Published in final edited form as:

Neurobiol Dis. 2012 June ; 46(3): 625–634. doi:10.1016/j.nbd.2012.02.012.

8OHdG as a marker for Huntington disease progression

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Abstract

Leukocyte 8-hydroxydeoxyguanosine (8OHdG) is an indicator of oxidative stress, impaired metabolism, and mitochondrial dysfunction, features that have been implicated in Huntington disease (HD). Increased levels of 8OHdG have been reported in the caudate, parietal cortex, and peripherally in the serum and leukocytes, in patients diagnosed with HD. However, little is known about levels in prodromal patients and changes that might occur as the disease progresses. To address these issues, 8OHdG was tracked over time for a subset of participants enrolled in the PREDICT-HD study. Participants were stratified into four groups based on proximity to HD diagnosis at study entry: *Controls* (gene-negative individuals), *Low* (low probability of near future diagnosis), *Medium*, and *High*. Blood samples were analyzed using Liquid Chromatography Electrochemical Array, and for comparison purposes, a separate cross-sectional sample was analyzed using liquid chromatography coupled with multiple-reaction-monitoring mass spectrometry. Longitudinal data analysis showed that initial status (at study entry) and annual rate of change varied as a function of proximity group, adjusting for sex, education, age at study entry, and site effects. Overall levels were lowest for the *Control* group and highest for the *High* group, and the rate of increase varied in a similar manner. The finding that 8OHdG concentrations increased as a function of proximity to projected disease diagnosis and duration indicates support for the continued assessment of 8OHdG as a robust clinical HD biomarker.

Keywords

8OHdG; Huntington disease; Biomarker; Oxidative stress; Mitochondrial dysfunction; PREDICT-HD

Introduction

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder of the brain caused by the trinucleotide cytosine–adenine–guanine (CAG) expansion in the gene of the

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¹See the Acknowledgments section for a complete list of PREDICT-HD Investigators and Coordinators.

The authors report no conflicts of interest.

protein huntingtin. There is currently no cure and the few treatments available are for symptom modification. Clinical diagnosis or phenoconversion is often based on the presence of motor symptoms consistent with the disease phenotype and most commonly occur during the fourth decade of life (Langbehn et al., 2004). In addition to motor deterioration, declines in cognition and psychiatric deficits are common (Duff et al., 2007; Paulsen et al., 2008; Stout et al., 2011; Tabrizi et al., 2009).

Identification of the gene responsible for HD (The Huntington's Disease Collaborative Research Group, 1993) has made it possible to identify prodromal research participants who are gene-positive, but are not yet exhibiting clinical motor manifestations sufficient for clinical diagnosis. Research emphasis has since shifted to the development of clinical trials for possible disease-modifying treatments in prodromal persons (Paulsen et al., 2010b), prior to the disease consequences of functional decline, brain atrophy, and adverse clinical symptoms. Investigations with prodromal HD participants allow researchers to gain valuable insight into pathogenesis through phenoconversion and beyond, the understanding of which could potentially lead to intervention measures preventing disease manifestation (Ross and Tabrizi, 2011). Despite ample advances in the characterization of clinical and imaging changes in prodromal HD, few studies have identified possible bio-markers that might be useful in successfully monitoring therapeutic intervention efficacy. Aggressive research in the identification and validation of possible biomarkers for HD is critical to efforts to devise treatments for this relentless brain disease.

One biomarker of interest is leukocyte 8-hydroxydeoxyguanosine (8OHdG), which is an indicator of oxidative stress, impaired metabolism, and mitochondrial dysfunction. Such features have been implicated in the neuronal dysfunction associated with HD (Kikuchi et al., 2002). 8OHdG is a compound formed through hydroxylation of the guanine base by radical oxygen species (ROS). Following oxidation, damaged DNA is repaired by cellular mechanisms, and the hydroxylated guanine is excreted via bodily fluids. Consequently, available levels of 8OHdG in the blood and urine correlate with the degree of internal DNA damage and have been used as markers for such purposes in disease modeling (Valavanidis et al., 2009).

Disruptions to mitochondrial function and metabolism and an increase in oxidative stress through the production of free radical molecules have been implicated in HD (Browne and Beal, 2006; Klepac et al., 2007; Sorolla et al., 2008). Increased levels of 8OHdG have been reported in the caudate and parietal cortex (Browne et al., 1997; Polidori et al., 1999), as well as peripherally in the serum (Hersch et al., 2006) and leukocytes (Chen et al., 2007) of patients diagnosed with HD. Additionally, the R6/2 mouse model, which expresses exon 1 of the human HD gene and an expanded CAG repeat, shows increased levels of 8OHdG in urine, plasma, and striatal microdialysates (Bogdanov et al., 2001). These studies provide support for the utility of 8OHdG measurement in HD and strengthen the rationale for therapeutic strategies that either potentiate antioxidant defenses or avoid oxidative stress generation to delay disease progression. The relationship between prodromal HD individuals and 8OHdG levels, however, has remained unexplored until now.

