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Striatal connectivity in premanifest Huntington disease is differentially affected by disease burden

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Background: Different amount of cumulative exposure to the toxic mutant form of the huntingtin protein might underlie distinctive pattern of striatal connectivity in premanifest Huntington's disease (pre-HD). Aim of this study is to investigate disease burden-dependent cortical- and subcortical-striatal loops in different pre-HD stages.

Methods: Sixteen pre-HD participants and 25 controls underwent magnetic resonance exam to investigate striatal structural and functional connectivity. Pre-HD individuals were stratified into far and close to disease onset groups according to the disease-burden score. Cortical-striatal and subcortical-striatal functional connectivity was investigated through seed-ROI and ROI-to-ROI approaches, respectively. Integrity of white matter pathways originating from striatal seeds was investigated through probabilistic tractography.

Results: In far-to-onset pre-HD, the left caudate nucleus showed cortical increased functional connectivity in brain regions overlapping with the default mode network, and increased coupling connectivity with the bilateral thalamus. By contrast, close-to-onset individuals showed increased fractional anisotropy (and mean diffusivity) in the right caudate nucleus and widespread striatal atrophy. Finally, we reported an association between cortical-caudate functional connectivity and caudate structural connectivity, although not surviving multiple comparison correction.

Conclusions: Functional reorganization of the caudate nucleus might underlie plasticity compensatory mechanisms which recede as premanifest individuals approach clinical symptoms onset and neurodegeneration.

Introduction

Huntington's disease (HD) is an autosomal genetic disorder caused by a pathological expansion of cytosine-adenine-guanine (CAG) repeat in the huntingtin gene, leading to a mutant form of the huntingtin protein (mHTT). This mutation is responsible of the progressive functional disability [1]. Mutation gene carriers before the clinical diagnosis of the disease (i.e. premanifest stage, pre-HD) exhibit subtle cognitive deficits and brain alterations [2]. Striatal atrophy can be observed up to 10 years before diagnosis in pre-HD [3]. However, pre-HD individuals are able to maintain a normal level of cognitive functioning to perform usual activities of daily living [2], suggesting that compensatory mechanisms occur over time [4]. Recently, studies investigating brain functional connectivity through resting-state functional magnetic resonance imaging (rs-fMRI) suggested a

compensation model in pre-HD, through an initial increase in cortical connectivity and subsequent decline. According to this model, the reduction of gray matter will lead to elevated cortical connectivity, which in turn will give rise to stable behavioral performances [5,6]. These mechanisms also have been reported in several neurodegenerative diseases, such as Alzheimer's disease (AD) and frontotemporal dementia (FTD). Specifically, compensatory responses have been linked to increased brain connectivity in preclinical/mild stages, when brain functional organization might be relatively resilient to the incoming structural damage [7-9]. By contrast, progressive degeneration in the AD/FTD continuum is associated with an early breakdown of functional connections, related to disease severity [10,11].

Similarly, stage-dependent increased functional connectivity was observed in the HD's continuum, suggesting early compensatory mechanisms to counterbalance the incoming neurodegeneration [12]. Indeed, pre-HD stage refers to a heterogeneous stage reflecting non-linear longitudinal trajectories changes in brain connectivity in the presence of linear neuronal degeneration [6]. Recently, several studies stratified pre-HD into close-to-onset or far-to-onset - according to the disease burden score (DBS), an index conveying the toxicity exposure to the mHTT protein [13] - reporting disease burden-dependent reorganization of brain connectivity pathways [14]. However, although striatum represents a core brain region for this pathology, few studies investigated striatal connectivity across pre-HD stages, reporting contrasting results. Gorges et al. [15] investigated caudate connectivity in far-to-onset pre-HD compared to controls reporting null results. By contrast, Kronenbuerger and colleagues [16] reported functional connectivity (FC) alterations between the striatum and several cortical areas in both close- and far-to-onset pre-HD. Therefore, it is still unclear whether striatum might represent a key gate for compensatory mechanisms and at what stage within the pre-HD's continuum. Moreover, to date, no studies investigated whether subcortical-striatal circuitries are differentially affected within different pre-HD stages.

