Striatal metabolism and psychomotor speed as predictors of motor onset in Huntington’s disease

ABSTRACT

The clinical diagnosis of Huntington’s disease (HD) is based on the motor symptoms, although these can be preceded by cognitive and behavioral changes. Biomarker studies have shown that structural imaging modalities are useful biomarkers of HD onset, while functional imaging measures have been studied less often for this purpose. Our aim was to investigate the combined value of 18-fluorodesoxyglucose (FDG)–PET and cognitive measures as biomarkers of HD onset. Twenty-two premanifest mutation carriers of HD (PMCs) and 11 healthy controls were assessed twice with FDG–PET scan, neurological and neuropsychological assessments over a 2-year interval. Seventeen PMCs had an additional third neurological evaluation, 10 years after baseline. Disease load was defined as the probability of motor onset within 5 years. Metabolism in putamen, caudate and pallidum of PMCs was significantly lower than that of controls, at both assessments. Almost half of the PMCs had converted to manifest HD 10 years later and all converters had low average or abnormal putaminal metabolism at 2 year follow-up. In contrast, all PMCs with normal putaminal metabolism at 2 year follow-up remained premanifest during the following 8 years. Furthermore, glucose metabolism of putamen explained a substantial part of the variance in disease load. A composite score of psychomotor tests contributed significantly to the prediction model as well, while cognitive performance was comparable for PMCs and controls. We conclude that in future clinical trials a combination of psychomotor tests and putaminal glucose metabolism may be used to identify PMCs close to motor onset of HD.
INTRODUCTION

Huntington’s disease (HD) is an autosomal-dominant neurological disease characterized by progressive impairment of motor function, cognitive deterioration and psychiatric and behavioral symptoms. The underlying genetic defect is an expansion of a CAG trinucleotide repeat in the Huntington gene on chromosome 4, making predictive testing of at-risk individuals possible. Currently, the clinical diagnosis of HD is based on the presence of characteristic and specific motor signs, confirmed by a positive genetic test result or a family history of HD. Up till now, gene mutation carriers without evident motor signs are considered to be in the premanifest stage of HD (pre-manifest mutation carriers: PMCs). Nevertheless, in PMCs cognitive impairment may be present before the onset of motor signs. Different HD phenotypes can already be distinguished in this premanifest stage, such as predominant cognitive impairment, behavioral impairment and cognitive preservation. The age of onset of motor symptoms is variable and inversely related to the CAG repeat length. The exact moment of conversion to clinical HD cannot be predicted for an individual mutation carrier, but the probability of onset of motor signs within 5 years can be calculated with the Langbehn formula. Although to date no cure is available, it would be helpful in identifying mutation carriers that are relatively close to clinical HD; thus, facilitating statistical power and efficacy of future disease-modifying intervention trials. Data from two large multisite biomarker studies have shown that cognitive, imaging, sensory, and motor measures can be used as quantifiable endpoints in such clinical trials. With respect to cognitive biomarkers, measures of emotion recognition, psychomotor speed, working memory, and executive function are most sensitive to early cognitive changes in PMCs, sometimes even up to a decade before the estimated motor onset. Structural brain imaging measures are most effective for detecting early changes in the brains of PMCs, revealing reduced volumes of striatum and white matter. Functional imaging changes such as reduced dopamine D2 receptor binding and alterations in brain glucose metabolism, even a specific HD related pattern of metabolism, may precede these structural changes in PMC brains. Increases in regional glucose metabolism may reflect compensation for early neuronal loss or dysfunction; decline in these measures may herald clinical onset. However, insufficient data are available about how exactly in PMCs functional imaging parameters change over time and whether cortical degeneration precedes or follows subtle striatal changes in HD. Tang et al found recently that metabolic network measurements provide a sensitive method to quantify disease progression in premanifest HD. The relationship between the onset of cognitive dysfunction and the underlying neuropathological and structural changes remains unclear. A combination of functional imaging and cognitive biomarkers may provide better prediction of HD onset than single measures. We combined 18-fluorodesoxyglucose (FDG)–PET measurements with cognitive assessments in a PMC cohort that was examined longitudinally. Previously, we could not detect a significant decline in striatal glucose metabolism or dopamine D2 receptor (raclopride) binding in these subjects over a 2-year
period, although both measures were lower than in controls\textsuperscript{19}. Now we reanalyzed the FDG-PET data in relation to the cognitive measures. We wanted to know which combinations of metabolic and cognitive measures best predict the probability of motor onset within 5 years for our cohort. In retrospect we were able to stratify our PMC cohort in those far from and those close to motor onset of HD at baseline; this could be achieved by a repeat neurological evaluation 10 years after the first assessments.

