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Functional and structural neuroimaging in Huntington's disease

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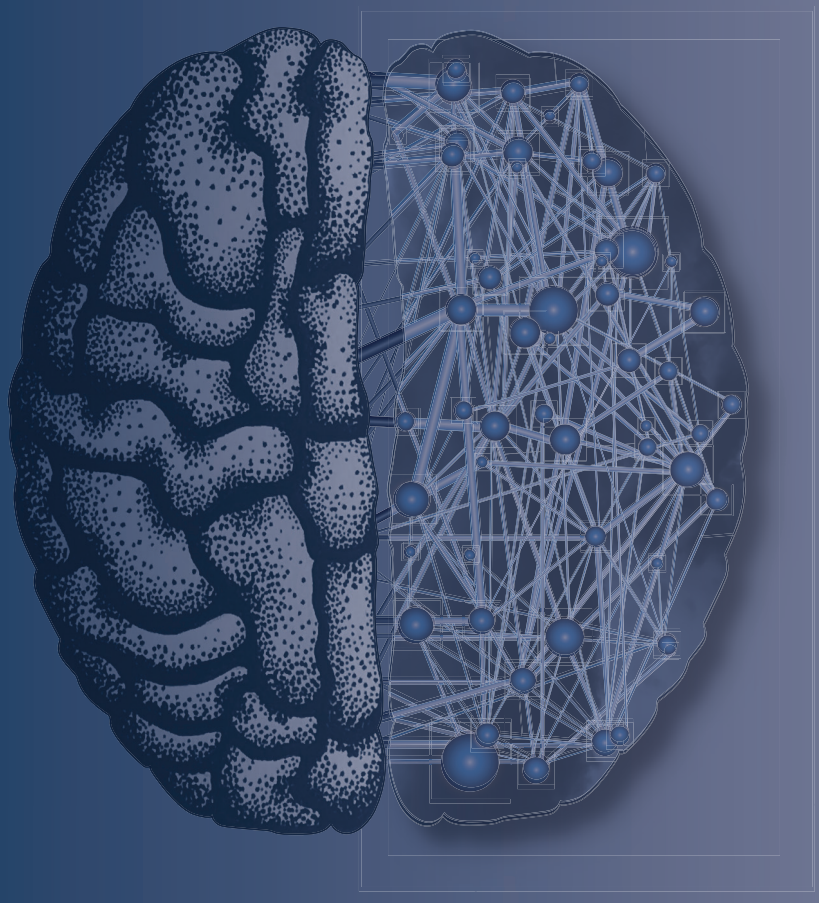

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Longitudinal resting state fMRI analysis in healthy controls and premanifest Huntington's disease gene carriers: a three-year follow-up study

CHAPTER 2

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Abstract

Background

We previously demonstrated that in the premanifest stage of Huntington's disease (preHD), a reduced functional connectivity exists compared to healthy controls. In the current study we look at possible changes in functional connectivity occurring longitudinally over a period of 3 years, with the aim of assessing the potential usefulness of this technique as a biomarker for disease progression in preHD.

Methods

Twenty-two preHD and 18 healthy control subjects completed resting state fMRI scans in two visits with 3 years in between. Differences in resting state connectivity were examined for eight networks of interest using FSL with 3 different analysis types: a dual regression method, region of interest approach and an independent component analysis. To evaluate a possible combined effect of grey matter volume change and the change in BOLD signal, the analysis was performed with and without voxel-wise correction for grey matter volume. To evaluate possible correlations between functional connectivity change and the predicted time to disease onset, the preHD group was classed as preHD-A if ≥10.9 years and preHD-B if <10.9 years from predicted disease onset. Possible correlations between burden of pathology score and functional connectivity change in preHD were also assessed. Finally, longitudinal change in whole brain and striatal volumetric measures was assessed in the studied cohort.

Results

Longitudinal analysis of the RS-fMRI data revealed no differences in the degree of connectivity change between the groups over a period of 3 years, though a significantly higher rate of striatal atrophy was found in the preHD group compared to controls in the same period.

Conclusions

Based on the results found in this study, the provisional conclusion is that RS- fMRI lacks sensitivity in detecting changes in functional connectivity in HD gene carriers prior to disease manifestation over a 3-year follow-up period.