In this study, we investigated cortical-striatal and subcortical-striatal loops in close- and far-to-onset pre-HD individuals. We assume a dynamic adaptation of striatal FC among different pre-HD stages, showing increased striatal connectivity in the earliest stage, while reversing the directionality in close-to-onset pre-HD, as neurodegeneration becomes evident. Moreover, we expect to report a significant association between structural and functional connectivity, confirming compensatory

mechanisms overcoming neurodegeneration.

Methods

Participants

We recruited 41 participants between May 2015 and July 2017. Sixteen were HD mutation gene carriers evaluated with the Unified Huntington's Disease Rating Scale (UHDRS) [17]. They were at the premanifest stage, reporting a total functional capacity score of 13 and a total motor score below 5 [18]. Moreover, the composite UHDRS (cUHDRS) score, which combines total functional capacity, total motor score, symbol digit modality test and the Stroop word reading test, was computed [19]. Twenty-five participants were healthy controls (HC). Participants had no neurological or psychiatric disorders other than HD in the mutation carriers.

Pre-HD participants were stratified into far and close to disease onset groups based on the group median split of DBS, computed as $CAG \times age \text{ product} = [(age \text{ at MRI}) \times (CAG \text{ repeats} - 35.5)]$, which is a validated index of disease burden in pre-HD [13].

This study was performed in accordance with the Declaration of Helsinki. Participants were recruited from an observational clinical biomarker study (NCT01412125) in outpatients approved by the ethics committee of Henri Mondor Hospital (Créteil, France). All participants gave written informed consent and were tested at Henri Mondor Hospital (Créteil, France) and at the CENIR (Paris, France).

MRI data acquisition and analysis

Participants underwent MRI scanning on a Siemens Prisma 3-T scanner (CENIR, Paris, France), including eye-closed rs-fMRI, diffusion, and structural scans. The MRI protocol is described in the Supplementary Information.

Structural data were processed using FreeSurfer. Subcortical volumetric analysis was limited to the bilateral caudate nucleus, putamen and nucleus accumbens. These structures were normalized to the subject's intracranial volume (ICV) using the following formula: $\text{volume norm} = \text{volume raw} \times \text{group mean ICV} / \text{subject ICV}$ in mm^3 . Additionally, we investigated both cortical thickness and whole brain volumetric alterations (see Supplementary Information).

A probabilistic tractography approach to reconstruct striatal white matter pathways was performed using the FMRIB Diffusion Toolbox (FDT version 3.0) included in FSL. The analysis was focused on fiber bundles starting from each striatal region-of-interest (ROI) and terminating within the cortex, without using *a priori* masks. Fractional anisotropy (FA) values within striatal structural pathways were computed. FC analysis was focused on the caudate nucleus, putamen and nucleus accumbens, bilaterally, and based on the corresponding FreeSurfer segmentation.

Two different approaches using the Conn toolbox [20] were implemented to investigate both cortical-striatal and subcortical-striatal FC. For detailed description of preprocessing and analysis steps of structural and functional connectivity, see Supplementary Information.

Statistical analysis

Group differences in sociodemographic features and clinical/cognitive outcomes were assessed with Kruskal-Wallis or χ^2 tests, as appropriate ($p < 0.05$). Within each hemisphere, Kruskal-Wallis p-values for ROI subcortical atrophy and FA were corrected for multiple comparisons with Bonferroni correction ($n = 3$ striatal ROIs; $p < 0.017$). All the analyses were performed using the SPSS package release 23.0 (SPSS Inc. Chicago, IL). Moreover, FA across groups was compared through a voxel-wise ANOVA (“far-to-onset pre-HD vs. HC”; “close-to-onset pre-HD vs. HC”; “far-to-onset pre-HD vs. close-to-onset pre-HD”) based on permutation testing at a threshold-free-cluster enhancement level of $p < 0.05$ corrected for family wise error [FWE].