The PREDICT-HD study is a longitudinal observational study aimed at identifying clinical and biological markers of HD in prodromal and diagnosed individuals who have undergone genetic testing for the HD gene mutation (Paulsen et al., 2006). The study has previously reported the prodromal existence of mild cognitive impairment (Duff et al., 2010), behavioral symptoms (Beglinger et al., 2008), psychiatric symptoms (Duff et al., 2007), motor deficits (Biglan et al., 2009), and neurostructural abnormalities (Nopoulos et al., 2011; Paulsen et al., 2010a). The current study aims to build on these findings by examining

possible biochemical changes occurring in HD, specifically those related to mitochondrial dysfunction.

The purpose of the current study is to examine the levels of 8OHdG in plasma samples taken from prodromal HD participants who vary in their disease progression at study entry. The primary goal is to evaluate if 8OHdG is sensitive to change over time and whether such change is a function of disease progression. In addition to change, overall differences among individuals varying in disease progression are of interest. These goals are addressed using two methods of blood specimen analysis.

Methods

Participants

Three separate samples of participants already enrolled in PREDICT-HD were considered for the analysis. Table 1 shows descriptive information of the three samples for a number of demographic variables. Each sample had two types of participants, those who tested positive for the expanded CAG repeat length (cases), and those who tested negative (controls). All participants had undergone genetic testing for the CAG repeat expansion prior to the study. Inclusion criterion for PREDICT-HD and this study was that participants had to be “prodromal” at the first visit, meaning expansion-positive participants did not meet the traditional definition of HD diagnosis as determined by question 17 of the Unified Huntington's Disease Rating Scale (UHDRS). Question 17, also known as “diagnostic confidence level” (DCL), is a 5-point scale on which investigators rate their level of confidence that an individual fulfills the criteria for a clinical motor diagnosis based on the presence of an otherwise unexplained movement disorder. A score of zero indicates that no motor abnormalities are present, whereas a score of 4 corresponds to a confidence level 99% that an individual is displaying unequivocal signs of HD. Participants with DCL=4 at the first visit were excluded from the analysis. Additional details regarding recruitment procedures in PREDICT-HD are provided by Stout et al. (2011).

The first sample was longitudinal (L) consisting of $N_L=77$ participants with up to three repeated measures over visits 1–3. The longitudinal participants had their blood specimens processed according to the first method to be discussed below (LCECA analysis). The longitudinal participants were sampled from 13 sites.

The second sample was cross-sectional (C) consisting of $N_C=80$ participants with observations mainly collected over visits 3–6. The cross-sectional participants had their blood specimens processed according to the second method presented below (LC-MS/MS analysis). The cross-sectional participants were sampled from 5 sites.

The third sample was cross-sectional and consisted of eighteen participants who overlapped (O) the two previous samples. That is, $N_O=18$ participants were common among the previous two samples, but only had cross-sectional observations. The overlapping participants had their blood specimens processed by both methods discussed below and were sampled from 4 sites.

The participants were sampled so as to represent various levels of disease progression at time of entry into PREDICT-HD. All expansion-positive participants were classified into groups according to their predicted proximity to HD diagnosis at study entry based on the CAG length/age product (CAP) variable (Zhang et al., 2011). CAP groups are *Low*, *Medium*, and *High*, with the labels reflecting levels of cumulative toxicity of mutant huntingtin at study entry. The *Control* group consists of the expansion-negative individuals who will never develop HD (all individuals had $CAG<31$). Table 2 shows the frequency (%)

of total) of CAP group membership by type of sample. As seen in Table 2, the *High* group was the largest for all the samples.

Validity evidence of the CAP groups for the larger PREDICT-HD data set is provided by Zhang et al. (2011), who show group differences on a large number of clinical and behavioral phenotypes. For present purposes, limited phenotypic information is provided for the longitudinal sample, as this was the sample for the primary analysis (see below). Means and SDs are provided for key phenotypic variables at study entry by CAP group in Table 3, along with the appropriate omnibus test statistic in the last column (F for means and chi-squared for proportions, testing any type of difference among the groups). The variables are striatal volume to total volume $\times 100$ (Striatum), UHDRS Total Motor Score (TMS; possible range 0–100 with higher scores reflecting greater symptoms), UHDRS Functional Assessment (FAS; range 0–25 with lower scores reflecting worse functioning), Symbol Digit Modalities Test (SDMT; minimum=0 with lower scores indicating greater impairment), Beck Depression Inventory (BDI; range 0–63 with higher scores indicating greater depression), and the control variables years of education, age at study entry, and proportion of males (Sex). The last row shows the proportion who received a DCL motor diagnosis (i.e., phenoconverted) at some point over the observed duration for the sample (larger values reflect greater disease progression).