Introduction

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder characterized by motor, cognitive and psychiatric symptoms with a mean age at onset between 30-50 years.[1] It is caused by an expanded CAG trinucleotide repeat in the huntingtin (*HTT*) gene on the short arm of chromosome 4.[2] Magnetic Resonance Imaging (MRI) studies in HD have revealed extensive brain atrophy, most notably in the striatum.[3-9]

A current challenge in HD research is establishing reliable biomarkers for measuring disease progression in HD, both before and after disease manifestation. This is crucial for assessing the efficacy of future proposed therapies. Several large longitudinal studies are currently being conducted for the purpose of establishing such biomarkers.[10-13] Using MRI, these studies have shown that atrophy of different structures in the brains of premanifest gene carriers (preHD), and of the caudate nucleus in particular, is correlated with the estimated years to disease onset (YTO) as calculated by the formula of Langbehn et al.[10-14] This is of particular interest, as these subjects have yet to present clinically with the hallmark motor symptoms of HD.

As the correlations found up to this point only partially predict the rate of clinical deterioration, combining imaging modalities might increase the predictive validity of a potential biomarker. With Resting State functional MRI (RS-fMRI) interregional correlations of blood oxygenation level dependent (BOLD) signal fluctuations between brain regions that are spatially distinct, are measured in the wakeful brain, without challenging it with a particular task. The patterns acquired with this technique are usually referred to as "functional connectivity". RS-fMRI has the theoretical potential of revealing changes occurring in the brain before changes on the structural imaging level are evident, which could be important in targeting the disease in its earliest stages. It may in addition help to unravel compensatory mechanisms responsible for apparently normal brain function despite ongoing neurodegeneration. The technique has already been shown to be a valuable marker for tracking disease progression in Alzheimer's disease, and in mild cognitive impairment.[15,16]

In a previous report, our group has reported functional connectivity differences between controls, preHD and manifest HD subjects, cross-sectionally. The results showed preHD subjects already exhibiting altered functional connectivity with different structures in the brain compared to the matched control group. Importantly, this was still valid after correction for atrophy.[17] The first report detailing reduced cortico-striatal functional connectivity findings in preHD when compared to controls was by Unschuld et al.[18] A recent report by Poudel et al. further confirms findings of functional connectivity reductions in both preHD and manifest HD subjects.[19]

In the current longitudinal study we aim to assess the potential usefulness of this technique as a biomarker for disease progression in the premanifest stage of the disease. We investigate possible changes in functional connectivity occurring longitudinally over a follow-up period of 3 years. With the aim of having a comprehensive interpretation of the acquired data, three separate data analysis methods were applied.

Methods

Subjects

Of the 28 premanifest HD carriers (preHD) and 28 healthy age-matched control subjects who completed RS-fMRI scans during their first visit at the Leiden University Medical Center (LUMC) study site of the TRACK-HD study,[7] 23 preHD and 20 control subjects completed the resting state scans at the second visit, with a 3 year interval between visits. Excluded from analysis were 1 preHD subject due to missing scan volumes and 2 control subjects due to excessive motion artifacts (maximum motion during scan < 4 mm).[20] This resulted in 22 preHD and 18 healthy control subjects that were included in this study (Table I).

Inclusion criteria for study participation for preHD subjects comprised of a positive genetic test with ≥ 40 CAG repeats, the absence of motor disturbances on the total motor score (TMS) of the Unified Huntington's Disease Rating Scale (UHDRS) of more than 5 points and a burden of pathology score greater than 250 ((CAG repeat length - 35.5) x age).[7,21] Age- and gender-matched gene-negative relatives of HD gene carriers and spouses were included as healthy controls. Exclusion criteria for all participants included significant previous head trauma, any neurological or major psychiatric disorder or unwillingness to undergo MRI scanning.[7] Medical history taking, an interview-based assessment and questionnaires were used to ascertain that no major psychiatric disorder could be classified at the time of inclusion and scanning. Consequently, the use of neuroleptic medications or antidepressants was sparse and considered to be of no influence.

For preHD subjects the estimated number of years until disease onset was calculated based on their current age and the CAG repeat length, by means of the formula developed by Langbehn et al.[14]

As previously applied by Tabrizi et al.,[7] for a second analysis, the preHD group was divided at baseline according to the median (10.9 years) for the predicted years to onset into preHD-A (≥10.9 years from predicted onset) and preHD-B (<10.9 years). This resulted in two groups each consisting of 11 subjects (Table II). In a further analysis performed within the preHD group, possible associations between functional connectivity change and burden of pathology score were assessed.