MATERIALS AND METHODS

Participants

The participants are part of the original cohort of 27 PMCs and 14 controls (Cs) that has been described in detail earlier\textsuperscript{15, 19}. All subjects underwent predictive genetic testing for HD at the University Medical Center Groningen (UMCG) or the Leiden University Medical Center (LUMC) and all gave their written informed consent. Those subjects with a CAG repeat length of 36 or more, without HD motor signs (motor score below 5) on the Unified Huntington’s Disease Rating Scale (UHDRS)\textsuperscript{20} were considered PMCs. For PMCs the probability of motor onset within 5 years, given a person’s current age and CAG repeat length was obtained from the Langbehn formula\textsuperscript{2}:

\[
S(\text{Age,CAG}) = \left(1 + \exp\left(\frac{\pi [-21.54 - \exp(9.56 - 0.146CAG) + \text{Age}]}{\sqrt{35.55 + \exp(17.72 - 0.327CAG)}}\right)\right)^{-1}.
\]

This ‘disease load’ at baseline was on average 0.21 with a median of 0.16; at follow-up this had only slightly increased towards 0.25 (Table 1). Individuals from HD families without the HD mutation participated as controls (Co). At baseline, all participants had a normal neurological examination and MRI scans were normal. Furthermore all participants had an mini mental status examination score higher than 24\textsuperscript{21}. The educational level was scored

<table>
<thead>
<tr>
<th>Table 1 Demographic characteristics of participants at baseline</th>
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<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Gender: women n (%)</td>
</tr>
<tr>
<td>Age at NPA (years)</td>
</tr>
<tr>
<td>Educational level\textsuperscript{a}</td>
</tr>
<tr>
<td>CAG repeat length</td>
</tr>
<tr>
<td>Disease load\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (range) or frequency (percentage)

\textsuperscript{a} na not applicable. \textsuperscript{b} NPA neuropsychological assessment, PMCs ‘far’/‘close’ preclinical mutation carriers far from/close to motor onset, based on the UHDRS DCL score at 10 years after baseline

\textsuperscript{1} Verhage classification

\textsuperscript{2} Probability of motor onset within 5 years
with a Dutch classification scale\textsuperscript{22}.

Of the original group of 41, 33 participants (22 PMCs and 11 Co) were seen after 2 years (see Fig. 1). The reason for drop out was self-withdrawal (n = 4) or technical failures in obtaining repeat PET-scans (n = 4). We analyzed the baseline (T0) and follow-up (T1) results from the FDG–PET scan and neuropsychological assessment (NPA). Data were complete for nearly all participants, except for 2 PMCs who had missing data for NPA at T0 and two Co by whom FDG–PET scans could not be carried out successfully.

5 of the 22 PMCs did not have a neurological examination 10 years after baseline; 3 persons could not be contacted anymore and 2 PMCs declined further participation. 8 of the remaining 17 PMCs (47\%) had converted to the clinical (motor) stage of HD (UHDRS motor score: M = 11.9; SD = 4.2) after 10 years. The other 9 PMCs did not have significant motor symptoms (UHDRS motor score: M = 4.8; SD = 3.5) at that time. Those PMCs that had converted to HD during the 10 years are hereafter designated as PMCs close to motor onset of HD (PMC ‘close’), the other 9 PMCs are designated as PMCs far from motor onset of HD (PMC ‘far’). The PMCs and Co were comparable with respect to age, educational level and gender (Table 1). The two PMC subgroups (‘close’ and ‘far’) had comparable demographic characteristics as well (Table 1). The disease load for PMCs ‘close’ was higher than for PMCs ‘far’, but this did not reach significance. UHDRS total functional capacity at baseline was comparable for both PMC subgroups (scores 12 or 13).

**Procedure**

Neurological assessment and assessments of FDG–PET scans were done by a single blinded movement disorder neurologist at the UMCG (JvO). The NPA took place in a fixed order and
was carried out by trained neuropsychologists, either at the UMCG (MHD) or the LUMC (MNW, CKJ). All subjects were tested and scanned twice, with a mean interval of about 2.5 years. For most subjects the NPA was performed prior to the FDG–PET scan on 1 or 2 subsequent days, with a maximum interval of 3 months. At baseline this could unfortunately not be realised for a subgroup of our participants (median interval = 9 months, range 0–21 months). Neurological assessment 10 years after baseline was carried out by a movement disorder neurologist.