To assess surface cortical-striatal FC differences across groups, the following contrasts were performed through a vertex-wise ANOVA test: “far-to-onset pre-HD vs. HC”; “close-to-onset pre-HD vs. HC”; “far-to-onset pre-HD vs. close-to-onset pre-HD”. Significance threshold for each contrast was set to $p < 0.05$ with a vertex-level false discovery rate [FDR]-correction with a more conservative cluster-level threshold of $p < 0.01$ FWE-corrected. An affine approach was then used for mapping surface coordinates in Montreal Neurological Institute space. Subcortical ROI-to-ROI FC results were reported at $p < 0.05$ FDR corrected at the analysis level and FWE at the network level using Network Based Statistics [21]. To control for motion, the mean framewise displacement was included as covariate in all the analysis.

Moreover, for each individual and striatal ROI, a mean global cortical-striatal FC map was

computed as follow: we extracted corresponding network spatial maps on the pooled sample of HC and pre-HD individuals. Positive and negative correlational maps were separately assessed through a one-tailed, one-sample t-test ($p < 0.05$ FDR-corrected; cluster-level $p < 0.01$ FWE-corrected). Positive correlation maps were then used as masks to compute mean FC.

Finally, a general linear model was used to investigate the interaction effect of DBS severity (far- and close-to-onset pre-HD) on the mean FC and cUHDRS for each bilateral striatal ROI. The same model was applied to investigate the interaction effect on the three groups (HC, far-to-onset pre-HD and close-to-onset pre-HD) on functional and structural connectivity (i.e. mean FC and mean FA) within each bilateral ROI. P-values were corrected for multiple comparisons within each hemisphere ($n = 3$ striatal ROIs; $p < 0.017$) to control for type I error with Bonferroni correction.

Results

Based on median DBS across pre-HD, 8 participants were included in the far-to-onset pre-HD group (below the DBS median) and 8 individuals were stratified in the close-to-onset pre-HD group (above the DBS median). The pre-HD groups and controls were matched for age (HC: 44 ± 9 ; far-to-onset pre-HD: 41 ± 14 ; close-to-onset pre-HD: 43 ± 6 ; $p = 0.91$), education years (HC: 15 ± 2 ; far-to-onset pre-HD: 13 ± 2 ; close-to-onset pre-HD: 16 ± 2 ; $p = 0.09$) and sex ($p = 0.66$) (See Table 1). Neither total functional capacity score nor total motor score differed across pre-HD groups ($p > 0.1$) (Table 1). Likewise, symbol digit modality test, Stroop test, verbal fluency, and cUHDRS were similar across groups ($p > 0.5$) (Table 1).

No participant was excluded from the analysis due to segmentation failures. Supplementary Figure S1 illustrates subcortical ROIs across imaging modalities included in the present analysis.

Group comparison revealed differences across groups in bilateral volume of the caudate nucleus (left: $p = 0.003$; right: $p = 0.002$), putamen (left: $p < 0.001$; right: $p < 0.001$), and nucleus accumbens (left: $p = 0.002$; right: $p = 0.003$) (Table 1; Figure 1A). Post-hoc analyses revealed reduced subcortical volumes in close-to-onset pre-HD compared only with HC. All these differences survived multiple comparison correction. Between-group differences did not reach statistical significance in far-to-onset pre-HD compared with both close-to-onset pre-HD group and HC ($p > 0.05$) (Table 1; Figure 1A). Results were confirmed by the whole brain volumetric group analyses

(Figure 1B). Compared to controls, close-to-onset pre-HD group showed bilateral atrophy limited to the striatum. By contrast, far-to-onset pre-HD group did not show regions with reduced grey matter compared with both controls and close-to-onset pre-HD group. Neither differences between pre-HD groups, nor increased grey matter in pre-HD groups compared to controls were found. Similarly, only close-to-onset pre-HD individuals showed reduced cortical thickness compared to controls, mapping in the superior/middle occipital gyrus (MNI $x = 24$; $y = -86$; $z = 22$) (Figure 1C). We did not observe differences in cortical thickness between far-to-onset pre-HD and close-to-onset pre-HD, nor thickness increased among pre-HD groups compared to controls.

