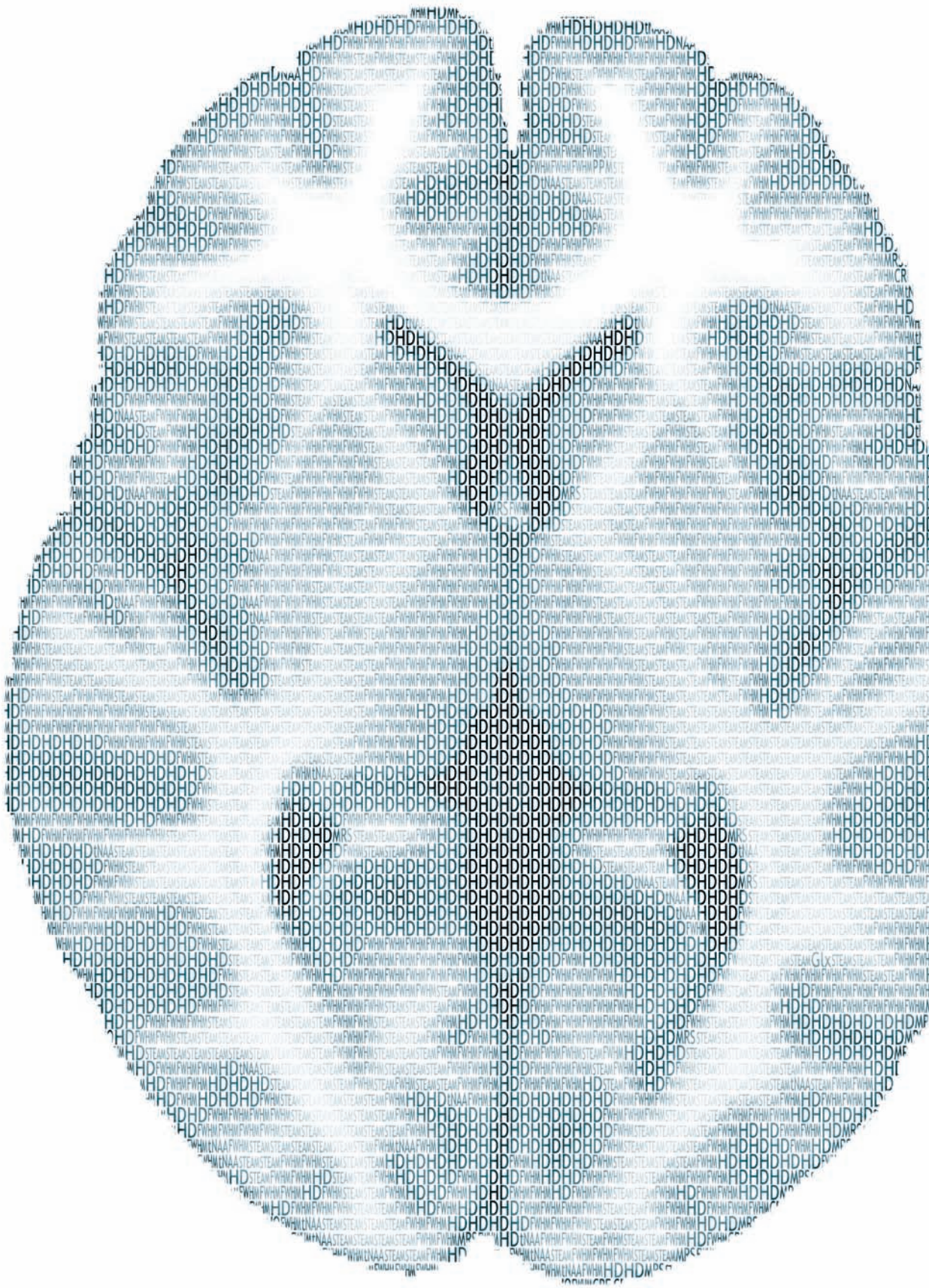

Chapter 9

From premanifest to manifest Huntington's disease: a two year follow up study with Magnetic Resonance Spectroscopy at 7 Tesla

Simon J.A. van den Bogaard[1], Eve M. Dumas[1], Wouter M. Teeuwisse[2], Hermien E. Kan[2], Andrew Webb[2], Mark A. van Buchem[3], Raymund A.C. Roos[1], Jeroen van der Grond[3]