The study was approved by the ethics committee of the LUMC and written informed consent was obtained from all participants following a complete description of the study and procedures. For full details of study parameters, see Tabrizi et al.[7]

Table I. *Group characteristics and clinical scores*

	Healthy controls	preHD (A and B)
N	18	22‡
Gender M/F	7/11	10/12
Age in years (V1), mean (SD)	46.7 (6.9)	43.3 (8.5)
Handedness R/L	18/0	18/4
Level of education (ISCED), median (range)	4 (3)	4 (3)
DART-IQ, mean(SD)	105.3 (9.3)	100.3 (11.6)
BMI in kg/m^2 (V1) , mean (SD)	26.9 (6.6)	24.9 (4.1)
CAG repeat length, mean (SD)	n/a	42.6 (2.6)
Estimated years to onset (YTO), mean (SD)	n/a	11.6 (4.4)
Total functional capacity, mean (SD)		
V1	13.0 (0.0)	12.7 (0.8)
V2	13.0 (0.0)	12.6 (0.9)*
UHDRS-TMS, mean (SD)		
V1	2.4 (2.5)	2.4 (1.5)
V2	2.2 (3.0)	5.4 (5.7)*
SDMT, mean (SD)		
V1	53.7 (8.9)	48.7 (9.7)
V2	58.4 (8.0)	49.4 (10.5)*
BDI-II, mean (SD)		
V1	4.4 (6.3)	5.1 (5.7)
V2	4.8 (5.1)	5.3 (6.0)
Between-scan interval in months, mean (SD)	35.6 (1.20)	35.3 (0.94)

N = number of participants, SD = Standard deviation, n/a = not applicable, ISCED = International Standard Classification of Education, DART-IQ = Dutch Adult Reading Test Intelligence Quotient, CAG = Cytosine-Adenine-Guanine, UHDRS-TMS = Unified Huntington's Disease Rating Scale Total motor score, SDMT = Symbol Digit Modalities Test, BDI-II = Beck Depression Inventory-II, BMI = Body Mass Index, V1 = visit 1, V2 = visit 2.

** Indicates a significant difference at $p < 0.05$.*

‡ Including four subjects progressing to the manifest stage during the three year follow-up period.

Clinical measures

To monitor disease state, the following clinical measures were collected longitudinally for all groups: Unified Huntington's Disease Rating Scale Total Motor Score (UHDRS-TMS), Total Functional Capacity (TFC), Symbol Digit Modalities Test (SDMT) and Beck Depression Inventory-II (BDI-II) scores. The UHDRS-TMS is the traditional measure which defines disease state in HD. The SDMT in particular has been shown to be a sensitive longitudinal cognitive measure in HD, independent of disease related motor effects.[22]

MRI acquisition

MRI acquisition was performed on a 3-Tesla whole body scanner (Philips Achieva, Healthcare, Best, The Netherlands) with an eight channel receive array head coil. An anatomical T1-weighted scan was acquired using an ultrafast gradient echo 3D acquisition sequence with the following imaging parameters: repetition time (TR) = 7.7 ms, echo time (TE) = 3.5 ms, field-of-view = 24 x 24 x 16.4 cm^3, matrix size 224 x 224, with a duration of 9 minutes. For post-processing registration purposes, a high resolution T2*-weighted scan, with the following parameters was collected: repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, field-of-view = 220 x 220 x 168 mm^3, flip angle = 80°, matrix size = 112 x 109 mm^2, with a duration of 46 s. A RS-fMRI scan with the following parameters was obtained: 200 EPI volumes, repetition time (TR) = 2200 ms, echo time (TE) = 30 ms, field-of-view = 220 x 220 x 10.4, resolution = 2.75 x 2.75 x 2.75, no slice gap, flip angle = 80°, matrix size 80 x 79, with a duration of 7.5 minutes. No background music was played during the RS-fMRI scan and to ensure a wakeful disposition participants were asked to keep their eyes open with normal background light.

Pre-processing of resting state data

RS-fMRI images were analysed using FSL 5.0 (fMRIB Software Library; available at *www.fmrib.ox.ac.uk/fsl)*. Pre-processing consisted of motion correction,[23] removal of non-brain tissue,[24] spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM) and high-pass temporal filtering equivalent to 100 s (0.01 Hz). After pre-processing, the functional images were registered to the high-resolution T2*-weighted images. These high-resolution images were subsequently registered to the anatomical T1-weighted images. Finally, the anatomical scan was registered to the 2 mm isotropic MNI152 standard space image.[23] These three registration matrices were combined to obtain a matrix for transforming fMRI data from native space to standard space and its inverse (from MNI space to native space). Visual quality control was performed by two qualified raters to ensure correct registration.

