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Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression

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Abstract

24S-hydroxycholesterol (24OHC) is involved in the conversion of excess cholesterol in the brain, and its level in plasma is related to the number of metabolically active neuronal cells. Previous research suggests that plasma 24OHC is substantially reduced in the presence of neurodegenerative disease. Huntington disease (HD) is an inherited autosomal dominant neurodegenerative disorder caused by a cytosine-adenine-guanine (CAG) triplet repeat expansion in the coding region of the huntingtin (HTT) gene. The current study focused on the relative importance of 24OHC as a marker of HD progression. Using mass spectrometry methods, plasma 24OHC levels were examined in three groups of gene-expanded individuals (Low, Medium, High) characterized by their progression at entry into the parent PREDICT-HD study, along with a group of non-gene-expanded controls (total $N = 150$). In addition, the correlation of 24OHC with a number of motor, cognitive, and imaging markers was examined, and effect sizes for group differences among the markers were computed for comparison with 24OHC. Results show a progression gradient as 24OHC levels decreased as the progression group increased (Low to High). The effect size of group differences for 24OHC was larger than all the other variables, except striatal volume. 24OHC was significantly correlated with many of the other key variables. The results are interpreted in terms of cholesterol synthesis and neuronal degeneration. This study provides evidence that 24OHC is a relatively important marker of HD progression.

Introduction

Huntington disease (HD) is an inherited autosomal dominant neurodegenerative disorder caused by a cytosine-adenine-guanine (CAG) triplet repeat expansion in the coding region of the huntingtin (HTT) gene (The Huntington Disease Collaborative Research Group, 1993). The mutation results in an elongated stretch of glutamine residues located in the NH₂-terminal of HTT (Walker, 2007). Neurodegeneration of the striatum and the cortex is a pathological hallmark of HD with a substantial loss of brain mass, in the order of 30% by

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the time of death (Vonsattel et al., 1998). This massive neurodegeneration is associated with a progressive striatal and cortical atrophy as measured with MRI (Aylward 2007; Henley et al., 2009) evident 10–15 years before motor onset in prodromal (also called pre-manifest) gene-positive participants (Paulsen et al., 2008;). The identification of mutation carriers provides the opportunity to investigate early disease mechanisms and, ideally, to find therapeutic strategies to halt disease progression prior to symptom onset (Weir et al., 2011).

In the early stages of the disease course, a progressive neuronal dysfunction is associated with cognitive, sensory and motor impairment. Later on, progressive cell death in vulnerable regions of the brain becomes correlated with motor signs and other functional disabilities (Ross and Tabrizi, 2011). Subtle and minor motor signs of HD are evident several years before a formal diagnosis. Together with measurable cognitive impairments these clinical findings link with neurobiological changes such as striatal atrophy (Ross and Tabrizi, 2011). PET blood flow analyses and functional MRI studies in prodromal individuals reveal reduced activation patterns in the basal ganglia (Harris et al., 1999) and cingulate cortex (Aylward et al., 2004), in the absence of volumetric losses, as well as reduced neural activation (Zimbelman et al., 2007). Reduction in raclopride (D2 dopamine receptor) binding at PET scanning has also been shown in asymptomatic mutation carriers (Weeks et al., 1996). In total, these findings suggest that abnormalities in cell function may be detectable much earlier than objective signs of cell death.

Cholesterol is an essential structural and regulatory component of brain cells and membranes. It is involved in the maturation of the CNS, signal transduction, neurotransmitter release, synaptogenesis, and membrane trafficking (Björkhem et al, 2006). All the brain cholesterol is locally synthesised (Dietschy and Turley 1994). Excess cholesterol in the brain is converted by the neuronal-specific cholesterol 24-hydroxylase (CYP46A1), into the more polar 24S-hydroxycholesterol (24OHC) which is released from the brain into circulation (Lütjohann et al., 1996).