Probabilistic tractography showed consistent striatal white matter pathways across individuals (Supplementary Figure S2). Group comparisons did not identify differences in mean FA pathways originating from striatal ROIs (left caudate: $p = 0.28$; right caudate: $p = 0.84$; left putamen: $p = 0.08$; right putamen: $p = 0.65$; left nucleus accumbens: $p = 0.44$; right nucleus accumbens: $p = 0.24$) (Figure 2A). By contrast, voxel-wise analysis showed increased FA in close-to-onset pre-HD individuals compared with both HC and far-to-onset pre-HD in a small portion of the right caudate streamline (Supplementary Table S2, Figure 2B). A post-hoc analysis assessed that increased FA in close-to-onset pre-HD was accompanied by increased mean diffusivity (MD) within the right caudate streamline (Supplementary Table S2, Supplementary Figure S4).

FC surface maps were spatially different across striatal structures (Supplementary Table S1 and Supplementary Figure S3). Group comparison showed increased FC in the far-to-onset pre-HD group compared to HC in the left caudate-cortical functional pathway (Figure 3; Table 2). Specifically, increased connectivity was found within the posterior cingulate/precuneus and the right inferior parietal lobe. Similarly, when we compared left caudate nucleus connectivity in far-to-onset pre-HD vs. close-to-onset pre-HD individuals, in the former group we found increased connectivity in the posterior cingulate/precuneus and the inferior parietal lobe, bilaterally (Figure 3; Table 2). No significant differences were found between close-to-onset pre-HD and HC. No differences were reported between pre-HD and controls considering FC of right caudate nucleus, bilateral putamen and bilateral nucleus accumbens.

Subcortical ROI-to-ROI analysis showed increased coupling connectivity in far-to-onset pre-HD individuals compared to HC between left caudate and bilateral thalamus (Figure 4). Between-

group comparison revealed no significant differences between far-to-onset and controls and across pre-HD groups.

There was no significant interaction between cUHDRS scores and pre-HD groups on mean FC of any striatal ROI (caudate left: $W = 0.658$, $p = 0.42$; caudate right: $W = 0.707$, $p = 0.40$; putamen left: $W = 0.552$, $p = 0.46$; putamen right: $W = 0.683$, $p = 0.41$; accumbens left: $W = 1.16$, $p = 0.28$; accumbens right: $W = .009$, $p = 0.93$).

Finally, a significant interaction effect was reported in the left caudate nucleus between groups and FA on FC ($W = 7.99$, $p = 0.018$), although not surviving multiple comparison correction (Supplementary Figure S5). No significant interactions were reported for the right caudate nucleus ($W = 2.432$, $p = 0.18$), putamen (left: $W = 0.022$, $p = 0.99$; right: $W = 4.021$, $p = 0.13$) and nucleus accumbens (left: $W = 0.103$, $p = 0.95$; right: $W = 1.330$, $p = 0.51$).

Discussion

These findings suggest different patterns of brain reorganization during pre-HD stages, where enhanced caudate nucleus FC is one of the earliest signs of neural reorganization in pre-HD's continuum, a pattern which disappears as the disease progresses and as striatal degeneration becomes evident. Functional reorganization of caudate nucleus in far-to-onset pre-HD mapped to brain regions showing consistent pattern of positive connectivity among HC (Supplementary Figure S4). Specifically, brain regions mapping to the default mode network, precuneus and inferior parietal lobe, showed increased connectivity in far-to-onset pre-HD. Moreover, in far-to-onset pre-HD the left caudate nucleus showed increased FC with the bilateral thalamus. This loop receives cortical inputs which are integrated within the striatum and thalamus before being projected back to the cortex [22]. Increasing evidence suggests that subcortical structures may serve as critical hubs integrating cortical functional networks via cortical-striato-thalamic loop. The caudate nucleus and thalamus showed functionally integration between neural networks supporting attention and top-down control and default mode network, suggesting that these subcortical regions may act as a cognitive integration zones [23]. Taken together, these results might suggest a compensatory interplay between plasticity mechanisms reinforcing pre-existent functional pathways and segregating anti-correlated neuronal processes through a reorganization of the cortical-striato-thalamic loop in far-to-onset pre-HD. This

very early compensatory pattern is supported by previous animal studies reporting compensatory mechanisms for the loss of striatal function in the pre-manifest stages of HD [24,25]. Recently, Cabanas et al. [26] reported a significant increase of neuronal activity in the dorsomedial striatum of pre-HD mice, which disappeared as soon as the motor symptoms became manifest. Similar compensatory mechanisms have been described in preclinical progranulin mutation carriers [9]. However, not significant associations between caudate nucleus FC and cognitive functioning were reported, indirectly supporting the idea that compensation is likely to be effective at the behavioral level.