1. Department of Neurology, Leiden University Medical Centre, Leiden, the Netherlands
2. C.J. Gorter Centre for High Field MRI, Leiden University Medical Centre, Leiden, the Netherlands
3. Department of Radiology, Leiden University Medical Centre, Leiden, the Netherlands

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Abstract

Background

Previous cross-sectional magnetic resonance spectroscopy (MRS) studies in Huntington's disease (HD) have demonstrated differences in metabolite concentrations compared to controls in several regions of interest, especially the putamen and caudate nucleus. It has been suggested that metabolite changes could be used as biomarker in future therapeutic trials. The aim of the present study was to assess metabolite changes in both premanifest and early HD over a two year follow up period using MRS at 7 Tesla.

Methods

In 13 HD gene carriers (10 premanifest and 3 manifest HD) proton MRS was performed at baseline and after 24 months. At follow up, four of the premanifest HD gene carriers had progressed into manifest HD, as assessed by clinical measures. 7T MR proton spectroscopy was performed in three regions of interest; the caudate nucleus, putamen and prefrontal cortex. Six metabolites were quantified for each region at each time point. Statistical analysis was performed using paired t-tests.

Results

In the caudate nucleus a decrease in creatine (p=0.032) and myo-inositol (p=0.006) concentrations was observed. A significant decrease in the putamen was seen in the total N-acetylaspartate (tNAA) (p=0.022) and choline concentrations (p=0.007). Premanifest HD converters showed higher rates of tNAA decrease in the putamen (p<0.003) than non-converting premanifest HD.

Conclusion

Over a period of 2 years we have demonstrated metabolite changes in the caudate nucleus and putamen of HD gene carriers around disease onset. This demonstrates the potential of MRS for providing a biomarker of disease progression and for evaluating future therapeutic interventions.

Introduction

Huntington's disease (HD) is a devastating neurological disorder causing widespread neuronal damage throughout the brain[1-3]. Atrophy of the striatum is regarded as the hallmark of the pathologic findings in HD[4] and is consistently reported as the earliest brain structure affected, well before clinical symptoms arise[1-3;5]. Almost all other brain regions show structural changes at later stages of the disease, which can be examined in vivo with MRI[2] and post-mortem[4].

The pathophysiological mechanism leading to neuronal damage still remains unclear. The genetic defect is located at chromosome 4 in the Htt-gene, leading to a mutant huntingtin protein[6]. Several pathophysiological hypotheses have been postulated. First, direct toxic effects of the aggregated and misfolded huntingtin protein could lead to neuronal damage. Second, transcriptional dysregulation of multiple genes is reported with associated functions in neurotransmitter receptors, enzymes and proteins involved in neuron structure, stress responses and axonal transport. Furthermore, there is evidence for impaired energy metabolism due to mitochondrial disturbances and disrupted dynamic intracellular processes, such as trafficking of intracellular vesicles and synaptic disruptions. Finally, the excitotoxicity hypothesis has gained a lot of support, which describes an increased sensitivity to neurotransmitter mediated stimulation, causing overstimulation and cell death[7-9]. Magnetic resonance spectroscopy (MRS) can be used in HD research either to explore pathophysiological mechanisms of the disease and/or in the search for a HD biomarker. Both objectives are closely linked as a robust biomarker should have a basis in the pathologic mechanisms of the disease. In HD, there is a need for objective biomarkers both for disease monitoring purposes and for evaluation of future therapeutic compounds. Novel compounds targeting specific pathogenic pathways, such as disturbed energy metabolism and those related to excitotoxicity theory, can potentially be monitored by means of MRS.

Previous studies have consistently demonstrated reduced N-acetylaspartate (NAA) and creatine levels in the caudate nucleus and/or putamen in manifest HD[10-12]. Whether these reductions in metabolite concentrations start in the premanifest phase of the disease or at a later disease stage remains a matter of debate. Lower concentrations of NAA and creatine in premanifest HD have been demonstrated[12], although not all studies agree on this finding[10;13]. In addition to NAA and creatine, changes in choline[14], glutamate[11;15], lactate[16] and myo-inositol[10] concentration in HD have been reported. Choline was found to be reduced in the frontal lobe and was related to cognitive deficits[14]. Glutamate levels have been reported to be either increased[15] or decreased[11] in the striatum in manifest HD. This discrepancy could possibly be explained by the use of a glutamate-to-creatine ratio in one

publication[15], against a glutamate concentration calculation using water as a concentration standard in the other[11]. Increased lactate in the occipital cortex and basal ganglia has been reported, suggestive of impaired energy metabolism[16]. Finally, a myo-inositol increase in the putamen has recently been reported in a large cohort from the TRACK-HD study[10].

