will be taken at face value for this test, and it will not be included in the false discovery corrections.

Hypothesis Testing

Hypothesis 1: There is evidence of a disease-related measurable phenotype ~20 years from expected symptom onset in: a) brain imaging; b) cognition; c) neuropsychiatric function; and d) biofluid measures.

A repetition of the cross-sectional analyses performed in HD-YAS 1.0 will be performed, using the additional statistical power provided by the repeated or new subject

measurements collected in HD-YAS 2.0. Unless otherwise noted, outcomes are continuous or pseudo-continuous measures. By default, we will use general least-square linear models to evaluate possible overall group differences and age interactions between the young adult premanifest HD gene carriers and controls. Within these same models, we will control and test possible CAG-length-driven differences within premanifest HD gene carriers. Covariates will include age, gender, site and age interactions with gender. The main hypothesis of group differences will be evaluated by least-square means.

The primary cross-sectional analyses model will have a nearly identical generic form to that used in the original baseline analysis:

$$y = b_0 + I_{pHD} * (b_1 + b_2 * CAG + b_3 * age + b_4 * CAG * age) + b_5 * age + b_6 * sex + b_7 * age * sex + b_8 * NART + b_9 * EducLevel + r_{subject} + e$$

where *y* is an outcome variable, *IpHD* = 1 if a participant is from the premanifest HD gene carrier group and 0 otherwise, the *bi*'s are linear regression coefficients, $r_{subject}$ is a subject-specific random effect applicable to participants who have had two measurements, and *e* is the residual random error term Both $r_{subject}$ and *e* are assumed to be independently, identically normally distributed among the participants with 0 mean. (See above for contingencies when these distributional assumptions prove empirically untenable.) Note that NART score (a proxy for estimated baseline IQ) and education level will only be used for cognitive outcomes.

Hypothesis 2: There is evidence of disease-related *longitudinal* change ~20 years from expected symptom onset in: a) brain imaging; b) cognition; c) neuropsychiatric function; and d) biofluid measures.

The longitudinal analyses will build on the above model by adding these further regression terms:

$$a_0 * \textit{follow-up} + I_{pHD} * \textit{follow-up} * (a_1 + a_2 * CAG + a_3 * age + a_4 * CAG * age) + \textit{follow-up} * (a_5 * age + a_6 * sex + a_7 * age * sex + a_8 * IQ,)$$

where the regression coefficients *a.* estimate the longitudinal influence of follow-up time with the covariates listed in the original cross-sectional model. One other difference between the cross-sectional and longitudinal analyses is that the cross-sectional analysis will use age at each visit as a covariate, whereas the longitudinal analysis will use at baseline. This is because changes of age with follow-up and length of follow-up are redundant measures. A readily interpreted assessment of observed longitudinal change with follow-up requires that the age term in the model remains constant.

Boundary Shift Intervals (BSI) measures of MRI volumetric change will require a modification to the above longitudinal model, since these are a direct estimate of change between two MRI scans. Boundary shift volumes will be divided by time between the MRI scans, and the resultant statistic will be used as a volume change rate. This volume change rate will be the outcome variable, y, subjected to analysis.

$$y = b_0 + I_{pHD} * (b_1 + b_2 * CAG + b_3 * age + b_4 * CAG * age) + b_5 * age + b_6 * sex + b_7 * age * sex + b_8 * IQ + e$$

The age term in this BMI model again represents baseline age for the participants, to avoid confounding of changing age and follow-up time in the study. Since there will at most a single BMI measure per participant, the model will contain only a simple residual error term. No subject-specific random effects are estimable.

Hypothesis 3: Early pathological events lead to impairment in isolated functional domains such as cognitive flexibility.

Statistical analyses will only examine potential associations between early pathological events and functional impairment. Issues of causality will remain the subject of logical scientific conjecture. Analyses will be limited to combinations of pathological measures and functional measures that demonstrate differences between the HD gene carriers and controls, per the mixed model analyses described above to address Hypotheses 1 and 2.

For the functional measures used, we will assess both cross-sectional and lagged associations with pathological measures using the combined data from HD-YAS 1.0 and HD-YAS 2.0. To test cross-sectional associations, we will add the contemporary pathological measures as additional potential predictors in the cross-sectional models of the form defined above for hypothesis 1. These analyses will be limited to only the HD gene carriers and will therefore not include terms for HD-carrier versus control status or interactions of that genetic status.

We will further test for a potential lag between pathological events and measurable impairment using only the cross-sectional data impairment data from HD-YAS 2.0. Analyses will be limited to those from the HD-YAS 1.0 who also participated in HD-YAS 2.0. With single HD-YAS 2.0 functional observations per participant, we will estimate ordinary least squares regression models using both the baseline and follow-up (HD-YAS 2.0) pathology measures. (Subject to potential adjustments for violation of mathematical modeming assumptions as discussed in the General Methods section above). We will test for an association between HD-YAS 1.0 pathology measures and HD-YAS 2.0 functional measures. We will further estimate the joint associations of the HD-YAS 1.0 and HD-YAS 2.0 pathology measures by including both as joint predictors of the functional outcome. We will also refit the models by re-parameterizing the 1.0 and 2.0 values as the 1.0 value and the change in the pathology measure between 1.0 and 2.0. Other covariates in these models will be the same as described for the previous analyses (age, sex, CAG length, CAG by age interaction, and in the case of cognitive functional measures, baseline NART and Education level scores).