Table 3 illustrates the baseline progression differences among the CAP groups. Striatal volume, SDMT, and FAS means decreased as disease progression increased, though FAS group differences were not statistically reliable ($F(3,74)=1.48$, $p>0.05$). TMS, diagnosis proportion, and BDI means increased as disease progression increased, though BDI group differences were not statistically reliable ($F(3,74)=1.86$, $p>0.05$). Additional analyses not presented suggested that differences among the *Control* and *Low* groups were attributable to differences in mean age and years of education. For additional CAP group comparisons and additional validation with the larger PREDICT-HD cohort, see Zhang et al. (2011).

Blood specimen collection protocol

Un-fasted blood specimens were obtained from the cubital vein into EDTA-coated tubes, inverted multiple times, and then centrifuged at 4000 rpm at 4 °C for 10 min. Resultant plasma was divided into 1.5–2 mL aliquots, then frozen and stored at –80 °C until analysis.

LCECA analysis

For the longitudinal participants ($N_L=77$), blood specimens were analyzed using a long gradient Liquid Chromatography Electrochemical Array (LCECA) (Bogdanov et al., 1999; Matson et al., 1984, 1987). The LCECA survey method isolates ca. 1500–2000 responses from plasma components to levels of ca. 200 pg/mL. A mixed standard of 60 components of the tyrosine, tryptophan, and purine pathways, sulfur amino acids, and markers of oxidative stress, were used to determine the expression levels of previously reported known compounds.

At the initiation of the study, a pool of all samples was created from sub-aliquots of each sample and was used for both quality assessment and normalization of the analytical data. Samples were randomized for analysis sequence. Samples were run in the sequence order: mixed standard, pool, eight samples, mixed standard, pool, eight samples, etc. All individual samples were time-normalized to a pool in the middle of the study. The data were then exported as digitized values (digital maps) capturing all information from the analytical platform. The digital maps were analyzed in raw form using principle component analysis to determine the extent of any analytical drift over the study, and subsequently normalized to the average pool value for the entire study against the pools bracketing each sample

sequence of eight. The digital maps were then trimmed to contain only values below the noise threshold of the instrument.

LC–MS/MS analysis

For the cross-sectional participants ($N_C=80$), 8OHdG was assessed using reverse phase C-18 liquid chromatography coupled to a multiple-reaction-monitoring (MRM) mass spectrometer (LC–MS/MS). This assay was developed and performed by Pharmaceutical Product Development (PPD) and used a stable-heavy-isotope-labeled form of 8OHdG.

Sample processing

The 80 human plasma specimens were received from Coriell Institute in dry ice. The average volume of a specimen was 1.2 mL. A pool produced from human plasma QC samples was obtained from the Stanford University Blood Bank and maintained in a -80°C freezer before processing. For each sample, 500 μL plasma was mixed with 500 μL water, and 5 μL $^{15}\text{N}_5$ -8OHdG was added to the mixture. 150 μL 1 M ammonium acetate (NH_4Ac , pH 5.25) was added to the solution and the solution was vigorously vortexed for 10 min on a shaker. The solution was centrifuged at $15,800\times g$ at RT for 2 min, because precipitates were observed in many specimens, which can cause a variety of processing problems. The supernatant was loaded onto Seppak tC18 solid-phase-extraction cartridge (200 mg, Waters, MA) that was preconditioned with 1 mL methanol and 1 mL water. The cartridges were then washed with 3 mL water before the fraction containing 8OHdG was eluted with 1 mL 40% methanol.

The eluent was dried under centrifugation and vacuum and then reconstituted with 100 μL buffer A (10 mM NH_4Ac in water, pH 4.75 adjusted by formic acid). Reconstituted solution was filtered with a pre-cleaned 10KD MWCO centrifugal device (wash: 200 μL for 15 min; sample: 100 μL for 12 min, both centrifuged at $15,800\times g$) and stored in a -80°C freezer for LC–MS/MS analysis.