All of the above described studies have been cross-sectional in design. In terms of identifying potential biomarkers, following changes in metabolite concentrations in longitudinal studies is imperative. Our study population consists of predominantly premanifest HD participants, which show a high conversion rate, meaning progression from the premanifest to manifest HD. This specific study group is of particular interest given the fact that if any potential therapeutic compound should prove effective, it is in this premanifest stage of the disease, when neuronal loss is still limited, that one would start with interventions. Therefore, the aims of our study were to assess metabolite changes in both premanifest and early HD over a two year follow up period and how potential changes relate to clinical outcome measures. The current study consists of the follow up data of previously reported 7T MRS results in manifest and premanifest HD[11].

Materials and methods

Subjects

Recruitment of participants was performed as described previously[11]. In short, participants were recruited from the outpatient neurology clinic of the Leiden University Medical Centre. The presence of a positive genetic test with a CAG repeat ≥ 39 and the absence of significant comorbidity were mandatory inclusion criteria for both participant groups. The premanifest HD group was defined by the absence of motor disturbances on the Unified Huntington's Disease Rating Scale '99 (UHDRS), with a total motor score (TMS) < 5. Early manifest HD was defined as a TMS of ≥ 5, a Total Functional Capacity (TFC) ≥ 7 and a diagnostic confidence level of 4 on the UHDRS. In total thirteen gene carriers were available for a follow up scan after two years. The study was approved by the local Medical Ethical Committee of the Leiden University Medical Centre. All participants provided written informed consent.

Clinical measures

At both time points the same set of clinical measures was performed. This included the UHDRS motor scale (score 0-124) and the TFC, a global scale of

impairment in daily life activities, score 0-13. In addition, a short cognitive battery was administered, consisting of the Mini Mental State Exam (MMSE), Stroop word reading card (Stroop), the Symbol Digit Modality Test (SDMT) and the Trail Making Test part B (TMT-B), Behavioural disturbances were evaluated with the Beck Depression Inventory 2nd version (BDI-II) and the Problem Behaviour Assessment short version (PBA-s). Predicted years to onset were calculated from current age and CAG repeat length using the formula by Langbehn *et al.*(2004)[17].

MR data acquisition:

The MR protocol performed at the baseline time point has been described previously: it consisted of a T1-weighted MRI and an MRS sequence performed on a Philips 7 Tesla Achieva whole body scanner (Philips Healthcare, Best, The Netherlands) with a NOVA Medical quadrature transmit coil and 16 channel receive coil array. A three-dimensional, high resolution, T1-weighted gradient echo (GRE) scan was acquired (TR/TE = 11/5.4 ms, voxel size 0.44 x 0.44 x 0.84 mm, total scan time 1:46 mins) for accurate planning. Localized proton spectra were acquired using a stimulated echo acquisition mode (STEAM) sequence with the following scan parameters: TR/TE/TM = 2000/19/25 ms, BW 4 kHz, 2048 complex data points and 128 signal averages. Water suppression was performed using a frequency-selective RF pulse and gradient spoiling, six saturation bands were additionally applied to suppress signal from surrounding tissue. All scans included a reference scan without water suppression for quantification. The voxel was placed within the region of interest with the maximum volume containing only tissue from the intended structure, minimizing the contribution from surrounding tissue, and also partial volume effects. Regions of interest consisted of the caudate nucleus, putamen, and the prefrontal white and cortical grey matter. For the prefrontal voxel, positioning included approximately 50% white and 50% cortical grey matter.

In the follow up studies minor changes were made to improve the signal to noise ratio (SNR), without changing the basic imaging and spectroscopy sequences which remained identical; volume-selective rather than global power optimization was implemented[18], the first- and second-order shims were adjusted using a B0 map[19] rather than a pencil-beam shimming routine, and water suppression was achieved using variable-power RF pulses with optimized relaxation delays (VAPOR)[20;21] rather than frequency selective excitation and spoiling.