In HD, however, additional factors might contribute to 24OHC homeostasis. The mutant huntingtin protein has been shown to diminish brain cholesterol by inducing transcriptional downregulation of a series of sterol regulatory element-regulated gene products that are essential for cholesterol biosynthesis (Valenza et al., 2005; Katsuno et al., 2010). Decreased amounts of 24S-hydroxycholesterol have also been reported in transgenic and knock-in mouse model of HD (Valenza et al., 2010). In this context, brain cholesterol metabolism in the YAC transgenic HD mice appeared to be related to the repeat length since disease-related decline of brain lathosterol, cholesterol, and 24OHC content were found to be proportional to the length of the CAG repeat (Valenza et al., 2007b). Also, in a knock-in HD mice model, the heterozygous and homozygous animals had brain 24OHC reduced by 20% and 50%, respectively (Valenza et al., 2010). It seems likely from these studies that both the length of CAG repeat and the mHtt protein load have negative effects on brain cholesterol synthesis (reduced lathosterol), and cholesterol turnover (reduced 24OHC).

Plasma 24OHC was found significantly reduced in some human neurodegenerative diseases, such as Alzheimer disease (AD), Multiple Sclerosis (MS), and HD (Papassotiropoulos et al., 2000; Leoni et al., 2002; Kolsch et al., 2004; Shobab et al., 2005; Solomon et al., 2009). Because the final effect of a neurodegenerative process is a loss of active neural cells, a reduction in 24-hydroxylase activity with subsequent decline in the formation of 24OHC and its lower efflux from the brain into circulation, are likely outcome in these disorders. Also, it has been suggested that HD impaired cholesterol synthesis associated with mutant HTT toxicity and transcriptional alterations might further contribute to the observed alterations in 24OHC metabolism (Valenza et al., 2010; Katsuno et al., 2010). The reduction in 24OHC levels we previously observed in patients' plasma paralleled the reduction of MRI

caudate volumes, suggesting that the reduction of 24OHC might in fact reflect progressive neuronal loss in the gray matter. In the small group of pre-manifest participants studied (i.e. with no overt motor signs), overall plasma concentration of 24OHC was similar to controls, though the few participants who were closer to motor onset had low levels of plasma 24OHC, similar to those found in the HD manifest patients (Leoni et al., 2008).

The current study seeks to extend previous findings by examining 24OHC levels among several HD progression groups and controls. Emphasis is on the relative importance of 24OHC as a marker of HD progression. The importance of 24OHC is assessed by its correlation with other phenotypes and its ability relative to the other variables to distinguish among progression groups.

Material and methods

Patients

We studied $N=150$ individuals, 30 mutation-negative controls and 120 HD mutation-positive cases from five sites in the larger PREDICT-HD parent study (Paulsen et al., 2008). PREDICT-HD is a longitudinal, international, multi-site observational study following a large sample of prodromal cases along with controls who are offspring of parents with HD. All aspects of the study were approved by the Institutional Review Board at each participating institution, and all participants gave written informed consent.

Though PREDICT-HD is a longitudinal study, the analysis reported here was cross-sectional. A single plasma specimen was selected per participant in such a way to provide a variety of participant progression levels (see below). Thus, specimens were selected at different visits for different participants. In order to control for this variation, the length of time in the parent study prior to specimen collection was computed for use in the statistical modelling described below. Every participant had completed genetic testing for HD prior to (and independent from) PREDICT-HD study enrolment. Gene expansion status and lab-verified CAG repeat length were confirmed at the initial study visit. Participants were considered to have prodromal HD if their CAG repeat expansions were greater than 35. Thirty of the gene-expanded participants (20%) had a motor diagnosis at the time of their specimen collection for the current analysis. The motor diagnosis was based on the highest rating of the Diagnostic Confidence Level (DCL) of the Unified Huntington's Disease Rating Scale (UHDRS) (Huntington Study Group, 1996). The highest DCL rating indicates that the clinician is 99% confident that the patient is displaying motor abnormalities that are unequivocal signs of manifest HD.