**MEASURES**

**Neuropsychological assessment**

NPA took place based on the CAPIT-HD protocol\textsuperscript{23}. In the current study we analyzed the results of only those tests known to be sensitive to early cognitive change in premanifest HD: the stroop colour word test\textsuperscript{24}, the trail making test (TMT)\textsuperscript{25} and the symbol digit modalities test (SDMT)\textsuperscript{26}. In addition we analyzed scores on letter fluency (FAS)\textsuperscript{27} and some subtests of the Wechsler memory scale (WMS); logical memory (total immediate recall), digit span (forward and backward) and associate learning\textsuperscript{28}.

Test scores were analysed separately, but were also combined to represent three cognitive domains: psychomotor speed (PmS), memory (M) and executive function (EF). correct answers, were transformed to time scores (time needed to complete the whole test). Subsequently, all test scores were converted to z-scores based on the means and standard deviations of the control data. The PmS score was calculated as the non-weighted average z-score of time scores on the stroop colour naming, stroop word reading, SDMT and TMT part A. The same was done for the M score based on the WMS subtasks logical memory immediate recall, associate learning, and digit span forward, representing working memory and episodic memory. The EF score contains the non-weighted average z-score for the stroop interference index (interference card/colour card), the TMT index (part B/part A), the digit span backwards total correct span and the letter fluency (using the letters F-A-S; total correct answers). This combined EF score represents aspects of inhibitory control, cognitive flexibility, generating and manipulating information from memory.

**Imaging measures**

Static 18-fluorodesoxyglucose (FDG)-PET scans were performed and analyzed according to previously published protocols\textsuperscript{15, 19}, aiming to minimize variability due to operator and analysis factors. We expressed FDG–PET data as a normalized regional glucose metabolic index (GMI), indicating regional activity relative to average brain metabolism. The results are presented as means of left and right GMI. We analyzed GMI in the caudate nucleus,
putamen, pallidum, and dorsolateral prefrontal cortex (DLPFC). The latter was composed as an average score of the Montreal Neurological Institute (MNI) coordinates for superior and middle frontal gyrus.

**Statistical analyses**

Given the great individual variability in onset and presentation of first cognitive symptoms, we also calculated individual classification scores for PmS, M, and EF as well as GMI in each selected brain region. These classification scores were considered ‘abnormal’ for an individual when below two standard deviations (SD) of the control mean (M), ‘low average’ when between one and two SD below M and ‘normal’ above one SD below the control mean.

Data were analyzed with IBM SPSS statistics 20 for Windows. Nonparametric tests were used since the data were not normally distributed and the sample size is rather small. Group comparisons were made with Chi-squared tests and Mann–Whitney tests when appropriate. General linear model repeated measures Ancova analyses were carried out to analyze possible group, time, and interaction effects for the cognitive and imaging data. The age was included as a covariate to control for possible age-related differences within and between groups. For correlations between the measures, partial correlations were computed, controlling for the influence of age as well. Spearman’s Rho was computed when appropriate. Linear regression analyses were carried out to analyze the contribution of these variables to the prediction of disease load. We took one-sided alpha levels, since we expected the PMC group to perform worse on all measures as compared to Co. For post hoc multiple comparisons, the Bonferroni–Holm correction was applied.

**RESULTS**

**Group comparisons**

PMCs showed a significantly lower glucose metabolism than Co in putamen $F(1, 28) = 17.3$, $p = 0.0001$, caudate nucleus $F(1, 28) = 5.5$, $p = 0.01$ and pallidum $F(1, 28) = 5.3$, $p = 0.01$ at T0 as well as at T1 (Table 2). Frontal metabolism was comparable for both groups at both assessments. Furthermore, group analyses for the cognitive measures did not reveal any significant differences between PMCs and Co at both assessments. Additional comparisons between PMCs ‘far’ and PMCs ‘close’ did not show any significant differences as well, neither on imaging, nor on cognition measures. All scores remained stable during the 2 year follow-up period, for PMCs as well as Co (Table 2).
The frequencies of individual classifications of glucose metabolism and cognitive performance (normal, low average, abnormal) of both PMCs and Co are shown in Table 3. PMCs had significantly more abnormal putaminal GMI scores than Co at T0 ($p = 0.0006$) as well as at T1 ($p = 0.0002$). Fourteen PMCs (63.6 %) had an abnormal putaminal classification score at T0, while 3 PMCs (13.6 %) had a low average score and 5 PMCs (22.8 %) had a normal score. At T1, 11 PMCs (50 %) had an abnormal score for putaminal metabolism, while 5 PMCs (22.7 %) had a low average score and 6 PMCs (27.3 %) had a normal score. Further inspection of the data shows that 11 of the original 14 PMCs with abnormal putaminal
metabolism at baseline still had an abnormal score at follow-up. The other 3 PMCs with an abnormal putaminal score at baseline now had a low average putaminal score at T1. The caudate, DLPFC and pallidum showed comparable distributions of these scores for PMCs and Cs at both assessments.