MRS post-processing

MRS data were analyzed with LCModel[22;23] according to the procedure described previously[11]. The unsuppressed water was used as an internal reference to calculate the concentrations of the metabolites of interest: choline, creatine, glutamine + glutamate (Glx), total NAA (N-acetylaspartateglutamate + NAA), myo-inositol and lactate. Data processing was identical for both time points, with a Cramer-Rao Lower Bound (CRLB) of 20% or less as calculated by LCmodel required for all spectra as inclusion criteria for all metabolites with the exception of lactate for which we followed the guidelines of Tkac *et al.*(2009)[24]. For lactate CRLB above 100% were excluded from further analysis. If lactate was not quantified with a CRLB <100% in at least 50% of analyzed spectra, the analysis of lactate was not performed. For inclusion in the statistical analysis the participant had to have spectra of sufficient quality at both time points. After this quality check, longitudinal data of 9 participants was included for the caudate nucleus, 7 data sets for the putamen and 9 for the prefrontal cortex.

Statistics

All statistical analysis was performed with SPSS for Windows (version 17.0, SPSS Inc, Chicago, IL, USA). Paired t-tests were applied to assess differences between the baseline visit and follow up for clinical variables as well as for metabolite concentrations. The differences in clinical measures and metabolite concentrations between the two time points were calculated. Pearson's correlation was used to explore the relationship between changes in metabolite concentrations and clinical measures. This correlation analysis was only performed for those metabolites that demonstrated statistically significant longitudinal change. Finally, paired t-tests were used to examine two separate subgroups; premanifest gene carriers that progressed to manifest HD as opposed to those premanifest HD participants that did not undergo this conversion. Again this procedure was only performed for those metabolites demonstrating significant longitudinal changes.

Results

Demographics and clinical measures

The gene carrier group consisted of 10 premanifest and three early manifest participants. During the two year follow up period four premanifest participants converted to manifest HD as defined by their UHDRS TMS. At baseline the mean age of the group was 43.5 years with a standard deviation (SD) of 10.6 years and the mean CAG-repeat length was 44.0 (SD 3.2). The group consisted of 5 males and 8 females. The premanifest HD group was mean 7.8 years (SD 4.3) to

predicted years to onset. The clinical characteristics at baseline visit and follow up are shown in Table 1. Over the 24 month period a significant decrease in the BDI-II and Stroop performance was observed.

Table 1: Clinical measures at baseline and follow up of 13 gene carriers

		Mean	SD	t	p-value
TMS	Baseline	4.69	5.60	-1.639	0.127
	Follow up	7.31	6.59		
TFC	Baseline	12.38	0.65	0.322	0.753
	Follow up	12.31	1.03		
PBA-s	Baseline	10.92	13.10	1.194	0.256
	Follow up	8.31	10.40		
BDI-II	Baseline	7.85	6.54	2.386	**0.034**
	Follow up	5.62	4.23		
TMT-B	Baseline	74.62	30.00	-0.978	0.347
	Follow up	85.69	50.65		
SDMT	Baseline	45.54	9.66	-0.892	0.390
	Follow up	46.54	10.08		
Stroop	Baseline	91.23	17.27	3.014	**0.011**
	Follow up	84.31	18.89		
MMSE	Baseline	28.77	1.54	-0.433	0.673
	Follow up	28.85	1.68		
Total gene carriers			N = 13		
Total premanifest at baseline (includes 4 converters during follow-up)			N = 10		
Total manifest at baseline			N = 3		

TMS = total motor score, TFC = total functional capacity, PBA-s= problem behaviour assessment short version, BDI-II: becks depression inventory, second version, TMT-B= trail making test, part B, SDMT=symbol digit modality test, MMSE=mini mental state exam, SD = standard deviation, n = number of participants

Metabolite analysis

All metabolite concentrations at both baseline and follow up are shown in Table 2. In the caudate nucleus a decrease in creatine (p=0.032) and myo-inositol (p=0.006) concentrations was observed. In addition, in the putamen significant decreases in total N-acetylaspartate (p=0.022) and choline concentration (p=0.007) were observed. No metabolite concentration differences were found in the prefrontal region. Figure 1 shows typical examples of spectra from the follow up visit.