In previous collaborative studies with participants free of cholesterol-odifying therapy, we found that the overall plasma levels of 24OHC were significantly reduced in HD patients compared with healthy individuals (Leoni et al., 2008; Leoni et al., 2011). Being aware that plasma levels of 24OHC might be influenced by factors as lipoproteins transport and liver clearance (Leoni and Caccia, Biochimie 2012), we excluded from our present analysis participants with dyslipidemia-metabolic syndrome, or chronic/acute liver dysfunction. Also, potential effects on 24OHC levels of lipid lowering therapies (such as cholestiramine and statin treatments or phytosterol intake), were taken into account, and participants under statin therapy were excluded. In this context, it is important to take note of the literature report showing that 6 months treatment with pravastatin 40mg/day in healthy volunteers resulted in irrelevant changes of plasma levels of 24OHC (Thelen et al., 2006).

Characterizing disease progression at study entry is essential for proper inferences, as individuals with different CAG expansion have varied exposure times based on their age. Previous research suggests that CAG repeat length, age, and their interaction, are all

important predictors of onset (Langbehn et al., 2010; Penney et al., 1997). Therefore, progression groups were based on the CAG-Age product or CAP score (Zhang et al., 2011), computed as $CAP = (Age \text{ at PREDICT-HD entry}) \times (CAG - 33.66)$. CAP is similar to the genetic burden score developed earlier by Penney et al. (1997). Cutoffs for three CAP groups (Low, Medium, and High) were based on an optimization algorithm using all available PREDICT-HD participants (Zhang et al., 2011). The descriptors of Low, Medium, and High refer to the level of cumulative toxicity of mutant huntingtin at study entry. CAP also relates to the probability of a motor diagnosis within 5 years. In addition to the CAP groups, there was a Control group of mutation-negative individuals. For additional details, including internal validation of the CAP groupings, see Zhang et al., 2011.

Isotope dilution mass spectrometry

Plasma 24OHC was measured by isotope dilution mass spectrometry. A screw-capped vial sealed with a Teflon-septum 250 μ l of plasma was added together with 100 ng of D3-24S-hydroxycholesterol, 50 μ l of butylated hydroxytoluene (5 g/l) and 50 ml of EDTA (10 g/l). Argon was flushed through the vial to remove air. Alkaline hydrolysis was allowed to proceed at room temperature (22°C) with magnetic stirring for 1 h in the presence of ethanolic 1M potassium hydroxide solution. After hydrolysis the sterols were extracted twice with 5 ml of cyclohexane. The organic solvents were evaporated under a gentle stream of argon and converted into trimethylsilyl ethers (pyridine:hexamethyldisilazane:trimethylchlorosilane 3:2:1 v/v/v).

Gas chromatography mass spectrometry (GC-MS) analysis was performed on an Agilent Technologies HP 5890 series II combined with a 5972 mass selective detector. The GC was equipped with a DB-XLB (30m $\times$ 0.25mm id $\times$ 0.25 mm film; J&W, Palo Alto, CA, USA) and injection was performed in the split less mode using helium (1 ml/min) as a carrier gas. The temperature programme was as follows: initial temperature of 180°C kept for 1 min, followed by a rise of 20°C/min up to 270°C and immediate rise by 5°C/min up to the end temperature of 290°C, kept for 10 min.

The mass spectrometer operated in the selected ion-monitoring mode. Peak integration was performed manually, and sterols were quantified from selected-ion monitoring analyses against internal standards using standard curves for the listed sterols. Additional qualifier (characteristic fragment ions) ions were used for structural identification (Leoni et al., 2008; Solomon et al., 2009).