Online HPLC–MS/MS (MRM) measurements were carried out chromatographically on an Agilent capillary 1100 binary pumping system (Agilent Technologies, Santa Clara, CA). Reverse phase-HPLC separations were achieved on a Zorbax® SB C18 capillary HPLC column (150.0.5 mm, particle size: 5 μm , pore size: 8 Å, Agilent Technologies, CA). LC flow rate was 15 $\mu\text{L}/\text{min}$, using 10 mM NH_4Ac in water adjusted to pH 4.6 with formic acid (buffer A) and 0.1% formic acid in MeCN (buffer B). Gradient elution was performed as follows: 0–2 min, 0–0% buffer B; 2–10 min, 0–50% buffer B; 10–12 min, 50–50% buffer B; 12–13 min, 50–0% buffer B; 13–20 min, 0–0% buffer B. MS/MS analysis was performed in positive ion mode, using a Q-Trap 4000 triple–quadrupole ion trap (ABI-Sciex, Foster City, CA). MRM acquisition mode was used; m/z 168 and 173 (the most abundant product ions resulting from the precursor ions $[\text{M}+\text{H}]^+$ at m/z 284 and 289, light and heavy) were utilized as quantifier ions with a dwell time 200 ms/cycle per ion.

Statistical analysis

The overall goal of the analysis was to examine CAP group differences in 8OHdG controlling for the covariates of years of education, age at entry, and sex. For the longitudinal participants the CAP group differences were in terms of initial status (intercept) and mean annual rate of change (linear slope). With the cross-sectional participants, the CAP group differences consisted of overall mean differences controlling for the covariates. The secondary goal of the analysis was to compare the 8OHdG processing methods for the $N_O=18$ overlapping participants.

Linear mixed effects regression (LMER) was used for the longitudinal analysis to account for dependency due to repeated measures and to accommodate missing data (Verbeke and Molenberghs, 2000). The time metric for the analysis was duration, defined as the age at observation minus the age at study entry. Thus, initial status reflected the level at duration=0. Site variation was a consideration, as the longitudinal participants were sampled from a relatively large number of sites (see Table 1). The LMER model had duration nested within participants, and participants nested within sites. Random intercepts and random slope terms were specified for duration, along with a random intercept for site. Preliminary statistical and graphical analyses not presented suggested that linear growth curves were adequate.

The first part of the longitudinal analysis consisted of fitting three models: (1) a model with no CAP group differences, i.e., the same initial status and annual rate of increase for all groups; (2) a model with initial status differences among the CAP groups, but equal rates of increase; and (3) a model with initial status and rate of change differences among the CAP groups. All models included the covariates of years of education, age at study entry, and sex. Details of the models are provided in the Appendix A.

Parameters for the LMER analysis were estimated using maximum likelihood methods. The models were compared with the likelihood ratio test (LRT) and Akaike's information criterion (AIC) (Akaike, 1973, 1974) corrected for small sample bias (AICc) (Hurvich and Tsai, 1989). Two scalings of the AICc were also computed, dAICc and wtAICc. dAICc is the AICc for a model minus the smallest AICc of the set. dAICc=0 for the best fitting model and dAICc>0 for the remaining models. Though no statistical testing is performed with dAICc, a rule of thumb is that dAICc>3 represents a “very strong” difference between models (Anderson, 2008). wtAICc is the weight of evidence, which is a probability scaling of the AICc, indicating the probability that a model is the most plausible given the set of models and the data (Burnham and Anderson, 2002; Long, 2012). The weight of evidence has the range 0 wtAICc 1, with larger values indicating better fit to the data and higher plausibility.

The second part of the longitudinal analysis focused on specific effects of the best fitting model. The *Control* group was the reference group for comparison with all the other groups.

The analysis of the cross-sectional sample was similar to that of the longitudinal data in the respect that three models were compared. Though the data were cross-sectional, there was variability in duration due to different individuals having different values (rather individuals having repeated duration observations). Consequently, it was possible to examine the relationship between 8OHdG and duration controlling for the covariates and test the cross-sectional analogs of the three models in the longitudinal analysis.

Preliminary LMER analysis for the cross-sectional data (not presented) indicated zero fitted model variance for sites. Therefore, site variability was not modeled and linear regression (LR) was used for the cross-sectional analysis. The three models were compared using the *F*-test, AICc, dAICc, and wtAICc.

The size of the overlapping sample was small ($N_O=18$). Therefore, no statistical analysis was performed other than to fit LR models of 8OHdG by duration for descriptive purposes. Graphs of the observed data with the fitted curves are presented below.

Results

Longitudinal sample

The results of the longitudinal data analysis indicate statistically reliable differences among the CAP groups in terms of initial levels of 8OHdG and differences in the mean rate of 8OHdG increase overtime. The nature of the effects is illustrated in Fig. 1, which is paneled by CAP group. The dashed lines depict observed levels of 8OHdG tracked over time (duration) for individuals, and the solid lines depict linear fits of the mean 8OHdG level over time for the groups. It is the starting point of the solid lines (i.e., the intercepts) and their slopes (i.e., rates of change in 8OHdG per year) that are the focus of the LMER analysis described above.