Table II. *preHD-A vs. preHD-B, visit 1*

	preHD-A	preHD-B
N	11	11
Gender M/F	3/8	7/4
Age in years, mean (SD)	43.8 (5.8)	43.0 (10.9)
Handedness R/L	9/2	9/2
Level of education (ISCED), median (range)	4 (3)	4 (3)
DART-IQ, mean (SD)	102.3 (9.9)	98.3 (13.2)
BMI in kg/m^2, mean (SD)	25.6 (3.0)	23.1 (2.3)
CAG repeat length, mean (SD)	41.5 (1.4)	43.8 (3.1)*
Estimated years to onset (YTO), mean (SD)	14.4 (4.5)	8.8 (1.6)*
Total functional capacity, mean (SD)	12.7 (0.7)	12.6 (0.9)
UHDRS-TMS, mean (SD)	1.9 (1.5)	2.9 (1.3)
SDMT, mean (SD)	51.6 (9.9)	45.9 (9.1)
BDI-II, mean (SD)	4.5 (6.0)	5.6 (5.7)
Between-scan interval in months, mean (SD)	35.6 (1.0)	34.9 (0.7)

N = number of participants, SD = Standard deviation, ISCED = International Standard Classification of Education, DART-IQ = Dutch Adult Reading Test Intelligence Quotient, CAG = Cytosine-Adenine-Guanine, UHDRS-TMS = Unified Huntington's Disease Rating Scale Total motor score, SDMT = Symbol Digit Modalities Test, BDI-II = Beck Depression Inventory-II, BMI = Body Mass Index.

** Indicates a significant difference at $p < 0.05$.*

Statistical analysis

Statistical analysis of group demographics and clinical measures was performed using IBM SPSS Statistics (version 20.0, IBM Corp., USA). Where appropriate either an independent samples t-test or chi-squared tests were applied. Potential longitudinal change in clinical measures between the groups was also investigated. Difference values were computed and independent samples t-tests on these delta-scores evaluated whether preHD subjects experienced a greater change from visit 1 to visit 2 than control subjects.

Striatal and whole brain volumes were obtained from the TRACK-HD study database.[7,13] These measures were calculated using the Iowa BRAINS method as previously described.[7,13,25,26] Assessment of possible longitudinal volumetric change was performed using a general linear model with age, gender and total brain volume (the latter only for assessing striatal volumes) as covariates in the model.

The functional connectivity analysis was performed in three ways using the dual regression method of FSL, a technique that allows a voxel-wise comparison of resting state functional connectivity.[27] To assess possible associations between the burden of pathology score and functional connectivity change, a regression analysis was preformed within the preHD group only.

Network of interest analysis

First, resting state functional connectivity was determined in terms of similarity of the BOLD fluctuations in the brain in relation to characteristic fluctuations in predefined resting state networks or networks of interest (NOIs). Our choice of resting state networks was based on high reproducibility of these networks from independent component analysis of different data sets.[28,29] These standardized resting state networks parcellate the brain into eight templates that represent over 80% of the total brain volume:[30] 1) medial visual network, 2) lateral visual network, 3) auditory network, 4) sensorimotor system, 5) default mode network, 6) executive control network, 7 and 8) dorsal visual stream networks (Figure 1).[28] To account for noise, a white matter (WM) and a cerebrospinal fluid (CSF) template were included in the analysis.[31-33]

Dual regression analysis (part of FSL 5.0) was performed to identify subject-specific time course and spatial maps. To create the average time course within each network for every subject, the eight resting state networks[28] and the two additional WM and CSF maps[31-33] were used in a linear model fit against each individual subject's fMRI dataset (spatial regression). Hence, WM and CSF activities were included in the regression model as proxy measures for non-neuronal noise. The personalized time courses were subsequently regressed back onto that subject's fMRI dataset to create personal spatial maps (temporal regression). This gives ten 3D images per individual per visit, with voxel-wise the z-scores of functional connectivity to each of the templates. The higher the absolute value of the z-score, the stronger the connectivity to a network.

Independent component analysis

In a second approach, large-scale patterns of functional connectivity were identified by independent component analysis (ICA) using probabilistic ICA as implemented in the MELODIC tool of FSL.[28,34] The original concatenated 4D RS-fMRI dataset was decomposed into sets of time courses and associated spatial maps, to identify different activation components without any model being specified.[34,35] The number of components was fixed to 25 to limit independent component splitting into subcomponents.[15,27]

Subsequently the dual regression analysis as described above was repeated for the group ICA results. This time the 25 independent components were used as spatial regressors, ultimately resulting in 25 z-score maps per individual per visit, reflecting the connectivity strength of each voxel in the brain to each of the 25 independent components.