Samples from fasted and non-fasted HD-gene-positive participants and controls in this study were analysed blindly in a unique analytical session. All samples were analysed in the Laboratory of Clinical Pathology and Medical Genetics, Foundation IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

MRI

All MRI scans were obtained at the same visit as the clinical measures (see below), using a standard protocol that included an axial 3D volumetric spoiled gradient echo series and a dual echo proton density/T2 series. Most sites used General Electric 1.5 Tesla scanners, though two sites used Siemens 1.5 Tesla scanners. Scans were processed at The University of Iowa using AutoWorkup (Pierson et al., 2011), an automated procedure implemented in BRAINS (Magnotta et al., 2002) and artificial neural networks. There were three volumetric imaging variables: striatum (putamen + caudate), global grey matter, and global white matter. Each was expressed as the ratio to intracranial volume $\times$ 100.

Clinical Variables

In addition to the imaging variable, a number of clinical variables from the motor and cognitive domains were selected to contrast with 24OHC concentration. The variables were selected based on previous research to represent the major domains of deterioration in HD (motor, cognitive, imaging). The first motor variable was the total motor score (TMS), which is the sum of the items on the Motor Assessment scale of the UHDRS (higher scores indicate greater impairment) (Huntington Study Group, 1996). The second motor variable was speeded tapping, measured as the mean inter-tap interval over a block of trials for the non-dominant hand (in milliseconds).

The following cognitive variables were used: the Hopkins Verbal Learning Test-Revised (HVLT-R) (Brandt & Benedict, 1991), three Stroop tests (color, word, and interference) (Stroop, 1935), self-paced tapping (Rowe et al., 2010), the University of Pennsylvania Smell Identification Test (UPSIT; Doty, Shaman, Kimmelman, & Dann, 1984), and the Symbol Digit Modalities Test (SDMT) (A. Smith, 1982). The HVLT assesses verbal learning and memory (score is number of correct); the Stroop word and color tests measure basic attention, and the interference task measures inhibition of an overlearned response (score is number correct); self-paced metronome tapping is an index of psychomotor performance measured as the precision (reciprocal of the standard deviation) among a block of trials for alternating thumbs; the UPSIT measures olfactory functioning (score is the percentage of correctly identified scents); and the SDMT measures working memory, complex scanning, and processing speed (score is number correct).

Statistical Analysis

There were three components of the statistical analysis: (1) comparison of 24OHC concentration among the groups (Control, Low, Medium, High) controlling for a number of covariates; (2) comparison of the 24OHC global effect size from the first analysis with the effect sizes of the other clinical variables in similar analyses; and (3) examination of the correlation of 24OHC with the other clinical variables.

A concern for the first two analysis components was the nesting of participants within sites. Site variability was expected, due to scanner idiosyncrasies, protocol administration vagaries, and geographical diversity (PREDICT-HD sites are in six countries on three continents). It has been argued that in multi-site studies, site variability should be modelled to help ensure proper statistical inferences (e.g., Localio et al., 2002). To account for the site variability due to nesting, linear mixed models (LMMs) for clustered data were used for the group comparisons (Pinheiro & Bates, 2000). Each LMM included a number of covariates to adjust for pre-existing group differences. The covariates were years in the PREDICT-HD study prior to specimen selection (duration), gender, years of education, and age at specimen collection. Two LMMs were fit for 24OHC concentration, a reduced (null) model that had no group mean differences and a full (alternative) model that included group mean differences (both models adjusted for the covariates). The models were estimated using maximum likelihood methods. All analyses were performed using the R statistical software (R Core Team, 2013). The lme4 package was used for the LMM analysis (Bates, Maechler and Bolker, 2012). Follow-up pairwise comparisons were performed with the multcomp package (Hothorn, Bretz, and Westfall, 2008).