As Fig. 1 shows, the slopes of the solid lines varied as a function of CAP group, with the *Control* group having a slight decrease in 8OHdG level per year, and the *High* group having the fastest increase in 8OHdG per year. The graph also shows intercept differences in levels of 8OHdG, with the *Control* group having the lowest mean level at duration=0, and the other groups having higher initial levels.

The results of the global model comparison are shown in Table 4. As seen in the table, Model 3 had substantially better fit than the other two models (wtAICc=0.92). Recall that Model 3 had unequal mean intercepts and unequal mean slopes among the CAP groups, similar to the solid lines in Fig. 1.

Because Model 3 was the best fitting, additional details are presented. Table 5 shows the estimates of the intercepts (upper portion) and the slopes (lower portion) for each CAP group, adjusting for the covariates. The inferential statistics (*SE*, *z*, *p*) reference the difference between the *Control* group and the remaining CAP groups (*Low–Medium–High*), thus they are omitted for the *Control* group rows in Table 5. The *Control* group slope estimate ($\hat{\alpha}_{0C} = 1.05$) had an associated $z = -0.40$ indicating the slight increase in 8OHdG level after controlling for the covariates was not statistically reliable. The slight increase of the model-based estimate in Table 5 is in conflict with the slight decrease shown in Fig. 1 due to the inclusion of the covariates (Fig. 1 is not adjusted for the covariates). The covariates had relatively small effects compared to CAP group differences, with age at entry having the strongest initial status effect ($z = -1.49$; younger participants had a weak tendency to start at higher levels), and sex having the strongest slope effect ($z = 1.04$; males had a small tendency to increase at a faster rate).

Focusing first on the CAP group slopes, Table 5 shows that the 8OHdG level increased at a faster rate as the CAP group increased (from *Control*, $\hat{\alpha}_{1C} = 1.05$, to *High*, $\hat{\alpha}_{1H} = 3.99$). The *z*-ratio column shows that the *High* group had the largest increase relative to the *Control* group ($z = 3.38$), followed by *Medium* versus *Control* ($z = 1.38$), and *Low* versus *Control* ($z = 0.35$).

Turning to the mean intercept differences (at duration=0), the *Control* group had the smallest estimated initial 8OHdG level ($\hat{\alpha}_{0C} = 19.61$) and the *High* group had the largest estimated level ($\hat{\alpha}_{0H} = 24.20$). In terms of effect size, the largest difference was between the *Control* versus *High* group ($z = 3.18$), followed by *Medium* versus *Control* ($z = 1.81$), and *Low* versus *Control* ($z = 1.42$).

Cross-sectional sample

The results indicate no statistically reliable differences among the CAP groups for the cross-sectional sample. The individual observed points and the fitted linear curves are shown in Fig. 2, with the panels representing CAP groups. The reader is reminded that the data in Fig.

2 are cross-sectional as the variability in duration is due to different individuals rather than repeated measures within individuals.

The results of the LR analysis are shown in Table 6. The table shows that the model with no CAP group differences was the best fitting model (Model 1; wtAICc=0.93). Parameter estimates for Model 1 not presented showed there was a strong effect for sex ($z=3.67$), with males having a substantially higher mean 8OHdG level than females.

Overlapping sample

As previously mentioned, there were $N_O=18$ participants whose blood specimens were processed using both methods. The observed points and fitted linear curves are shown in Fig. 3.

Each vertical cross-section of duration has two points, an open circled and a filled circle, indicating the two types of processing for the same individual. As can be seen in Fig. 3, the fitted curves are similar for the *High* and *Medium* groups, but not for the *Low* and *Control* groups.

Discussion

The aim of this study was to examine 8OHdG expression levels as a function of HD disease progression. Disease progression was indexed by CAP group (*Low*, *Medium*, *High*), which is based on a proxy variable of time to diagnosis (Zhang et al., 2011) and showed initial differences on several key phenotypic variables for the participants in the primary analysis (see Table 3). Three samples of participants were considered, a longitudinal sample with LCECA blood analysis, a cross-sectional sample with LC-MS/MS analysis, and an overlapping sample that used both methods. The results of the longitudinal sample show that plasma concentrations of 8OHdG varied as a function of CAP group controlling for numerous covariates. Specifically, there were intercept differences (at duration=0) among the groups, with the mutation-negative participants (*Control* group) having the lowest level, and the participants with the most advanced disease progression (*High* group) having the highest level. The rate of annual increase in 8OHdG level also varied as a function of disease progression. The mutation-negative participants had no appreciable change over time, similarly for the *Low* CAP group. The *Medium* group had a slight increase over time, and the *High* group had the fastest increase over time (see Fig. 1).