Within the premanifest HD gene carrier group, we will determine how well the clinical and neuroimaging variables are predicted by CSF levels of mHTT and by CSF and plasma levels of NfL. Analyses will be performed one biomarker one at a time and have the general form:

$$y = b_0 + b_1 * biomarker + b_2 * age + b_3 * gender$$

$$+ b_4 * age * gender + b_5 * IQ + r_{subject} + e$$

Additionally, we will assess evidence of longitudinal prediction of change in HD measures by adding the additional terms to the above model.

$$\textit{follow-up} * (a_1 + a_2 * CAG + a_3 * age + a_4 * CAG * age) +$$
$$\textit{follow-up} * (a_5 * baseline\ biomarker) + a_6 * biomarker\ change\ from\ baseline,)$$

Hypothesis 4: Individuals with higher levels of somatic CAG-repeat expansion are more vulnerable to early striatal atrophy, breakdown of corticostriatal connections and cortical pathology.

We will analyse potential relationships between somatic expansion and other HD measures using the same models described immediately above for hypothesis 3.

Hypothesis 5: Measures identified as showing disease sensitivity in hypotheses 1 and 2 have utility for future trials of disease-modifying therapy in premanifest HD gene carriers.

We will calculate nominal sample size estimates for longitudinal measures showing relationship to HD gene expansion vs. controls or longitudinal relationships to the severity of combined age and CAG genetic risk. We will perform such calculations for measures with longitudinal change below the 15% estimated false discovery rate. We note however, that this screening for evidence of disease sensitivity will tend to optimistically bias such estimates towards smaller sample sizes. The resultant sample sizes will therefore represent an estimated lower bound.

We will consider the following 5 biomarkers as primary outcomes for these analyses, as previous data, including baseline data from YAS 1 have suggested cross-sectional relationships between these measures and early HD disease pathology:

1. Putamen volume change
2. Caudate volume BSI
3. CSF NfL levels
4. Plasma NfL levels

5. CSF mHTT levels

Estimates of sample size requirements will be based upon the estimated longitudinal effect sizes (mean slope divided by between-subject slope standard deviation) calculated from mixed effect longitudinal. The models will be limited to HD gene-expanded participants and follow-up time will be the sole fixed effect. (The resultant estimates of natural history change will thus represent the relevant average natural history progression among the entire group of CAG-expanded participants. Additional effects of potential stratification are discussed shortly below.) Initial calculations will assume 5- and 10-year trials with annual follow-up, 50 and 70% slowing due to treatment, no placebo effect, and use of slopes rather than net change of outcome from the beginning to end of the trials. We will assume no drop-out and that the within-subject outcome variance does not increase over time. These conditions will yield best-case estimates for idealized trials. For any measure, should the resultant sample sizes appear hypothetically feasible, we will perform follow-up calculations assuming 2-, 3-, and 4-year trials with similar and smaller treatment effect sizes.

Potential trial enrichment: For the potential trial outcomes used in the above analyses, we will further test whether the possible stratification criteria of HD-ISS stage of 1 or greater (versus 0) or CAP100 score of 100 or greater are significantly associated with different rates of natural history longitudinal change. We will repeat the sample size analyses described in the previous paragraph with the data limited to the subset of CAG-expanded participants meeting the stratification criterion in question.

Table 1: Outcome variables by modality

Neuroimaging	**Regional brain volume** • Whole brain (cross-sectional volume and change YAS 1-2) • Putamen (plus HD-ISS classification) • Caudate (cross-sectional volume and change YAS 1-2) (plus HD-ISS classification) • Ventricles (cross-sectional volume and change YAS 1-2) • Grey matter (cross-sectional volume and change YAS 1-2) • White matter (cross-sectional volume and change YAS 1-2) **Structural brain connectivity** • 6 cortico-striatal connectome • 14 corticocortical connectome **NODDI** • Fractional Anisotropy • Mean Diffusivity • Radial Diffusivity • Orientation Dispersion Index • Neurite Density Index **MPM** • R1(iron & myelin) • R2* (iron/oligos) • MT (myelin)
Cognitive/emotional function	**Cognitive Flexibility** • IED ED Shift Errors • IED Failures at Stage 8 **Processing Speed** • SDMT (total correct) (plus HD-ISS classification) **Verbal Fluency** • Animals (total correct) **Interference** • Stroop Interference (total correct) **Inhibition** • Stop Signal Reaction Time **Emotion Cognition** • Intensity Morphing Decreasing (Negative Emotions only) • Moral Emotions Agent Guilt Score **Planning** • OTS Minimum Move solutions

	Learning • IED pre-ED Errors • IED ED reversal errors **Attention** • RVP A' • RVP Mean Latency (for correct trials) **Memory** • PAL Total Errors Adj • SWM between errors **Motivation** • Goals Prior Assay Task Slope
Neuropsychiatric function	**State/Trait Anxiety (STAI)** • State anxiety • Trait anxiety **Zung self-rating depression scale (SDS)** • Total score **Barratt Impulsivity scale (BIS-11)** • Total score **Frontal systems behaviour scale (FrSBE)** • Apathy • Disinhibition • Executive function **Apathy motivation index (AMI)** • Total score **Obsessive-Compulsive Inventory (OCI-R)** • Total score **Pittsburgh Sleep Quality Index (PSQI)** • Total score **MOS 36-Item Short-Form Health Survey (SF-36)** • Physical functioning • Limitations due to physical health • Limitations due to emotional problems • Energy/fatigue • Emotional well-being • Social functioning • Pain

	• General health
Clinical	• Total Motor Score (TMS)
Functional	• Total Functional Capacity (TFC) • Independence Scale (IS)
Biofluid markers	**CSF** • Mutant HTT (logfM) • Total HTT (log fM) • NfL • Tau • YKL-40 • IL-6 • IL-8 • GFAP • UCHL-1 **Plasma** • NfL • (also GFAP, Tau, UCHL-1 included in Quanterix Neurology 4PlexA with NfL) **Blood** • DNA - somatic CAG-repeat expansion (YAS 1 and YAS 2)