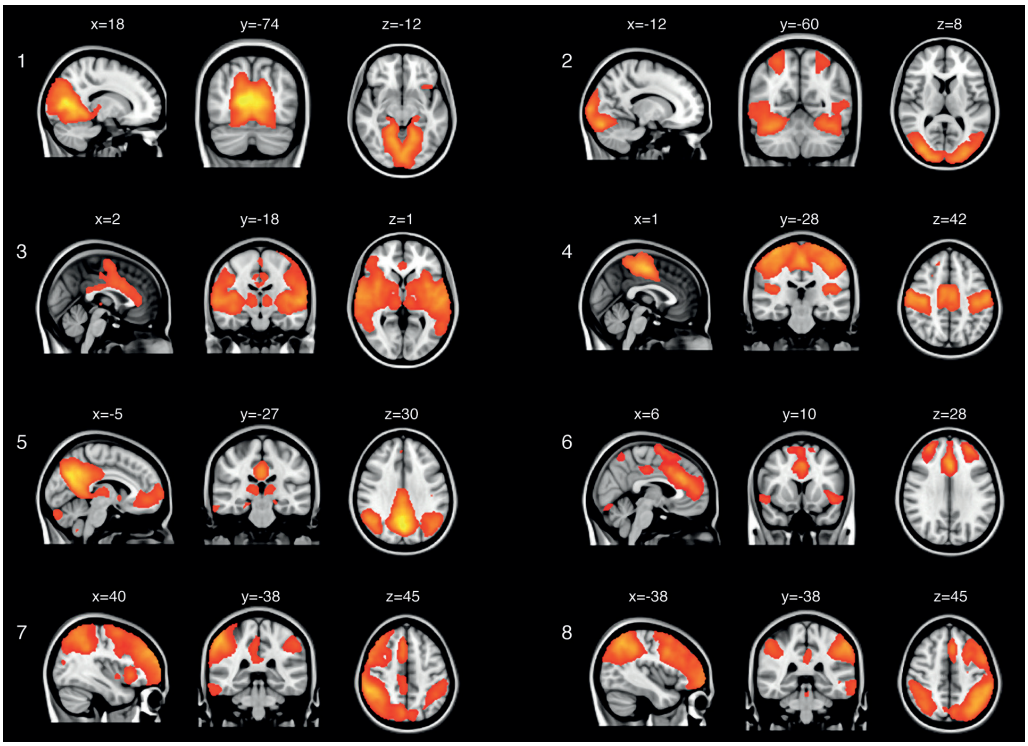

Figure 1. *Saggital, coronal and axial views of the dominant BOLD fluctuations within the eight predefined networks of interest [Beckmann et al., 2005]. All images have been coregistered into the MNI152 standard space template. Numbers at the top of the images denote the MNI coordinates (xyz) and images are shown in radiological orientation.*

Region of interest analysis

Given the overwhelming volume of evidence indicating the striatum as the prime and earliest region affected within the brain in HD, we chose the striatum as a region of interest (ROI) in our analysis. A mask was created to analyse the change in connectivity with the eight NOIs and the 25 independent components of the voxels within this ROI. The mask was based on the probabilistic atlas incorporated in FSL provided by the Harvard Center for Morphometric Analysis and contained the striatum from both hemispheres (Figure 2).[36-39]

Longitudinal change in connectivity per subject and per predefined network/independent component was the main parameter of interest. To assess this change, the individual functional connectivity maps (z-score) from the second visit were subtracted from the corresponding functional connectivity maps from the first visit.

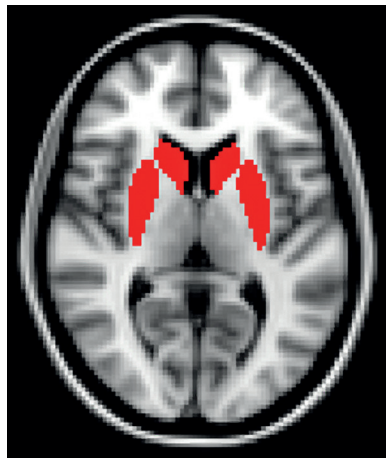

Figure 2. *Axial view of the region of interest (ROI) mask of the striatum shown superimposed on a MNI152 standard image.*

For the between-group analysis, the z-score maps created by dual regression and the maps containing the differences in z-score were collected across subjects into single 4D maps (one per NOI or original independent component, with the fourth dimension being subject identification) and submitted to voxel-based statistical testing. To obtain group averages of maps containing the differences in z-score, a one-sample non-parametric t-test was used and a two-sample t-test was applied to obtain group differences for each of the 8 NOIs and each independent component, using a general linear modelling (GLM) approach as implemented in FSL. Age and gender were included as covariates in the model. To statistically account for potential effects of local structural differences within and between the two groups, grey matter volume of each voxel was included as subject wise and voxel-wise covariates in the GLM design.[40] To evaluate a possible combined effect of grey matter volume change and the change in BOLD signal, the analysis was also performed without voxel-wise correction for grey matter volume.