The longitudinal sample results are consistent with previous observations of irregular mitochondrial functioning and increases in DNA damage caused by oxidative stress. Although previous investigators were able to show a similar increase in 8OHdG associated with severity of disease symptoms in diagnosed patients (Chen et al., 2007; Hersch et al., 2006), this is the first study to demonstrate elevated oxidative DNA damage stress with prodromal individuals. 8OHdG concentrations increased as a function of proximity to projected disease diagnosis, indicating support for the continued assessment of 8OHdG as a robust clinical HD biomarker.

This study showed a substantial difference between mutation-negative individuals and mutation-positive individuals who were relatively far in their disease progression. However, this comparison does not establish 8OHdG as a biomarker specific to HD. 8OHdG has been implicated in various forms of cancer (Marnett, 2000), and in different types of neurodegenerative diseases (Sayre et al., 2001). Additional research, possibly with comparison groups representing multiple diseases, is needed to investigate the extent to which 8OHdG is a reliable biomarker for HD.

Due to the cross-sectional nature of the LC–MS/MS data, it was not possible to evaluate if 8OHdG values produced by this method were sensitive to changes over time. Though the 8OHdG by duration relationship was examined by CAP group (see Fig. 2), this provided only indirect information regarding change, and the focus was on overall CAP group differences. The cross-sectional sample did not show CAP group differences in overall level (average level) or in 8OHdG growth rate (see Table 6).

The discrepancy between the results of the longitudinal LCECA analysis and the cross-sectional LC–MS/MS analysis needs to be interpreted in light of participant characteristics and differences in the blood processing. Regarding the first point, the cross-sectional LC–MC/MS sample consisted of individuals who had been in the study longer on average than the longitudinal LCECA sample and were recruited from fewer sites (see Table 1).

Regarding blood processing, there was an increased level of handling, freeze/thawing and transport of specimens to the PPD laboratory for the LC–MS/MS analysis, and all the specimens were processed after the LCECA analysis and nothing was excluded from analysis. The lab used for the LCECA analysis had a larger sample size due to the repeated measures, a more rigorous exclusion criteria, and faster access to samples (less freeze/thaw). The absolute 8OHdG values of the LC–MS/MS analysis were lower than the LCECA analysis and may represent a decreased sensitivity of this assay. The LCECA analysis produced 8OHdG results similar to those previously reported in the literature (Hersch et al., 2006), and the lab used for the LCECA is more familiar with analytical procedures specific to this compound (Bogdanov et al., 1999).

The 18 participants who had their blood specimens analyzed by both methods potentially illustrate the differences in the assay sensitivity. For the *Low*, *Medium*, and *High* groups, the LCECA method produced 8OHdG values larger than the LC–MS/MS method for each individual (see Fig. 3). This was not the case for every individual in the *Control* group, however. In terms of 8OHdG change over time, the regression lines were very similar for both methods in the *High* group, which had the largest number of participants (seven). On the other hand, the regression lines for the *Control* group (six) were dissimilar (see Fig. 3).

One limitation of the current study, as with any research involving prodromal participants, is the classification of groups in regard to proximity to diagnosis. Exclusion criteria for the PREDICT-HD study disqualify individuals clinically diagnosed with HD from enrolling in the study. Therefore, the initial selection of participants for the longitudinal sample was performed following participants' third annual assessment. Subsequently, this retrospective grouping may have inflated the 8OHdG increase seen in participants approaching predicted clinical diagnosis.

The development of robust biomarkers for HD is of the utmost importance. Due the genetic nature of the disease and the ability to identify participants before manifestation of clinical symptoms, there is a unique window for intervention, whereby the course of the disease could theoretically be arrested. Traditional clinical measures, such as the TMS and FAS are often too insensitive to detect subtle changes years prior to phenoconversion. As such, the development of suitable markers of progression, such as 8OHdG, is integral to the formation of earlier therapeutic interventions, as well as assessments of their efficacy.

The findings for the longitudinal sample show that 8OHdG, a biochemical compound easily isolated from multiple bodily fluids, increases with HD disease progression and is sensitive to individual change over time. Though a larger sample is preferred, our results suggest that plasma 8OHdG levels could be employed clinically as a tool to help measure disease progression in gene positive individuals prior to the appearance of overt motor dysfunction. It is unlikely that a single chemical compound would be employed for such purposes, but the

current findings could contribute to the development of a combination of robust measures with other biochemical biomarker candidates, as well as complement multiple other marker modalities, such as neuroimaging measures and cognitive and motor assessments to aid in the development and evaluation of interventional HD treatments.

Steering Committee

Role of the funding sources: The funding sources had no involvement in the study design; in the collection, analysis, or interpretation of the data; in the writing of the report; nor in the decision to submit the article for publication.

This work was supported by the National Institutes of Health, the National Institute of Neurological Disorders and Stroke [NS40068 to J.S.P.] and the CHDI Foundation, Inc. We thank the PREDICT-HD sites, the study participants, and the National Research Roster for Huntington Disease Patients and Families.