Relationship to clinical measures

Table 3 shows the association between cognitive changes over time and (significant) metabolite changes in the putamen and caudate nucleus. A decrease in depressive symptoms as measured by the BDI-II was related to a decrease in myo-inositol (p=0.020). Furthermore a decrease in cognitive performance as measured by the Stroop test was correlated to choline concentration decrease in the putamen (p=0.048).

Conversion from premanifest to manifest HD

The premanifest HD group that converted to manifest HD showed longitudinal changes in concentrations in both caudate nucleus and putamen for three metabolites, namely myo-inositol (p=0.019), choline (p=0.025) and NAA (p=0.003), as shown in table 4. The premanifest non-converter group showed a significant longitudinal change in creatine in the caudate nucleus (p=0.040).

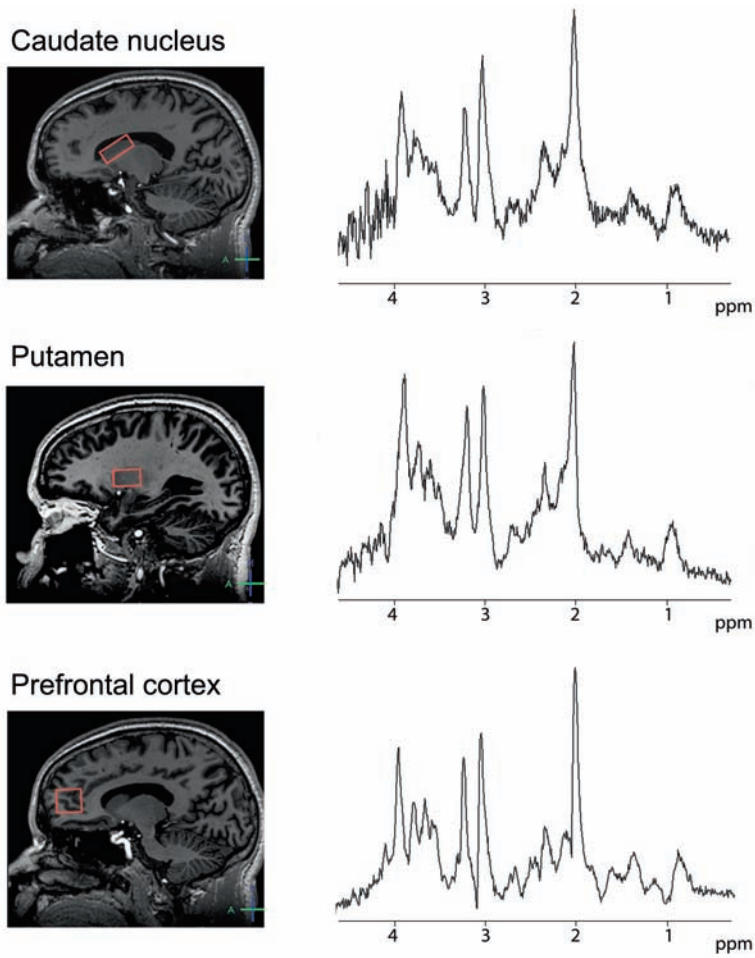

Figure 1: Typical example spectra and LCModel fit from 3 regions of interest in Huntington's disease gene carriers from the follow up visit of 3 premanifest participants. On the left the actual voxel in the sagital plane. Actual planning was performed in 3 directions. ppm=part per million

Table 2: Metabolite concentrations at baseline and follow up in 3 regions of interest in Huntington's disease gene carriers