Changes in connectivity were reported only for the left caudate nucleus, which might suggest an hemispheric asymmetry in very early HD, consistent with previous literature [27,28]. A divergent involvement of caudate nucleus was confirmed by the voxel-wise FA (and MD) analysis, in line with a recent meta-analysis reporting increased FA in the caudate nucleus of pre-HD (and increased MD in HD patients) [29]. Currently, there are different interpretations of increased striatal FA in HD. Douaud and colleagues suggested that it expresses a preferential loss of the striatal connections along specific radiating directions, while some other are relatively spared, transforming the striatum into a more organized structure [30]. Alternatively, it has been suggested that increased FA could result from pathological mechanisms that modify tissue integrity [31]. We found increased FA (and MD) in the right caudate nucleus of close-to-onset pre-HD, which might represent a marker of loss of connections in pre-HD approaching clinical symptoms, congruently with grey matter neurodegeneration observed in these individuals. Taken together, these results suggest that increased connectivity of the left caudate nucleus might temporally compensate the very subtle incoming structural damage, while the right caudate nucleus might exhibit an early vulnerability (becoming significant in the close-to-onset group), overcoming the functional compensation.

Alternative explanations are also possible. The inclusion of a higher number of right-handed individuals might explain this asymmetry, as the caudate nucleus of the dominant hemisphere might exhibit the maximal dynamic compensatory reorganization. Future studies should investigate striatal connectivity in left-handed HD individuals to directly address the influence of handedness.

Moreover, significant interaction between groups for the association between FC and FA of the left caudate nucleus was found, although not surviving multiple comparison correction.

Specifically, a negative relationship was reported between FC and FA in far-to-onset pre-HD individuals, while the relationship faded out in both HC and close-to-onset pre-HD ($R^2 < 0.07$). This result may reinforce the assumption of a dynamic reorganization of functional pathways linked with a stage-dependent structural organization of caudate.

In close-to-onset pre-HD we confirmed previous studies investigating neurodegeneration [32]. Moreover, the pattern of morphology alterations in close-to-onset pre-HD is congruent with recent data from a meta-analysis reporting volume reduction in bilateral striatum and right middle occipital gyrus in pre-HD (Figure 1C) [33]. While these structural changes in close-to-onset individuals suggest disease progression, we did not find FC reduction, although, as expected, a trend towards reduced subcortical-striatal connectivity was observed. This pattern might suggest that the underlying FC effect of close-to-onset pre-HD is relatively small. If there are subtle effects of close-to-onset pre-HD on FC, these might be detected with larger sample sizes or improving signal-to-noise ratio. Furthermore, it is also possible that FC in close-to-onset differs in some other brain regions.

Alternative explanations are still possible. The lack of FC differences in close-to-onset pre-HD may suggest that increased cortical-caudate FC is due to aberrant pruning of inhibitory synapses, while the excitatory inputs that the striatum receives from the cortex might be spared in the early stage of disease [34]. In pre-HD individuals closer to the clinical symptoms, neurodegeneration may subsequently involve excitatory synapses, restoring the balance between synapses. Moreover, Zullo et al. [35] reported an association between longevity and activity of genes involved in downregulation of neural excitation. These results might suggest a detrimental effect of the increased caudate-cortical connectivity, which might in turn trigger the clinical symptoms of HD. Future studies should disentangle whether hyper-connectivity is linked with compensatory or maladaptive mechanisms. This information will help to guide new treatments, including brain stimulation interventions aimed to normalize FC [36].