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Appendix A

This Appendix provides details of the statistical models considered in the analysis. As mentioned, the LMER analysis considered three models. The most complex model is considered here, as the simpler models omit terms included in the full model.

The time metric for the LMER models was duration (dur), defined as the current age minus the age at study entry. Suppose that age_{hij} is the current age for the i th participant ($i=1, \dots, 77$) in the h th site ($h=1, \dots, 13$). Then $dur_{hij} = age_{hij} - age_{hi1}$. The most complex Slope model had intercept and slope effects for CAP group and included the same for the covariates of age at entry (age_{hi1}), years of education ($educ_{hi1}$), and gender (gen_{hi1}). Define dummy variables for the CAP groups as $capL_{hi}=1$ if in the *Low* group and zero otherwise, $capM_{hi}=1$ if in the *Medium* group and zero otherwise, and $capH_{hi}=1$ if in the *High* group and zero otherwise. If y_{hij} is the 8OHdG value for the i th participant in the h th site at the j th time point, then Model 3 (see Table 4 and text) is:

$$\begin{aligned}
y_{hij} = & \beta_0 + \beta_1 (dur_{hij}) \\
& + \beta_2 (age_{hi1}) \\
& + \beta_3 (educ_{hi}) \\
& + \beta_4 (gen_{hi}) \\
& + \beta_5 (capL_{hi}) \\
& + \beta_6 (capM_{hi}) \\
& + \beta_7 (capH_{hi}) + \beta_8 (dur_{hij} \\
& \times age_{hi1} + \beta_9 (dur_{hij} \\
& \times educ_{hi} + \beta_{10} (dur_{hij} \\
& \times gen_{hi} + \beta_{11} (dur_{hij} \\
& \times capL_{hi} + \beta_{12} (dur_{hij} \\
& \times capM_{hi} + \beta_{13} (dur_{hij} \\
& \times capH_{hi}) + a_h + b_{0hi} + b_{1hi} (dur_{hij}) + \varepsilon_{hij}.
\end{aligned}$$

The intercept CAP group effects are indexed by 5, 6, and 7, whereas the slope effects are indexed by 11, 12, and 13. The intercept for the *Control* group is $\beta_0 = 0$, for the *Low* group it is $\beta_0 = \beta_0 + \beta_5$, etc. The slope for the *Control* group is $\beta_1 = 1$, for the *Low* group it is $\beta_1 = \beta_1 + \beta_{11}$, etc. In the model, a_h is the random effect for site assumed to be normally distributed with a mean of zero and variance σ_a^2 ; b_{0hi} is the random intercept, and b_{1hi} is the random slope. The random intercept and slope are assumed to have zero-means with variance–covariance matrix G , a joint-normal distribution, and orthogonal to a_h . Finally, ε_{hij} is random error assumed to have zero-mean, a normal distribution, constant variance σ^2 , and orthogonal to the random effects. In the LMER analysis, Model 2 omits all the duration interaction terms for the CAP groups, and Model 1 omits all the CAP group terms. The LR models for the cross-sectional data omit the random effects.

Corrigendum

At the request of CHDI, the authors agree to the corrigendum stated below:

The data generated at PPD/Capriion using the LC-MS/MS-based assay and directed and founded by CHDI Foundation that are reported here were included without the permission of CHDI. Further, CHDI researchers were not consulted about the presentation, analysis, or interpretation of those or any other data included in this article or any conclusions expressed here. Both of these omissions were inadvertent and are regretted.

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Abbreviations

8OHdG	8-hydroxydeoxyguanosine
HD	Huntington disease
CAG	cytosine–adenine–guanine
ROS	radical oxygen species
UHDRS	Unified Huntington's Disease Rating Scale
DCL	diagnostic confidence level
CAP	CAG length/age product
LCECA	Liquid Chromatography Electrochemical Array
MRM	multiple-reaction-monitoring
PPD	pharmaceutical product development
LMER	linear mixed effects regression
LRT	likelihood ratio test
AIC	Akaike's information criterion
LR	linear regression

Fig. 1. 8OHdG levels over time (duration) for individuals (dashed lines) and groups (solid lines). The individual curves are observed 8OHdG levels and the group curves are 8OHdG levels based on linear fits.

Fig. 2. Cross-sectional sample observed 8OHdG values (filled circles) and linear fitted curves (solid lines) by CAP group.

Fig. 3. Observed 8OHdG for two methods of processing by duration and CAP group. Open circles and gray lines indicate LCECA processing; filled circles and solid lines indicate LC-MS/MS processing.

Table 1

Descriptive statistics for the three samples used in the analysis.