		Mean	SD	t	*p*
Caudate nucleus (N=9, 7 PGMC (incl. 2 converters), 2 MHD)					
Creatine	Baseline	11.18	2.84	2.588	**0.032**
	Follow up	9.14	1.19		
Choline	Baseline	2.83	0.60	0.473	0.649
	Follow up	2.76	0.48		
Lactate	Baseline	1.08	0.98	0.018	0.986
	Follow up	1.07	0.74		
Myo-inositol	Baseline	7.34	1.88	3.705	**0.006**
	Follow up	4.85	1.48		
tNAA	Baseline	10.05	2.19	0.501	0.630
	Follow up	9.76	1.53		
Glx	Baseline	12.11	4.09	0.824	0.434
	Follow up	11.18	2.85		
Putamen (N=7, 6 PGMC (incl. 3 converters), 1 MHD)					
		Mean	SD	t	*p*
Creatine	Baseline	12.14	2.61	1.631	0.154
	Follow up	10.23	1.68		
Choline	Baseline	3.31	0.33	3.986	**0.007**
	Follow up	2.67	0.32		
Lactate	Baseline	1.13	1.48	1.005	0.354
	Follow up	0.46	0.46		
Myo-inositol	Baseline	7.40	5.00	1.011	0.351
	Follow up	5.05	1.78		
tNAA	Baseline	14.78	1.19	3.062	**0.022**
	Follow up	11.83	2.05		
Glx	Baseline	13.29	3.13	0.387	0.712
	Follow up	12.73	2.41		
Frontal (N=9, 7 PGMC (incl. 4 converters), 2 MHD)					
		Mean	SD	t	*p*
Creatine	Baseline	8.73	1.58	-0.092	0.929
	Follow up	8.76	1.35		
Choline	Baseline	2.30	0.66	-0.173	0.867
	Follow up	2.34	0.51		
Lactate	Baseline	1.23	0.81	1.231	0.253
	Follow up	0.77	0.69		
Myo-inositol	Baseline	10.13	4.49	1.742	0.120
	Follow up	7.54	1.69		
tNAA	Baseline	11.98	2.29	1.067	0.317
	Follow up	11.27	1.75		
Glx	Baseline	10.52	3.48	-0.005	0.996
	Follow up	10.53	1.71		

PMGC = premanifest gene carriers, MHD = manifest HD, tNAA=total N-acetylaspartate, Glx = Glutamate and Glutamine

Table 3: Correlation analysis between metabolite concentrations and clinical measures in Huntington's disease gene carriers

		TMS	TFC	PBA-S	BDI-II	TMT-B	SDMT	Stroop	MMSE
Caudate Nucleus									
Creatine	R	-.562	-.027	.255	.056	-.320	-.091	-.135	-.297
	p	0.116	0.944	0.507	0.887	0.400	0.817	0.730	0.437
Myo-Inositol	R	-.228	-.507	-.560	**-.749**	.329	.464	.404	.274
	p	0.555	0.164	0.117	**0.020**	0.387	0.208	0.281	0.476
Putamen									
Choline	R	.248	-.195	-.355	-.474	-.031	.236	**.759**	-.143
	p	0.593	0.675	0.435	0.282	0.948	0.611	**0.048**	0.760
tNAA	R	.721	-.489	-.413	-.678	.030	.613	.324	.087
	p	0.067	0.265	0.358	0.094	0.949	0.143	0.478	0.853

TMS = total motor score, TFC = Total Functional Capacity, PBA-s= problem behaviour assessment short version, BDI-II: becks depression inventory, second version, TMT-B= trail making test, part B, SDMT=symbol digit modality test, MMSE=mini mental state exam, tNAA=total N-acetylaspartate

Table 4: Metabolite concentrations in the conversion from premanifest to manifest HD versus non-conversion

			Baseline		Follow up		*p*-value
Caudate nucleus		N	Mean	SD	Mean	SD	
Creatine	non converters	5	11.31	2.23	9.28	1.62	**0.040**
	converters	2	14.11	2.03	9.23	0.52	0.137
Myo-inositol	non converters	5	6.83	1.69	4.98	1.89	0.108
	converters	2	8.01	1.81	4.56	1.66	0.019
Putamen							
Choline	non converters	3	3.19	0.28	2.86	0.19	0.353
	converters	3	3.57	0.22	2.67	0.11	**0.025**
tNAA	non converters	3	14.05	1.13	13.79	1.01	0.457
	converters	3	15.83	0.26	10.81	0.42	**0.003**

SD = standard deviation, tNAA=total N-acetylaspartate

Discussion

The main findings of this study show that over a period of two years, significant metabolite changes were observed in the progression of HD. More specifically, these changes occurred in a predominantly premanifest gene carrier group in which four participants converted from premanifest to manifest HD. The premanifest HD participants who converted to manifest HD seem to show a more rapid decline in NAA in the putamen than those who did not convert.