Voxel-wise non-parametric permutation testing was performed using FSL-randomise (5000 permutations).[41] All statistical maps were family-wise error (FWE) corrected using $p < 0.05$, based on the TFCE statistic image.[42]

Because multiple comparison correction method only corrects the results at the predefined network/independent component level, but does not adjust for the risk of Type 1 error (false positives) induced by increasing the number of components tested simultaneously at high model orders, additional correction for multiple comparisons was done using Bonferroni correction. The multiple comparisons consisted of two comparisons (either connectivity increase or decrease as compared to healthy controls) for 8 NOIs and 25 independent components.

Results

Group characteristics are shown in Table I. Age, gender, handedness and level of education did not differ significantly between controls and preHD subjects. At baseline, no differences were found in UHDRS-TMS, TFC, SDMT, BDI-II, and Dutch Adult Reading Test Intelligence Quotient (DART-IQ) scores. There also was no difference in Body Mass Index (BMI) at baseline. Repeated assessment at 3-year follow-up revealed significantly higher UHDRS-TMS and lower TFC and SDMT scores in the preHD group (Table I). Four of the twenty-two preHD subjects began to exhibit typical HD motor symptoms during the 3-year follow-up period, therefore reaching the definition of early manifest disease stage. The cross-sectional difference in UHDRS-TMS and TFC score between the groups at the second visit was negated after exclusion of these four converter subjects, yet the difference in SDMT score remained significant ($p = 0.07$, $p = 0.36$ and $p = 0.01$, respectively). The difference in SDMT comprised of higher mean scores within the control group when compared to their first visit, while the scores of the preHD group remained stagnant.

The longitudinal change in the UHDRS-TMS was significant when all participants were included ($p = 0.03$), yet this result was only reached as a result of outlier scores: when the four converters were excluded from analysis, this difference vanished ($p = 0.25$).

The longitudinal change in the SDMT score was significant when all participants were included ($p = 0.04$). While the mean SDMT difference in the preHD group remained essentially the same when the four converters were excluded (+0.64 vs. +0.67 difference points, respectively), statistical significance could no longer be reached ($p = 0.06$). See Table III for a view of the mean longitudinal change of the different measures.

No differences in any of the scores outlined above were found while comparing the preHD-A and preHD-B groups, neither at the first or second visit nor longitudinally. The CAG trinucleotide repeat count was significantly higher in the preHD-B relative to the preHD-A group ($p = 0.03$) (Table II; longitudinal change data not shown).

All scans were analysed with and without inclusion of the four converters. All scan analyses were also repeated with exclusion of the four left-handed subjects to avoid any possible lateralization effects. The reported results are with and without voxel-wise correction for grey matter volume, as described in the Methods section. No difference was found in the amount of motion between the groups.

RS-fMRI network analyses

In the eight designated NOIs, longitudinal analysis of the RS-fMRI data revealed no statistically significant differences in the degree of connectivity change between controls and the preHD group. There also were no statistically significant differences between controls and preHD-A and controls and preHD-B subjects. No association could be demonstrated between the degree of connectivity change in the different networks and the groups designated as *far* and *near* from expected onset of motor symptoms, nor with the burden of pathology score.

Table III. *Longitudinal change in clinical scores †, mean difference*

	Healthy controls	preHD (A and B)
N	18	22‡
Total functional capacity, MD (SD)	0.0 (0.0)	-0.1 (0.6)
UHDRS-TMS, MD (SD)	-0.2 (2.9)	3.0 (5.4)*
SDMT, MD (SD)	4.7 (5.7)	0.6 (6.1)*
BDI-II, MD (SD)	0.4 (3.6)	0.2 (5.1)
BMI in kg/m^2, MD (SD)	0.5 (2.3)	-0.4 (1.6)

N = number of participants, MD = mean difference, SD = Standard deviation, UHDRS-TMS = Unified Huntington's Disease Rating Scale Total motor score, SDMT = Symbol Digit Modalities Test, BDI-II = Beck Depression Inventory-II, BMI = Body Mass Index.