Statistical significance of the alternative model relative to the null model was assessed using the likelihood ratio test (LRT). The effect size of the comparison was based on Akaike's Information Criterion (AIC) (Akaike, 1973) a widely used index of statistical fit (smaller values indicate better fit). The difference of the AIC (ΔAIC) for the null and alternative fitted models was computed as $\Delta AIC = AIC(\text{reduced}) - AIC(\text{full})$. ΔAIC represents the

statistical distance between the null and alternative models and provides a scale-free index of effect size that can be used to compare relative effects (Burnham and Anderson, 2002; Long, 2012). Specifically for this analysis, ΔAIC was the statistical distance between the model having equal means among the CAP groups (the null model) and the model have unequal means among the CAP groups (the alternative model), adjusted for the covariates. Larger ΔAIC values indicate greater separation between the null and alternative models, meaning greater mean differences among the groups, and a larger effect size.

The goal of the second analysis was to provide context for interpreting ΔAIC for 24OHC concentration. That is, we wanted to see how strong the effect size for 24OHC was relative to the effect size for each of the clinical variables. Each clinical variable was analysed separately, and ΔAIC was computed for the same null and alternative models estimated with the covariates listed above.

For the third analysis, Pearson correlations among 24OHC concentration and the other variables were computed. Control participants were excluded and pairwise deletion was used to handle missing data. Each correlation was evaluated using a t -test with degrees of freedom (df) estimated from the data. For all analyses, the criterion of $p < .05$ was adopted for statistical significance.

Graphical and descriptive methods were used to assess consistency with statistical assumptions, such as normality of residuals. Results not presented revealed no evidence (e.g., outliers) that would preclude regular statistical interpretations.

As noted above, participants with a motor diagnosis were included in the sample. A supplemental analysis treating diagnosis as a subgroup showed no statistically significant effect. Therefore, no distinction was made between motor diagnosed participants and other mutation-expanded individuals.

Results

Descriptive statistics

Descriptive statistics for demographic and clinical variables are shown in Table 1. The demographic variables in the first six rows illustrate some pre-existing difference among the groups. The Low group had the smallest sample size, the lowest percentage of males, and the youngest average age. The Control group had the shortest average time in the larger PREDICT-HD study (duration) and the High group had the longest. The Control group had the highest average education and the Low group had the lowest. Participants with a motor diagnosis were in the Medium group (23.40%) and the High group (36.54%).

As for the clinical variables, 24OHC and the other variables showed a tendency for a progression gradient, with deterioration increasing from the Low through to the High group. The Control group sometimes had a better outcome than the Low group (e.g., TMS, Stroop color, striatal volume) and other times a worse outcome (e.g., SDMT, UPSIT, gray matter).

Group comparisons

A preliminary test was performed of the null hypothesis of no (zero) site variance using a bootstrap version of the LRT (Crainiceanu and Ruppert, 2004). The null hypothesis was rejected, bootstrap $X^2 = 5.02$, $p = .006$, confirming the need for the LMM approach for the group comparisons.

For the main group comparison of 24OHC, the LRT comparing the full and reduced model was statistically significant, $X^2(3) = 26.67$, $p < .001$, and $\Delta AIC = 20.67$. Figure 1 shows a

bar graph of the model-based group means (i.e., fitted means) and 95% confidence intervals (CI). The boxes on the bars indicate the results of the follow-up paired comparisons. Open boxes on the bars indicate statistically significant contrasts with the Control group, and filled boxes indicate significant contrasts with the Low group. As the boxes show, the p -values were very small ($p < .001$) for Control versus High and Low versus the more progressed groups.

Effect size comparisons

The second goal of the analysis was to assess the relative importance of 24OHC as a marker of disease progression. This goal was accomplished by comparing $\Delta AIC = 20.67$ for 24OHC with ΔAIC computed for all the clinical variables from Table 1 in separate analyses (each outcome was analysed separately). Figure 2 shows ΔAIC plotted for each measure, with the measures ordered by magnitude. Larger values indicate a greater effect size. The value for 24OHC is indicated by a filled circle. As the figure shows, striatal volume had the largest effect size ($\Delta AIC = 38.30$), followed by 24OHC. There was a step down to the next group of variables that had ΔAIC between 10 and 20. This group included several cognitive variables and the motor variables. HVLT, paced tapping, UPSIT, and global gray matter constituted a group that had especially small effect size values ($\Delta AIC < 5$).