To find out whether the classification scores of PMCs glucose metabolism and cognitive performance were correlated to each other, Spearman correlations were computed (the results are shown in Table 4). After Bonferroni–Holm correction, we did not find any significant correlations between the individuals imaging and cognitive Table 3

| Individual scores: | PMCs (all) | PMCs far T0 | PMCs close T0 | PMCs (all) | PMCs far T1 | PMCs close T1 | Controls T0 | Controls T1 | p  
|-------------------|------------|------------|--------------|------------|------------|--------------|-------------|-------------|-----
| Cognition scores  | T0 n = 20  | n = 9      | n = 8        | T0 n = 22  | n = 9      | n = 8        | T0 n = 11   | T0 n = 11   |     |
| PmS               | Normal 13 (65.0) 5 (71.4) 6 (62.5) 5 (56.2) 18 (81.8) 8 (88.9) 7 (78.5) 11 (100) 10 (90.9) **0.015**  
| Low average 3 (15.0) 1 (14.3) 1 (12.5) 2 (9.1) 1 (11.1) 0 0 0 0  
| Abnormal 4 (20.0) 1 (14.3) 2 (25.0) 2 (9.1) 1 (11.1) 0 0 0 0  
| Missing 2 2 0 0 0 0 0 0 0  
| Memory            | Normal 18 (90.0) 6 (85.7) 8 (100) 19 (86.4) 7 (77.8) 8 (100) 11 (100) 11 (100) ns  
| Low average 2 (10.0) 1 (14.3) 0 3 (13.6) 2 (22.2) 0 0 0 0  
| Abnormal 0 0 0 0 0 0 0 0 0  
| Missing 2 2 0 0 0 0 0 0 0  
| EF                | Normal 18 (90.0) 7 (100) 7 (87.5) 20 (91.0) 8 (88.9) 7 (87.5) 11 (100) 11 (100) ns  
| Low average 2 (10.0) 0 1 (12.5) 1 (4.5) 0 1 (12.5) 0 0 0  
| Abnormal 0 0 0 0 0 0 0 0 0  
| Missing 2 2 0 0 0 0 0 0 0  
| Imaging scores    | n = 22  
| DLPFC             | Normal 19 (86.4) 7 (77.8) 7 (87.5) 16 (72.8) 5 (55.6) 7 (87.5) 8 (88.9) 7 (77.8) ns  
| Low average 3 (13.6) 2 (22.2) 1 (12.5) 3 (13.6) 3 (33.3) 0 1 (11.1) 2 (22.2) ns  
| Abnormal 0 0 0 0 0 0 0 0 0  
| Missing 2 2 0 0 0 0 0 0 0  
| Caudate           | Normal 13 (59.1) 7 (77.8) 3 (37.5) 12 (54.5) 6 (66.7) 3 (37.5) 7 (77.8) 8 (88.9) ns  
| Low average 5 (22.7) 0 3 (37.5) 4 (28.2) 1 (11.1) 3 (37.5) 2 (22.2) 2 (22.2) 0.03 ns  
| Abnormal 4 (18.2) 2 (22.2) 2 (25.0) 6 (27.3) 2 (22.2) 2 (25) 0 0 0  
| Putamen           | Normal 5 (22.8) 2 (22.2) 1 (12.5) 6 (27.3) 4 (44.5) 0 7 (77.8) 7 (77.8) **0.0006**  
| Low average 3 (13.6) 2 (22.2) 1 (12.5) 5 (22.7) 2 (22.2) 3 (37.5) 2 (22.2) 2 (22.2) 0.0002  
| Abnormal 14 (63.6) 5 (55.6) 6 (75.0) 11 (50.0) 3 (33.3) 5 (72.5) 0 0 0  
| Pallidum          | Normal 9 (40.9) 4 (44.5) 2 (25.0) 15 (68.2) 6 (66.7) 6 (75.0) 7 (77.8) 8 (88.9) 0.03 ns  
| Low average 11 (49.1) 3 (33.3) 6 (75.0) 5 (22.7) 2 (22.2) 1 (12.5) 2 (22.2) 1 (11.1) ns  
| Abnormal 2 (10.0) 2 (22.2) 0 2 (9.1) 1 (11.1) 1 (12.5) 0 0 0  

Values are frequencies (valid percentage) and results of Mann–Whitney tests  
T0 baseline, T1 follow up, PmS psychomotor speed average z-score, M memory average z-score, EF executive function average z-score, DLPFC dorsolateral prefrontal cortex, ns not significant  
* Bold value indicates significant p value < Bonferroni–Holm corrected α (one-sided)  
a Score above one standard deviation (SD) below the controls mean (M)  
b Score between one and two SD below M  
c Score below two SD of M  
d p value for group difference between all PMCs and Co at T0  
e p value for group difference between all PMCs and Co at T1
As to cognitive performance, PMCs had significantly worse baseline scores on psychomotor speed (PmS) than Co (p = 0.015): 20 % of PMCs had an abnormal PmS score, 15 % had a low normal PmS score and 65 % had a normal PmS score, while all Co had normal scores. At T1 the distributions of PmS scores were comparable for PMCs and Co. Classification scores for executive function (EF) and memory (M) were comparable as well, at both assessments.

