Elevated Arteriolar Cerebral Blood Volume in Prodromal Huntington's Disease

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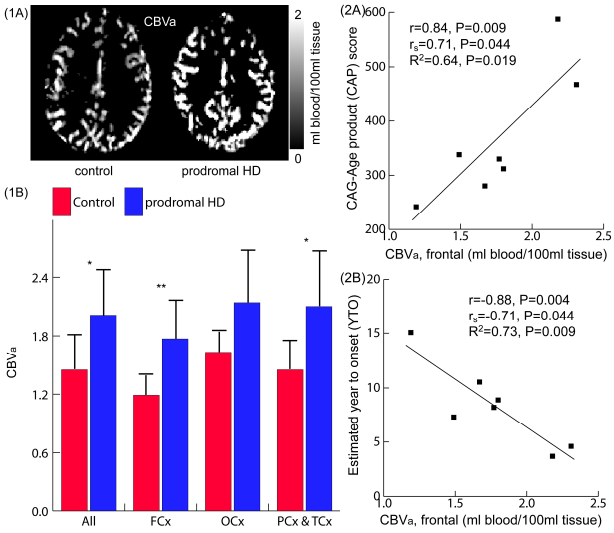
TARGET AUDIENCE: Neurologists, neuroscientists and physicists who are interested in neurodegeneration.

PURPOSE: Huntington's Disease (HD) is a neurodegenerative disorder caused by a CAG-repeat expansion of the *Huntingtin* gene. Mutation positive subjects can be identified decades before onset of motor symptoms. A major goal for the treatment of HD will be to design neuroprotective therapies during the prodromal (beginning about 15 years prior to onset) and early phases of the disorder. However, such therapeutic trials are hampered by the lack of sensitive and reversible early biomarkers for HD. Regional brain atrophy begins in striatum and cortex many years before the onset of diagnosable symptoms in HD. Functional brain changes suggest that neuronal dysfunction, perhaps related to neurovascular and metabolic alterations, may be early features of the disorder. It is therefore important to investigate potential microvascular abnormality in the prodromal period. Neurophysiology studies have shown that small arteries and arterioles are most responsive to changes in metabolism. In this study, we applied the recently developed inflow-based vascular-space-occupancy (iVASO) MRI technique, to investigate potential arteriolar CBV (CBVa) abnormality in cortical grey matter in prodromal HD patients, and compare it with brain atrophy in the same subjects.

METHODS: *Participants:* Seven prodromal HD patients and nine age and sex matched normal controls with informed consent were scanned. None of the subjects had other neurologic history or signs on exam, or history of vascular diseases. *MRI:* All scans were performed on a 7T Philips MRI scanner. A 32-channel phased-array head coil (Nova Medical) was used for RF reception and a head-only quadrature coil for transmit. Anatomical images were acquired with a 3D MPRAGE scan (TR/TE/TI=4.7/2.1/446ms, voxel=0.6mm isotropic). GM CBV_a was measured using iVASO MRI with a single slice oriented oblique axial and located slightly above the ventricle in order to sample the frontal, occipital, parietal and temporal lobes of cerebral cortex. For each subject, the slice was carefully placed at approximately the same location. iVASO parameters: TR/TI=10000/1626, 5000/1382, 3100/1081, 2000/797, 1667/691, and 1300/563ms; TE=17ms, voxel=2x2x4mm^3, crusher gradients of b=0.3s/mm^2 and V_{enc}=10cm/s on z-direction. *Image analysis:* SPM8 (UCL, UK) and other in-house code programmed in Matlab 6.0 (Mathworks, USA) were used for image analysis. Partial volume effects of WM and CSF on the iVASO signals in GM were corrected. *Statistical analysis:* Group difference was assessed using two-sample two-sided *t*-Tests for independent samples with unequal variances, and the Wilcoxon rank sum test (a.k.a. the Mann-Whitney test) for small sample size. Multiple comparisons were corrected for using the Benjamini-Hochberg false discovery rate (FDR) procedure. Effect size was estimated with Cohen's *d*. Correlations were tested using: 1) Pearson correlation coefficient (r); 2) Spearman rank correlation coefficient (r_s) for small sample size; 3) adjusted R^2 from simple linear regression for small sample size; 4) adjusted R^2 from multiple linear regression with age as a covariate.