Correlations

Correlations among all the variables are shown in Table 2. The correlation between 24OHC concentration and each variable appears in bold face in the first row. The t -test information (df and p) for the 24OHC correlations is shown at the bottom. As the table indicates, there was a statistically significant relationship between 24OHC and all the variables except paced tapping (P-tap), HVLT, UPSIT, global white matter, and global gray matter.

Discussion

We studied the modification of plasma 24OHC related to HD progression. Comparisons were made among three gene-expanded baseline progression groups (Low, Medium, High) and a gene-negative Control group. Results show that there was a significant global mean difference among the groups, and the effect size of this difference was larger than a number of cognitive, motor, and imaging variables (though not striatal volume; see Figure 2). The group differences for 24OHC concentration showed a progression gradient, decreasing in mean value as progression group increased (Low through High; see Figure 1). The highest progression group had the most substantial (and significant) difference relative to the controls and the lowest progression group. 24OHC was also significantly correlated with the majority of the other clinical variables (see Table 2).

Evidence of a progression gradient is consistent with the pattern of findings in other studies using several psychological measures, neurological tests, and MRI measurements of structural atrophy (Paulsen et al 2008; Ross and Tabrizi, 2011; Weir et al., 2011). Our results show that the effect size of the progression gradient for 24OHC was comparable to that of many other clinical variables, being second only to striatal volume. Notably, in the present study we found that striatum volumes correlated with several clinical variables, whereas global gray matter volumes did not (see Table 2). The detailed MRI investigations by Ross and Tabrizi (2010) among others, suggest that gray matter volume is not a reliable predictor of disease progression in HD. Rather, striatal volume and to a less extent, white matter volume, are the most reliable predictors.

Overall, our results suggest that 24OHC is a viable candidate as a marker of HD progression. The reduction of plasma 24OHC observed in the study does qualitatively mirror

the progression of striatum atrophy (see Table 1), and positively correlates with striatal volumes (see Table 2). This finding is arguably consistent with the hypothesis that plasma 24OHC levels depend to a large extent on the equilibrium between production by active neurons and liver clearance (Björkhem 2006; Björkhem et al., 2009). In agreement with this assumption, we observed significant reduction of plasma 24OHC in the Medium and High groups, with the Medium group having a statistically significant decline relative to the controls and to the Low group. The Medium group's lower bound of predicted time to motor onset was approximately 12 years (Zhang et al., 2011). Conversely, the patients in the Low group showed a mean plasma concentration of 24OHC statistically higher than that of the controls (see Figure 1). We speculate that it might be possible in an early stage of disease that a higher cholesterol turnover might ensue because of cyto-architectural and synaptic rearrangements in the HD brain. These rearrangements include the prodromal participants for whom the morphology is altered compared with the control participants possibly because of pre-existing developmental abnormalities (Nopoulos et al., 2007; Nopoulos et al., 2010). The rearrangements could associate with *de novo* cholesterol synthesis, and thus, result in higher brain efflux of 24OHC.

Along with disease progression into the neurodegenerative state, massive neuronal loss should manifest itself, arguably with the contribution of transcriptional abnormalities, leading to impaired cholesterol synthesis, with lower 24OHC levels in brain and plasma.

Because of the presence of the blood brain barrier, almost all the brain cholesterol is locally synthesised by astrocytes in adults (Diestchy and Turley, 2004). In order to maintain homeostasis, the excess of neuronal cholesterol in the adult brain is converted into 24OHC, which is able to cross the blood-brain barrier and enter the circulation (Lütjohann et al., 1996; Björkhem 2006). Under normal conditions, the enzyme responsible for formation of 24OHC (CYP46A1) is only present in neuronal cells, mainly in the cerebral cortex, hippocampus, dentate gyrus, amygdala, striatum, putamen and thalamus (i.e., only associated with gray matter)(Lund et al., 1999; Lund et al., 2003). The uptake of cholesterol by these cells may thus be balanced by the secretion of 24OHC (Bretillon et al., 2000; Lund et al., 2003).