In retrospect, when comparing on these classification scores PMCs ‘far’ and PMCs ‘close’, we only found a borderline significant difference for putaminal GMI (p = 0.04) at T1. More specifically, all PMCs ‘close’ then had an abnormal or low average putaminal score and none of them had a normal putaminal score, while 45 % of PMCs ‘far’ still had a normal putaminal GMI score. In other words, none of the PMCs with still a normal putaminal score at T1, had developed motor symptoms of HD 10 years after baseline (T2). PMCs ‘far’ and PMCs ‘close’ had comparable distributions on the other imaging and cognition classification scores at both measurements (see Table 3). To find out whether the classification scores of PMCs glucose metabolism and cognitive performance were correlated to each other, Spearman correlations were computed (the results are shown in Table 4). After Bonferroni–Holm correction, we did not find any significant correlations between the individuals imaging and cognitive classification scores, but at T0 a trend was seen for a positive correlation between glucose metabolism in caudate and PmS performance (p = 0.02). At T1 a comparable trend was found (p = 0.03) and additional even stronger trends were seen for positive correlations of PmS with pallidum and putamen metabolism (p = 0.01 and p = 0.02, respectively).

Correlations between disease load and other measures

Table 5 shows that the conditional probability of onset of motor symptoms within 5 years (disease load) from T0 was most strongly and negatively correlated with putaminal metabolism (p ≤ 0.001) and the PmS score (p ≤ 0.001), after correction for age. All the separate test scores that PmS consists of, as well as the WMS logical memory score, were

<table>
<thead>
<tr>
<th>Table 4 Correlations between the individual classification scores of imaging and cognition data for PMCs at T0 (n = 20) and T1 (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification scores</td>
</tr>
<tr>
<td>Putamen</td>
</tr>
<tr>
<td>Caudate</td>
</tr>
<tr>
<td>Pallidum</td>
</tr>
<tr>
<td>DLPFC</td>
</tr>
</tbody>
</table>

Values are Spearman’s Rho correlations

Bold value indicates close to significant p value < Bonferroni–Holm corrected alpha (one-sided)

T0 baseline, T1 follow up, PmS psychomotor speed average z-score, M memory average z-score, EF executive function average z-score, DLPFC dorsolateral prefrontal cortex
Table 5 Correlations between disease load and cognitive and imaging measures for PMCs at T0 (n = 20) and T1 (n = 22)

<table>
<thead>
<tr>
<th>Measures</th>
<th>Disease load T0</th>
<th>Disease load T1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cognitive measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PmS score</td>
<td>-0.69*</td>
<td>-0.83*</td>
</tr>
<tr>
<td>SDMTa</td>
<td>-0.62*</td>
<td>-0.75*</td>
</tr>
<tr>
<td>TMTb part A</td>
<td>0.54*</td>
<td>0.68*</td>
</tr>
<tr>
<td>Stroop colour</td>
<td>-0.55*</td>
<td>-0.53*</td>
</tr>
<tr>
<td>Stroop word</td>
<td>-0.53*</td>
<td>-0.52*</td>
</tr>
<tr>
<td>Stroop interference</td>
<td>-0.18</td>
<td>-0.48*</td>
</tr>
<tr>
<td>TMT part B</td>
<td>0.32</td>
<td>0.41*</td>
</tr>
<tr>
<td>EF score</td>
<td>-0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>TMT B/A indexd</td>
<td>-0.32</td>
<td>-0.13</td>
</tr>
<tr>
<td>Stroop i/c indexc</td>
<td>-0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Letter fluency FAS</td>
<td>-0.14</td>
<td>-0.35</td>
</tr>
<tr>
<td>DS bw</td>
<td>-0.08</td>
<td>-0.25</td>
</tr>
<tr>
<td>M score</td>
<td>-0.24</td>
<td>-0.39</td>
</tr>
<tr>
<td>WMS logical memf</td>
<td>-0.51*</td>
<td>-0.56*</td>
</tr>
<tr>
<td>WMS assoc learnf</td>
<td>-0.19</td>
<td>-0.37</td>
</tr>
<tr>
<td>DS fw</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Imaging measures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMI putamen</td>
<td>-0.69*</td>
<td>-0.70*</td>
</tr>
<tr>
<td>GMI striatum</td>
<td>-0.56*</td>
<td>-0.56*</td>
</tr>
<tr>
<td>GMI pallidum</td>
<td>-0.55*</td>
<td>-0.55*</td>
</tr>
<tr>
<td>GMI caudate</td>
<td>-0.36</td>
<td>-0.35</td>
</tr>
<tr>
<td>GMI DLPFC</td>
<td>-0.20</td>
<td>-0.21</td>
</tr>
</tbody>
</table>

Values are partial correlations, corrected for the influence of age.