Sample	Variable	Min	Med	Mean	Max	SD
Longitudinal ($N_L=77$, 13 sites)	Duration	0.00	1.03	1.05	2.72	0.85
	Entry age	26.15	42.21	42.91	75.85	9.20
	CAG	16.00	41.50	36.86	47.00	10.20
	Education	8.00	16.00	15.08	20.00	2.72
	Sex			0.55		
Cross-sectional ($N_C=80$, 5 sites)	Duration	0.00	2.95	2.73	4.26	1.06
	Entry age	19.15	41.73	41.83	62.59	8.39
	CAG	17.00	42.00	36.83	46.00	10.11
	Education	8.00	15.00	14.74	20.00	2.75
	Sex			0.50		
Overlapping ($N_O=18$, 4 sites)	Duration	1.91	1.99	2.05	2.36	0.13
	Entry age	26.84	39.02	39.10	61.17	8.42
	CAG	17.00	42.00	35.00	46.00	12.10
	Education	11.00	16.00	15.32	20.00	2.52
	Sex			0.53		

Note. Duration=current age-age at entry; sex = proportion of males.

Table 2

CAP group frequency (% of total) by sample.

Sample	Control	Low	Medium	High
Longitudinal	20 (26)	8 (10)	18 (23)	31 (40)
Cross-sectional	20 (25)	16 (20)	6 (8)	38 (48)
Overlapping	6 (23)	4 (21)	2 (11)	7 (37)

Table 3

Mean (SD) of key variables for the longitudinal sample by disease progression group at the first visit.

CAP group					
Variable	Control	Low	Medium	High	Test
Striatum	1.10 (0.13)	1.02 (0.13)	0.93 (0.16)	0.81 (0.14)	$F_{(3,47)} = 11.73$ ***
TMS	2.90 (2.49)	0.75 (1.49)	4.16 (4.68)	9.35 (6.74)	$F_{(3,74)} = 10.58$ ***
FAS	25 (0)	25 (0)	24.84 (0.37)	24.74 (0.68)	$F_{(3,74)} = 1.48$
SDMT	56.45 (5.77)	59.00 (11.74)	51.89 (13.50)	44.87 (10.55)	$F_{(3,74)} = 6.87$ ***
BDI	4.60 (5.58)	4.12 (5.00)	8.37 (8.78)	9.52 (10.24)	$F_{(3,74)} = 1.86$
Education	16 (2.15)	14.62 (2.39)	14.42 (2.97)	15 (2.92)	$F_{(3,74)} = 1.23$
Entry age	42.49 (9.09)	38.04 (8.55)	40.79 (9.63)	45.74 (8.63)	$F_{(3,74)} = 2.17$
Sex	0.55	0.5	0.63	0.52	$\chi^2_{(3)} = 0.74$
Diagnosis	0	0	0.21	0.39	$\chi^2_{(3)} = 13.52$ **

Note. Striatum=ratio of striatal volume to total brain volume $\times$ 100; TMS = Total Motor Score; FAS = Functional Assessment; SDMT = Symbol Digit Modalities Test; BDI = Beck Depression Inventory; Sex = proportion of males; Diagnosis = proportion who eventually phenocconverted.

**
p<0.01.

p<0.001.

Table 4

Results of the longitudinal sample model comparison.

Model	K	Chi sq	df	p	AICc	dAICc	wtAICc
1	11				1281.00	30.96	0.00
2	14	32.92	3	<0.0001	1254.93	4.89	0.08
3	17	11.96	3	0.0075	1250.04	0.00	0.92

Note. K = number of parameters.

Table 5

Estimated mean intercept and mean slope for the 8OHdG regression lines represented by Model 3.

Effect	CAP group	Estimate	SE ^a	z ^a	p ^a
Intercept	<i>Control</i>	19.61	4.52		
	<i>Low</i>	22.87	2.29	1.42	0.1543
	<i>Medium</i>	22.66	1.69	1.81	0.0706
	<i>High</i>	24.20	1.44	3.18	0.0015
Slope	<i>Control</i>	1.05	2.66		
	<i>Low</i>	1.56	1.45	0.35	0.7239
	<i>Medium</i>	2.40	0.98	1.38	0.1669
	<i>High</i>	3.99	0.87	3.38	0.0007

^aSE, z, and p refer to the difference between each group and the *Control* group. Results in this table are adjusted for years of education, age at study entry, and sex.

Table 6

Results of the cross-sectional sample model comparison.

Model	K	F	df ₁	df ₂	p	AICc	dAICc	wtAICc
1	9					442.97	0.00	0.93
2	12	0.82	3	69	0.4900	448.21	5.24	0.07
3	15	0.52	3	66	0.6734	455.20	12.23	0.00

Note. K = number of parameters.