** Indicates a significant difference at $p < 0.05$.*

† Longitudinal change denotes scores from visit 1 subtracted from scores from visit 2.

‡ Including four subjects progressing to the manifest stage during the three year follow-up period.

RS-fMRI ICA

Using the ICA method, 25 components were extracted from the data per person per visit and the differences between the two visits compared across the above outlined groups. There were no statistically significant differences in the degree of connectivity change between any of the groups. Dividing the preHD group according to the expected time of motor symptom onset again revealed no significant differences in the degree of connectivity change. Regression analysis using the burden of pathology score revealed no associations with the degree of functional connectivity change within the preHD group.

RS-fMRI ROI analysis

Using the described mask to assess the change of connectivity strength in the voxels within the striatum, no statistically significant differences could be demonstrated between any of the groups described above.

When comparing results from the outlined analysis methods, the ROI analysis provided the closest proximity to achieving a significant longitudinal reduction in functional connectivity in preHD when compared to controls. This was the case with the lateral visual network (NOI 2; $p = 0.08$) and default mode network (NOI 5; $p = 0.11$) (Figure 3). Power analysis using these results show that a minimum of 23 subjects per group would be needed to detect a significant longitudinal reduction in functional connectivity in 3 years within the striatum with the lateral visual network for preHD compared to controls (at 5% FWE rate with a power of 80%).

Table IV provides an overview of significance levels for longitudinal reduction of functional connectivity within the striatum over 3 years in preHD subjects compared to controls with the 8 NOIs.

Longitudinal volumetric analysis

In the 3-year follow-up period, no statistically significant difference in whole brain volume decline was found between controls (0.33%) and preHD (0.58%) ($p = 0.35$).

The striatal volume showed a significantly higher rate of decline over the 3-year period in preHD as compared to controls: 1.45% in the control group versus 7.29% in the preHD groep ($p < 0.001$). Striatal volume decline over the 3 years was significantly higher in both preHD-A (6.62%) and preHD-B (8.15%) when compared to controls ($p < 0.001$). The difference in striatal volume decline rate between preHD-A and preHD-B was not statistically significant over this time period ($p = 0.31$).

Discussion

This study showed no longitudinal difference in functional connectivity change between preHD and healthy control subjects over a period of 3 years. This was also the case when preHD subjects were divided in a preHD-A and preHD-B group based on the expected time to disease onset and when using burden of pathology score as a regressor for functional connectivity change. These conclusions are based on results obtained from three different analysis methods. Results remained the same with and without voxel-wise correction for grey matter volume and while running the analysis with the inclusion and/or exclusion of converters and left-handed subjects.

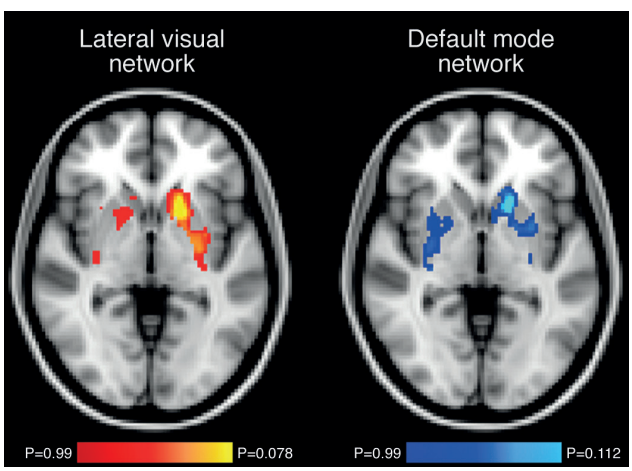

Figure 3. P-value maps of the nonsignificant longitudinal reductions in functional connectivity in preHD compared to controls in the striatum with the lateral visual and default mode networks in the 3-year study period.

Table IV. *Statistical parameters for longitudinal reduction of functional connectivity within the striatum over 3 years in preHD subjects compared to controls with the 8 networks of interest*

	Network of interest (NOI)	Minimal *P*-value	*x*	*y*	*z*	*t*-stat
1	Medial visual	0.356	39	68	42	3.527
2	Lateral visual	0.078	53	69	35	4.175
3	Auditory	0.686	36	56	49	3.280
4	Sensorimotor	0.804	54	62	45	2.491
5	Default mode	0.112	53	70	36	3.845
6	Executive control	0.734	31	69	32	2.327
7	Dorsal visual stream	0.502	59	66	39	3.355
8	Dorsal visual stream	0.262	36	68	29	3.754

x, y, and z denote MNI152 standard space coordinates.