This study has both strengths and limitations. The main strength is that we investigated for the first time both cortical-striatal and subcortical-striatal loops in pre-HD individuals stratified according to DBS. This design enabled to assess specific striatal alterations within different pre-HD stages. Among limitations, the relative small sample size of participants limits the generalization of the present findings and prevents examining the role of potentially modulatory factors, such as

handedness. Thus, our results should be considered as exploratory and interpreted with caution. It would be valuable in the future to longitudinally assess whether the pattern of increased connectivity would change within-subject as a function of time.

In conclusion, this study suggests early disease-burden dependent FC reorganization involving the left caudate nucleus in pre-HD. These findings could represent new insights to understand biomarkers and biological phenotypes linked with genotype, which would help the development of new therapies and treatment outcomes [37].

Data availability statement: both gene carriers and controls signed an informed consent form guaranteeing data confidentiality, and we were therefore unable to share our data through deposition in an open-access repository. However, the data are available from the last author, upon request and for research purposes only. The person requesting the data must sign a confidentiality agreement provided by Assistance Publique-Hôpitaux de Paris stipulating that they will make no attempt to identify the patients. Given the small size of the cohorts studied, the identification of the patients from center information is feasible. Thus, even with a signed confidentiality agreement, some of the information will be removed, to ensure that the patients cannot be identified.

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Table 1. Demographic, genetic, and subcortical variables of controls and premanifest HD groups.

Variables		Healthy controls (n=25)	Far-to-onset pre-HD (n=8)	Close-to-onset pre-HD (n=8)	p
Socio-demographic, clinical and genetic variables					
	Age	44 ± 9	41 ± 14	43 ± 6	0.91
	Education (years)	15 ± 2	13 ± 2	16 ± 2	0.09
	Sex (female)	11	5	4	0.66
	Handedness (right)	24	6	7	0.21
	Disease burden score	--	224 ± 44	364 ± 43	0.001
	CAG repeat	--	41 ± 2	44 ± 2	0.008
Clinical/Cognitive outcomes					
	Total Functional Capacity	--	13 ± 0	13 ± 0	1
	Total motor score	--	0 ± 0	0.5 ± 1	0.14
	Symbol digit modality test	--	53 ± 13	53 ± 8	1
	Stroop (word)	--	103 ± 11	99 ± 2	0.57
	Verbal Fluency (letters)	--	65 ± 17	66 ± 21	0.94
	Composite Unified Huntington's Disease Rating Scale [15]	--	17 ± 1	17 ± 1	0.53
Subcortical volumes					
Caudate	left	3402 ± 341	3205 ± 509	2498 ± 544 *	0.003
	right	3570 ± 385	3367 ± 482	2614 ± 535 *	0.002
Putamen	left	5002 ± 529	4652 ± 581	3774 ± 581 *	<0.001
	right	5045 ± 521	4654 ± 501	3839 ± 498 *	<0.001
Accumbens	left	607 ± 98	543 ± 89	460 ± 86 *	0.002
	right	561 ± 79	502 ± 71	428 ± 100 *	0.003

* Statistically different from HC

Table 2. Differences in functional connectivity between left caudate and cortical regions in far-to-onset premanifest HD compared to healthy controls and close-to-onset premanifest HD ($p < 0.05$ with a vertex-level FDR-correction and a cluster-level threshold of $p < 0.01$ FWE-corrected).

Region name	Side	Cluster mass	MNI coordinates				
			x	y	z	p-FWE	
Far-to-onset premanifest HD > healthy controls							
Precuneus/PCC	Left	10283	-3	-52	20	0.009	
	Right	18109	13	-50	38	0.005	
Inferior parietal lobe	Right	11216	38	-79	35	0.008	
Far-to-onset premanifest HD > Close-to-onset premanifest HD							
Inferior parietal lobe	Left	5375	-42	-60	48	0.005	
	Right	3961	42	-52	44	0.006	
Precuneus/PCC	Left	4338	-1	-45	28	0.006	

PCC: posterior cingulate cortex

A Subcortical region of interest analysis

B Whole brain voxel-based analysis

Healthy controls > Close-to-onset premanifest HD

C Cortical thickness analysis

Healthy controls > Close-to-onset premanifest HD

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A Mean fractional anisotropy analysis

B Voxel-based fractional anisotropy analysis

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