In the brain, the expression of CYP46A1 appears to be resistant to regulatory axes known to regulate cholesterol homeostasis and bile acid synthesis in whole body. The promoter region of CYP46A1 presents a high GC content, a feature often found in genes considered to have mainly a housekeeping function (Lund et al., 2003). Cholesterol 24S-hydroxylase is localized in the neuronal cells and since these cells depend on a flux of cholesterol from glial cells, it seems likely that substrate availability is an important regulatory factor for the enzyme under in vivo conditions, together with the gene expression (Björkhem I., 2006).

Previous studies in HD demonstrated that during the prodromal stage there is a progressive neuronal dysfunction that arguably precedes degeneration (Weir et al., 2011). Though in neurodegenerative stages reduced 24OHC levels in patients' plasma might just reflect the reduction in the number of metabolically healthy neurons within affected brain areas, a reasonable hypothesis is that the proper metabolism of cholesterol is also impaired in the brains of people with HD (Katsuno et al., 2010). Hence, because of previous evidence provided in HD patients' brain and in HD mice brain and blood (Valenza et al., 2005; Valenza et al., 2010), we speculate that reduced 24OHC levels seen in HD plasma might not be governed by the mere neurodegenerative process, but are also subject to a concomitant impairment of brain cholesterol synthesis in astrocytes, possibly paired with a weakened Apo-E-dependent transport to neurons (Katsuno et al., 2010; Valenza et al., 2010). Our data may therefore fit with the assumption that in HD, at difference with other neurodegenerative diseases, 24OHC levels depend on both the impressive neuronal loss ensuing in manifest

(motor) HD stages (Vonsattel et al., 1985; Leoni et al., 2008) and the impaired cholesterol synthesis (Valenza et al., 2010).

The correlations between 24OHC, MRI volumetric measurements and cognitive, sensory and motor dysfunctions, with their significant link to disease burden in HD gene-positive participants indicate a robust metabolic and predictive value for 24OHC determination. However, the prognostic value of 24OHC *per se* needs further examination by longitudinal analysis, in the same manner as reported for striatal volume decline in HD (Hobbs et al 2010).

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Highlights

- Mass spectroscopy analysis of 24OHC levels can be used as a marker for HD progression
- Results show that 24OHC levels indexed a progression gradient with highly progressed individuals showing the lowest values
- 24OHC levels were correlated with a number of other HD markers including striatal volume
- 24OHC was second only to striatal volume in its ability to distinguish among progression groups

Figure 1. Model-based means and 95% confidence intervals, and statistically significant pairwise comparisons; open boxes represent comparison with Control group ($\square = p < .05$, $\square\square\square = p < .001$), filled boxes represent comparison with Low group ($\blacksquare\blacksquare\blacksquare = p < .001$).

Figure 2.

Effect size indexed by ΔAIC as a function of measure; 24OHC concentration is denoted by a solid point. Abbreviations are the following: SDMT = Symbol Digit Modalities Test, Stroop-I = Stroop Interference, Stroop-W = Stroop Word, TMS = Total Motor Score, Stroop-C = Stroop Color, S-tap = Speeded Tapping, HVL = Hopkins Verbal Learning Test, P-tap = Paced tapping, UPSIT = University of Pennsylvania Smell Identification Test.

Descriptive statistics for demographic and clinical variables. Mean (SD) are reported except for sample size (*N*), percentage of diagnosed, and percentage of males.