SDMT = symbol digit modalities test, TMT = trail making test in seconds, WMS = wechsler memory scale, DS = digit span forward maximum span correct, DSbw = digit span backward maximum span correct, PmS = psychomotor speed average z-score, M = memory average z-score, EF = executive function average z-score, GMI = glucose metabolic index, DLPFC = dorsolateral prefrontal cortex, T0 = baseline, T1 = follow up.

* Bold value indicates significant p value < Bonferroni–Holm corrected α (one-sided).

a Number correct in 90 s
b In seconds
c Number correct in 45 s
d TMT part B/part A
e Interference card in seconds/colour card in seconds
f Subtest logical memory, average correct of stories 1 and 2
g Subtest associate learning, number correct
Given the small sample size the final prediction model was restricted to a maximum of three predictors that were significant and together explained 67% of the total variance in disease load; of the putaminal GMI and the PmS score, together explaining 68% of total variance in disease load.

Table 6 Results from the linear regression analyses for the prediction of disease load of PMCs at T0 (n = 20) and T1 (n = 22)

<table>
<thead>
<tr>
<th>Separate predictors</th>
<th>Adjusted $R^2$ T0</th>
<th>p T0</th>
<th>Adjusted $R^2$ T1</th>
<th>p T1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cognitive measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PmS score</td>
<td>0.45</td>
<td>&lt;0.001*</td>
<td>0.63</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SDMT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35</td>
<td>0.002*</td>
<td>0.54</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>TMT&lt;sup&gt;b&lt;/sup&gt; part A</td>
<td>0.26</td>
<td>0.007*</td>
<td>0.42</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Stroop&lt;sup&gt;c&lt;/sup&gt; colour</td>
<td>0.25</td>
<td>0.008*</td>
<td>0.23</td>
<td>0.008*</td>
</tr>
<tr>
<td>Stroop word</td>
<td>0.23</td>
<td>0.01*</td>
<td>0.21</td>
<td>0.01*</td>
</tr>
<tr>
<td>WMS log mem&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.22</td>
<td>0.01*</td>
<td>0.27</td>
<td>0.004*</td>
</tr>
<tr>
<td>TMT part B</td>
<td>0.05</td>
<td>0.09</td>
<td>0.12</td>
<td>0.03*</td>
</tr>
<tr>
<td>Stroop interference</td>
<td>−0.02</td>
<td>0.22</td>
<td>0.19</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Imaging measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMI Putamen</td>
<td>0.44</td>
<td>&lt;0.001*</td>
<td>0.46</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Striatum</td>
<td>0.23</td>
<td>0.007*</td>
<td>0.22</td>
<td>0.008*</td>
</tr>
<tr>
<td>GMI Pallidum</td>
<td>0.24</td>
<td>0.007*</td>
<td>0.24</td>
<td>0.006*</td>
</tr>
<tr>
<td>GMI Caudate</td>
<td>0.06</td>
<td>0.07</td>
<td>0.04</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Combined predictors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMI Putamen, PmS score</td>
<td>0.67</td>
<td>&lt;0.001*</td>
<td>0.68</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, PmS score, Stroop interference</td>
<td>na</td>
<td>na</td>
<td>0.67</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, PmS score, TMT part B</td>
<td>na</td>
<td>na</td>
<td>0.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, PmS score, GMI pallidum</td>
<td>0.65</td>
<td>&lt;0.001*</td>
<td>0.66</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, PmS score, WMS log mem</td>
<td>0.61</td>
<td>&lt;0.001*</td>
<td>0.68</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Striatum, PmS score</td>
<td>0.59</td>
<td>&lt;0.001*</td>
<td>0.63</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, WMS log mem</td>
<td>0.50</td>
<td>&lt;0.001*</td>
<td>0.54</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GMI Putamen, GMI pallidum</td>
<td>0.42</td>
<td>0.001*</td>
<td>0.44</td>
<td>0.008*</td>
</tr>
</tbody>
</table>

Values are individual and combined contributions of the measures to the regression model and p values

<sup>a</sup> Bold value indicates significant p value < Bonferroni–Holm corrected α (one-sided)

<sup>b</sup> Number correct in 90 s
<sup>c</sup> In seconds
<sup>d</sup> Number correct in 45 s
<sup>e</sup> Subtest logical memory, average correct of stories 1 and 2
also significantly correlated with disease load (p values between 0.003 and 0.01). In addition striatal and pallidal metabolism showed significant negative correlations with disease load (p = 0.004 and p = 0.005, respectively).