RESULTS: Representative CBV_a maps are shown in Fig.1A. GM CBV_a values in the whole slice (All), frontal (FCx), occipital (OCx), and parietal and temporal cortex (PCx&TCx) are illustrated in Fig.1B. The average GM CBV_a values in controls were all in normal range, providing validation for our measurements. The GM CBV_a values in all cortical regions were significantly greater (P<0.05) in prodromal HD patients (n=7) compared to controls (n=9) with relative changes of 30-50% and effect sizes of 1.4-2.1. This effect was the most significant in the frontal cortex, with the smallest p-value (P=0.008) and greatest relative change (48.9%) and effect size (2.11). Furthermore, significant correlations (adjusted R^2>0.5 and P<0.05) were found between the GM CBV_a values in the frontal cortex and genetic measures for disease progression including the CAG-Age product (CAP) score and estimated years to onset (YTO) of motor symptoms (Figs.2A,B). No significant impact from age was detected when performing multiple linear regression with both CBV_a and age as the independent variables. Similar trends of correlations between GM CBV_a values in other cortical regions and CAP/YTO were also observed, but did not reach statistical significance. Table 1 shows the volumes of various brain regions derived from the MPRAGE scans. No significant group difference was found with this small group of prodromal HD patients. The relative changes in volume between the two groups were 3-11%, except for in ventricles (34.7%, but P=0.45). The effects sizes ranged from 0.10 to 0.89. Both the relative differences and effect sizes were smaller than those of GM CBV_a. There was no significant correlation found between any of the brain volumes and genetic measures, nor between brain volumes and GM CBV_a.



DISCUSSION: It has been reported that blood vessels become smaller in diameter, but more numerous in post-mortem brain tissue from HD patients. A recent histological study[1] found similar results in the YAC128 mouse model of HD: slightly decreased microvessel diameter but significantly increased vessel density and length in the striatum. In addition, an elevated level of vascular endothelial growth factor (VEGF), a protein produced by cells that stimulates angiogenesis and regulates vessel growth, has been observed in mutant huntingtin striatal cells. More recently, using contrast-enhanced MRI, Cepeda-Prado et al [2] reported increased total CBV in the R6/2 mouse model of HD. Meanwhile, Lin et al [3] were able to visualize abnormal microvasculature in R6/2 mice with a contrast-enhanced MRA method and measured larger total CBV in neocortex and striatum compared to littermate controls. These literature data are all consistent with our finding of enlarged CBV_a in prodromal HD patients.

Table 1. Volumes (mL) of brain regions.	Control subjects (n=9)	Prodromal HD patients (n=7)	Relative change (%)	Effect Size	p-value (*t*-Test)
Sulcal CSF	190±21	181±23	-4.9	-0.45	0.42
Ventricle	18.1±10.0	24.4±18.8	34.7	0.46	0.45
Cerebellar GM	55.5±10.9	62.2±16.6	11.9	0.52	0.38
Cerebral GM	474±69	423±47	-10.8	-0.89	0.10
Caudate	8.53±1.11	8.27±4.05	-3.0	-0.10	0.88
Thalamus	20.2±1.7	19.6±2.0	-3.1	-0.36	0.53
Putamen	8.87±7.56	8.46±1.11	-4.6	-0.47	0.42
Brainstem	21.9±2.5	20.0±1.6	-3.9	-0.43	0.42
Cerebellar WM	20.9±2.7	22.0±2.0	5.1	0.46	0.39
Cerebral WM	463±73	442±46	-4.5	-0.35	0.50

CONCLUSION: We report significantly elevated cortical GM CBV_a in prodromal HD patients. This effect was the most pronounced in the frontal cortex, in which GM CBV_a values also significantly correlated with genetic measures. No significant brain atrophy was measured in these prodromal HD patients. The relative difference between patients and controls and effect size of cortical GM CBV_a substantially exceeded those of brain volumes. These results suggest potential value of CBV_a as a quantitative biomarker that can visualize onset of brain changes prior to prominent brain atrophy in the prodromal period of HD.

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