This result, taken together with clinical parameters like the UHDRS-TMS and SDMT showing longitudinal change between the included subjects, and significantly higher longitudinal striatal atrophy rate in preHD compared to controls, alludes to a lack of sensitivity of RS-fMRI in detecting concomitant changes in functional connectivity occurring longitudinally in preHD. This statement should be considered as tentative, as future studies with greater numbers of participants, improved signal-to-noise ratio, different analysis methods and/or a longer follow-up period might be able to demonstrate longitudinal differences in functional connectivity change. That being said, results from this study suggest that even if there is functional connectivity change occurring in the 3-year follow-up period, this is too small to detect with this technique using the highlighted methods with this cohort size, which is a relevant finding in light of longitudinal biomarker research in preHD.

Our study confirms the results found by Seibert et al.[43] Their study reported no change in functional connectivity over a 1 year period. The differences between the study of Seibert et al. and our own were the methodology used, where seeds instead of a priori spatial NOIs were used and subject-native space registration instead of the MNI152 standard space template was applied. The number of subjects examined in that report was higher than in our study: 22 controls and 34 preHD subjects.

Our earlier cross-sectional results suggested that functional connectivity, at the group level, was a fairly sensitive measure to differentiate preHD subjects from controls.[17] As such, we were quite hopeful to demonstrate a divergent longitudinal functional connectivity evolution between the groups, which in turn could serve as a measure for disease progression. We were however

unable to reproduce these results within our baseline cohort, most likely due to the smaller number of subjects that were included, as only those with scans at both time points could be assessed longitudinally. This study can therefore not account for the functional connectivity of the dropouts, as no data are available. Furthermore, the discrepancy in baseline findings might involve deteriorating health prompting more severely affected subjects to drop out prematurely of the study, thus leaving a relatively fitter group for this study. A such, selection bias disproportionately affecting subjects with the fastest rate of clinical deterioration is a possible reason for not finding different functional connectivities between the groups. This spurred using a more comprehensive approach and to base the hypothesis-driven part of the analysis solely by singling out the striatum as the primary region where possible changes in resting state activity are expected, given the fact that it is the region first affected in HD, as was again demonstrated by the volumetric study of the striatum within this cohort. Despite using three different analysis methods, no longitudinal change could be demonstrated in our cohort in a time frame of 3 years with two measurement points. The combination of a highly significant difference in striatal atrophy rate between preHD and controls with a total lack of significant difference in the rate of functional connectivity change between these groups strongly points to a lower sensitivity of RS-fMRI in demonstrating longitudinal change in the preHD population.

A similar sequence of results was found by the study of Wolf et al., where task-based fMRI showed significantly lower activity cross-sectionally in the left prefrontal cortex in preHD, yet failed to demonstrate a significant decline of that activity over a 2-year follow-up period.[44] In that study, the baseline and longitudinally examined cohort consisted of the same subjects. Despite the obvious differences in methodology and spatial parameters used in measuring the BOLD signals, the longitudinal study by Wolf et al. may further consolidate the notion of a lack of sensitivity in detecting BOLD signal changes occurring during a time frame that can be considered feasible for assessing the efficacy of an intervention in preHD.

The strength of our study lies in the application of three different analysis methods which allows for a more comprehensive interpretation of the data. This strength is complemented by the acquisition methodology used: the duration of the RS-scans (>6 min) and acquisition while the patients have their eyes open provide the most robust estimates of functional connectivity as demonstrated by different studies.[45,46]

A limitation of this study is the loss of power due to the expansive testing of various networks and independent components. This expansive testing is however justified given the goal of finding robust and specific functional connectivity changes in preHD for usage as biomarker candidate in a clinical trial setting. Other possible limitations include transforming the data to an atlas volume instead of subject-native space, the relatively small number of tested subjects and possible confounding effects of dropouts, the conceivably short follow-up period in the preHD stage setting and not accounting for possibly confounding covariables such as depression scores in the analysis model.

Based on the results found in this study, the provisional conclusion is that RS-fMRI seems to lack sensitivity in detecting changes in functional connectivity in HD gene carriers prior to disease manifestation over a 3-year follow-up period. This conclusion applies to this selective group of participants and the particular analysis methods used in this study. Results from future longitudinal studies, such as the ongoing Track-On HD study which has larger groups and more time points measured, should be awaited before articulating a definite recommendation on the possible utility of RS-fMRI as a biomarker tracking disease progression in preHD.

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