At T1, comparable and in part even stronger results were found (Table 5). In addition disease load was now significantly correlated with the colour word card of the stroop test (p = 0.01) and the TMT part B (p = 0.03).

**Prediction model**

The measures with the highest, significant, correlations with disease load at T0 (see Table 5) were included, one by one, in a linear regression analysis. Age did not contribute significantly to the model and was therefore left out of the analyses. The largest adjusted \( R^2 \) for the contributions of the separate measures to disease load were found for the PmS score (45 %) and putaminal GMI (44 %); \( F(1, 19) = 16.32, p \leq 0.001 \) and \( F(1, 21) = 17.73, p \leq 0.001 \), respectively (see Table 6). Most psychomotor (sub-) tests contributed significantly to the variance in disease load. The SDMT explained 35 % of total variance, TMT part A 26 %, stroop colour naming card 25 % and the stroop word reading card explained 23 % of total variance (corrected p values between 0.002 and 0.01). The WMS subtest logical memory explained 22 % of the variance in disease load (p = 0.01). Of the imaging measures the pallidal GMI accounted for 24 % of the variance (p = 0.007) but the caudate GMI did not contribute significantly to disease load.

Given the small sample size the final prediction model was restricted to a maximum of three predictors that were entered together, in a multiple linear regression model. The best model for predicting disease load at baseline consisted of the putaminal GMI and the PmS score, together explaining 67 % of the total variance in disease load; \( F(2, 19) = 20.06, p < 0.001 \), described in the following regression formula: disease load = 1.94–1.61 x GMI Putamen + 0.078 x PmS. The same regression analyses were carried out for the T1 data and resulted in comparable, even stronger findings. Putaminal GMI and PmS together explained 68 % of total variance in disease load \( F(2, 21) = 22.87, p < 0.001 \), now yielding the following equation: disease load = 1.19–0.89 x GMI Putamen + 0.11 x PmS.

**DISCUSSION**

We studied a well-defined group of premanifest mutation carriers of HD with imaging and cognitive assessment at two time points, 2 years apart, in order to identify the best combination of clinical markers to predict the probability of HD motor onset. This motor onset could be determined accurately as patients were reassessed 10 years after the initial examination. The combination of glucose metabolism in putamen and a composite score for
psychomotor speed accounts for two-thirds of variance in the calculated probability of motor onset within 5 years. This model holds true—and thus should be considered validated—for measurements obtained at 2-year-follow-up. The relevance of combined clinical measures in predicting disease onset and disease progression had been established before8, 9, 30, as well has the value of FDG–PET to detect early functional brain changes in PMCs14. Our study is the first to show that the combination of FDG–PET and cognitive (psychomotor speed) measures has significant predictive value.

Our functional imaging data replicate earlier findings on metabolic alterations in premanifest HD. We showed that putaminal metabolism was abnormal in almost two-thirds of PMCs at baseline and in half of PMCs at 2-year-follow-up. This slight paradoxical decrease in the number of abnormal putaminal metabolism scores may be explained by the phenomenon of ‘regression towards the mean’. We made a rather artificial classification for individual imaging and cognitive performances on the basis of z-scores. Three PMCs with an abnormal putaminal score at baseline (z-scores slightly below-2), ‘improved’ to the low average level at follow-up (with z-scores slightly above-2), while their absolute putaminal scores did not change that much. Furthermore, the average putaminal metabolism of the PMC group remained stable during follow-up. We also found abnormal caudate and pallidal metabolism in smaller subgroups of our cohort; however, these were not significantly different from Co. In line with the previous evidence, this illustrates that the striatum is one of the first brain regions affected in HD15, 16, 31. In contrast to some other studies11, we found metabolic alterations to be most profound in the putamen, whereas they found the caudate nucleus to show the first (structural) changes in HD. We did not find a significant difference in DLPFC metabolism for PMCs as compared to controls. This is in contrast with Wolf et al.32, who found left DLPFC hypo-activation in PMCs with increasing working memory load, while cognition was still preserved. An explanation for this may be that different paradigms were used, putting another demand on DLPFC function of PMCs; i.e., during task performance with event-related fMRI (the Wolf study) and in resting state with static FDG–PET (this study). Yet, Wolf et al. did not find any differences in striatal activation for PMCs and controls, while in our cohort the striatum is clearly affected in a substantial part of PMCs. Perhaps, the Wolf cohort was still farther from expected motor onset than our cohort. Their PMCs indeed seem on average slightly younger, with slightly lower CAG repeat lengths. It could be that the earliest functional changes in DLPFC are best demonstrated while the brain is confronted with higher cognitive demands; comparison of the two imaging paradigms in a larger PMC cohort may elucidate the discrepancy in findings on DLPFC function in PMCs.