This longitudinal study expands the knowledge on metabolite changes in HD by demonstrating changes in creatine concentration in the caudate nucleus and total NAA concentration in the putamen. This reduction occurs in a mixed premanifest and early manifest HD group (peri-manifest). In participants converting from premanifest to manifest HD, higher rates of decrease were particularly observed in the total NAA of the putamen. In our previous cross-sectional study, NAA and creatine were significantly decreased in both the putamen and caudate nucleus in manifest HD, but not in the premanifest group[11]. However, it was suggested that this decrease in concentration might already be visible in the premanifest group, but due to large intersubject variations in metabolite concentrations in combination with a small sample size, this finding did not reach statistical significance. The results of other cross-sectional results are in line with our longitudinal study[10;12]; one study reported decreases in NAA and creatine concentrations in the putamen in premanifest HD[12], whereas in another study lower total NAA and creatine in the putamen in manifest HD as well as a non-significant decrease in these metabolites in premanifest HD were reported[10]. In contrast to these findings, one study reported no altered metabolite concentration in premanifest HD[13], however relative metabolite concentrations as opposed to absolute ones referenced to water were used. In this respect, the use of metabolite ratio's should be approached with caution, since in HD it it has been demonstrated, cross-sectionally[10-12] and longitudinally, that both NAA and creatine concentrations change. Summing all the data, it seems that creatine in the caudate nucleus and especially NAA in the putamen are metabolites that show great potential for use as a biomarker.

Our study demonstrated a decrease in choline in the putamen over the two year follow up. One other study also reported decreased choline concentrations in the frontal region in premanifest HD[14]. A possible explanation for a reduced choline concentration in the frontal lobe is that membrane turnover slows down prior to neuronal death[14].

Myo-inositol concentration in the caudate nucleus was found to decrease over time in our study population. Abnormalities in myo-inositol concentrations have only been found by one other study reporting an increase rather than a decrease[10]. However, this increase in myo-inositol concentration was found only in the manifest stage of the disease as compared to controls with a cross-sectional design. It might be that the changes in the concentration of myo-inositol are non-linear and dependent on disease stage. It is possible that myo-inositol initially decreases in premanifest stages before increasing in manifest HD. However, this hypothesis must be investigated in larger longitudinal trials including multiple disease stages such as the TRACK-HD study[3;10].

Two changes in clinical measures were found to relate with lower metabolite concentrations. First, the decrease in depressive symptoms as measured by the BDI-II was significantly related to myo-inositol concentration decreases in the caudate nucleus. However, it must be noted that the clinical impact of this decrease in depressive symptoms is relatively small. The higher baseline BDI-II score could be explained by the fact that, before disease onset, anxiety to receive an imminent and expected diagnosis adds to psychological stress[25]. Reduced symptom awareness after disease onset may explain the lower scores during follow up[26]. Still, the relationship of BDI-II scores with myo-inositol, which is thought to be a glial marker, is not straightforward and these two changes could occur in parallel and be unrelated. The second relationship between changes in clinical measures and changes in metabolite concentrations is the simultaneous reduction in choline and Stroop performance. A similar neuropsychological disturbance relating to frontal choline concentrations has previously been reported[14]. As choline could possibly represent a pathogenic process related to neuronal loss or dysfunction in the putamen, the relationship to decline in executive functioning as measured by the Stroop seems plausible.

A limitation to our study is the relatively small sample size, which could obscure subtle changes. However, even with the relative small numbers statistically significant results are obtained for different metabolites in various regions of the brain, emphasizing the robustness of these findings and suggesting the high potential of MRS as a biomarker in HD. The sample size for the analysis of specifically premanifest converters is even smaller, hence findings should be treated with caution. However, again the differences observed between the premanifest group that converted to manifest HD versus the non-converter group are in line with other cross-sectional results and as expected, all metabolites show a higher rate of decrease in the progressive premanifest group.

In conclusion, we have demonstrated several metabolite changes in the putamen and caudate nucleus close to disease onset in HD over a two year period. This demonstrates the potential of MRS for providing a biomarker of disease progression and could be useful in evaluating future therapeutic interventions. Specifically the NAA in the putamen seems the best candidate biomarker.

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