Table 1

Variable	Group			
	Control	Low	Medium	High
<i>Demographic</i>				
N	30	21	47	52
Male (%)	50.00	38.10	48.94	55.77
Diagnosis (%)	0	0	23.40	36.54
Age (Yr)	45.75(11.40)	35.58(7.21)	44.85(10.08)	49.16(10.88)
CAG Expansion	20.33(3.68)	41.43(1.54)	41.77(2.15)	42.81(2.34)
Education (Yr)	15(2.36)	13.81(2.04)	14.15(2.72)	14.67(2.41)
Duration (Yr) ^a	0.83(1.40)	1.83(2.17)	1.49(1.87)	1.91(1.63)
<i>Clinical</i>				
24OHC (ng/mL)	58.95(8.26)	64.57(20.13)	52.17(9.84)	47.83(13.30)
Total Motor Score	2.50(2.57)	2.67(2.11)	8.19(10.25)	9.58(9.77)
Symbol Digit Modalities Test	55.4(7.52)	59.05(12.85)	50.21(11.98)	45.1(10.80)
Stroop Color	83.34(10.28)	82.57(11.83)	76.13(14.65)	70.71(12.52)
StroopWord	48.03(8.06)	51.81(8.83)	42.04(8.99)	39.98(8.72)
Stroop Interference	105.93(11.63)	104.38(16.58)	94.13(18.67)	88.92(17.71)
Speeded Tapping	222.6(24.69)	240.73(34.37)	251.67(68.02)	284.14(86.3)
Paced Tapping	2.71(0.67)	2.49(0.71)	2.31(0.85)	2.17(0.98)
Hopkins Verbal Learning Test	28.14(5.44)	27.93(5.68)	25.59(5.15)	25.82(7.49)
UPenn Smell ID Test	85.94(6.87)	86.47(7.45)	81.42(12.14)	75.23(16.42)
Striatum ^b	1.12(0.11)	1.09(0.17)	0.93(0.13)	0.82(0.11)
White Matter ^b	30.23(1.98)	29.68(2.46)	28.25(2.77)	26.43(2.67)
Gray Matter ^b	43.02(2.60)	44.57(2.67)	43.44(2.06)	43.97(2.33)

Note.

^aDuration refers to the years in the larger PREDICT-HD study prior to specimen collection;

^bRatio of structure volume to intracranial volume $\times 100$.

Table 2

Correlations among clinical variables. 24OHC correlations are in bold face; *df* and *p*-values at the bottom are for 24OHC correlations.

Variable	TMS	SDMT	Strp-C	Strp-W	Strp-I	S-tap	P-tap	HVLT	UPSIT	Striatum	White	Gray
24OHC	-0.27	0.27	0.30	0.31	0.26	-0.25	0.18	0.07	0.22	0.30	0.19	0.08
TMS		-0.47	-0.58	-0.56	-0.40	0.60	-0.51	-0.37	-0.38	-0.32	-0.40	-0.07
SDMT			0.59	0.49	0.61	-0.35	0.37	0.49	0.41	0.41	0.35	-0.02
Strp-C				0.83	0.65	-0.49	0.42	0.38	0.47	0.35	0.36	0.01
Strp-W					0.55	-0.59	0.38	0.44	0.43	0.31	0.37	0.00
Strp-I						-0.30	0.30	0.24	0.30	0.39	0.41	0.01
S-tap							-0.49	-0.28	-0.21	-0.44	-0.44	0.01
P-tap								0.22	0.25	0.23	0.26	-0.03
HVLT									0.42	0.29	0.02	0.14
UPSIT										0.49	0.16	0.08
Striatum											0.48	0.07
White												-0.36
<i>df</i>	118	118	118	118	118	84	84	73	84	60	60	60
<i>p</i> -value	< .01	< .01	< .01	< .001	< .01	< .05	0.17	0.44	0.07	< .05	0.22	0.12

Note. TMS = Total Motor Score, SDMT = Symbol Digit Modalities Test, Strp-C = Stroop Color, Strp-W = Stroop Word, Strp-I = Stroop Interference, S-tap = Speeded Tapping, P-tap = Paced tapping, HVLT = Hopkins Verbal Learning Test, UPSIT = University of Pennsylvania Smell Identification Test.