In our study psychomotor tasks are particularly sensitive to cognitive decline in premanifest HD, which is in line with the literature2–10. In addition, our data suggest that a composite score of several psychomotor tasks performance may have better predictive value than the test scores separately. Harrington et al.33 recently suggested that two factors, ‘motor planning/speed’ and ‘sensory-perceptual processing’, may be the best indicators of time
to diagnosis, after controlling for disease load and motor function. However, as the authors mentioned, the tests they employed are experimental and lack normative data. In contrast, the tests we included possess established clinical usefulness, already. It must be mentioned although, that some PMCs already had abnormal psychomotor speed before onset of overt motor symptoms, while others performed within normal range. These individual scores of psychomotor speed performance showed trends of meaningful correlations with regional striatal metabolism, which is in line with the earlier evidence from (structural) imaging studies. Nearly all PMCs had normal scores with respect to executive function and memory and this is in line with the existing evidence that alterations in information processing speed precede memory and executive dysfunction in PMCs. Cognitive performance did not change over the 2 years, in line with reports by others. Our results are in line with several studies that have shown a discrepancy between the progression of brain atrophy on structural MRI in the preclinical phase and stable, largely intact cognitive and clinical functioning. This means, that in a situation of progressive neuronal cell loss, compensatory mechanisms must be at play to minimize functional effects of these changes. In addition, during increasing exposure to mutant huntingtin, neuronal dysfunction probably precedes neuronal cell death. In this situation it may not be surprising, that a combination of functional imaging (i.e., FDG–PET) and NPA appears as the most sensitive combined measure for predicting HD motor onset in our study.

We had the unique opportunity to evaluate the neurological status of 17 of the 22 PMCs 10 years after baseline, thus enabling us to classify these subjects in retrospect as either far from or close to HD motor onset at study entry. Eight PMCs were diagnosed with manifest HD after 10 years and none had normal putaminal glucose metabolism scores at follow-up, about 8 years earlier. Of the other 9 PMCs, who did not develop motor symptoms during the study, 4 PMCs had normal putaminal glucose metabolism scores at follow-up. Statistical analyses showed a trend for worse putaminal metabolism at 2-year follow-up of PMCs close to motor onset as compared to PMCs far from motor onset. Cognition scores were comparable for both subgroups. Although one must be cautious given the small number of participants, a normal putaminal glucose metabolism score may imply that a PMC will not develop motor symptoms within 8 years.

The strengths of this biomarker study lie in the longitudinal design in which a well characterized cohort of PMCs was assessed using several clinical measures. Particularly the third neurological evaluation at 10 years after baseline provided us with important information about the actual closeness to motor onset of HD for most of our subjects, although we cannot say at what moment during follow they actually converted to HD. Limitations of our study are the small sample size, drop out, and incomplete data for a few subjects. Furthermore, radiation exposure, ligand production, and cost may limit the use of FDG–PET scanning. However, in recent years, FDG–PET has become more widely available, mainly through markedly increased demands for oncological patients. It will be important
to validate our results in a much larger cohort to make sure that these still hold within a heterogeneous population of PMCs.

In conclusion, we found a combination of functional imaging and cognitive measures to be of predictive value for motor onset of HD within 5 years. More specifically, the combination of glucose metabolism of the putamen and a composite score for psychomotor speed is a powerful and better predictor of motor onset of HD than each measure separately. The additional information about the real neurological status of most PMCs (half of them converted to manifest HD) 10 years after baseline, supports this conclusion. The presented model explained a large part of the variance in the motor onset measure at baseline, and an even higher amount after 2 years follow-up. Therefore, in future clinical trials a combination of the SDMT, TMT, stroop colour word test and putaminal glucose metabolism can be used to identify late-converter-PMCs.

ACKNOWLEDGMENTS

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ETHICAL STANDARDS

This study was approved by the local ethics committee and the research was conducted in compliance with institutional guidelines. All subjects gave their written informed consent.

CONFLICTS OF INTEREST

The research was conducted in compliance with institutional guidelines. There are no potential conflicts of interest in this study. This manuscript, or parts of it, has not been previously published elsewhere nor has it been submitted simultaneously for publication elsewhere.
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