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Evidence from the R6/2 Mouse Model of Huntington's Disease for Using Abnormal Brain Metabolism as a Biomarker for Evaluating Therapeutic Approaches for Treatment

Anna Parievsky, Carlos Cepeda, and Michael S. Levine*

Intellectual and Developmental Disabilities Research Center, Semel Institute for Neuroscience and Human Behavior and the Brain Research Institute, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA

Summary

Huntington's disease (HD) is an autosomal dominant genetic disorder characterized by a progression of motor abnormalities as well as cognitive and psychiatric symptoms [1]. Presently, there is no cure for HD and no treatment to reverse its course or prevent its onset. HD has been characterized primarily by significant degeneration of the striatum. In addition, imaging studies have shown alterations in extra-striatal regions including the cortex [2, 3], hippocampus, and hypothalamus [4]. Although previous functional magnetic resonance imaging (fMRI) studies in patients have yielded complex and heterogeneous findings, identifying functional alterations may serve as a useful tool for tracking the progression of HD and assessing the effects of therapeutic interventions. In a recent article Cepeda-Prado et al. use novel and groundbreaking fMRI methods to elucidate functional, structural, and metabolic alterations in the R6/2 mouse model of HD. Based on changes in relative cerebral brain volume (rCBV), neuronal activity, and glucose utilization, the authors suggest that R6/2 mice have impaired neurometabolic coupling. They propose the use of rCBV as a biomarker of HD progression, providing a basis for future research examining functional alterations in animal models.

Keywords

Huntington's disease; R6/2 mouse model; magnetic resonance imaging; functional magnetic resonance imaging; relative cerebral blood volume; local field potentials; seizure; [¹⁴C] 2DG uptake

Methods and Results

The R6/2 HD mouse model, expressing exon 1 of the human huntingtin gene [5], was used to examine brain metabolism and propensity for seizure activity. *In vivo* fMRI was

*Address Correspondence: Michael S. Levine, Ph.D. IDDRC Room 58-258 Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine 760 Westwood Plaza Los Angeles, CA 90095, Tel: 310 825 7595; Fax: 310 206 5060 mlevine@mednet.ucla.edu..

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performed to analyze rCBV maps in anesthetized R6/2 mice at early symptomatic (6 weeks) and late symptomatic (14 weeks) time points. fMRI methods generated rCBV maps of high spatial resolution allowing comparison across various brain regions, including the hippocampus (Hc), entorhinal cortex (EC), striatum (St), temporal cortex (TC), primary motor cortex (PMC), prefrontal motor cortex (PFC), cerebellum (Cb), thalamus (Th), nucleus accumbens (ACC), and hypothalamus (Hyp). Six-week R6/2 mice had significantly higher mean rCBV values in the PFC, PMS, TC, and St. These differences were further increased at 14 weeks and expanded to other areas such as the Th and Hc. Region of interest volumetric analysis was performed on the same images used for fMRI to compare structural changes of the lateral ventricles, Hc, Cb, St, and neocortex (Neo). Analyses did not reveal any structural abnormalities in 6-week R6/2 mice when compared to their WT littermates. However, 14-week R6/2 mice had significant volume loss in St, Hc, and Neo. Unlike previous publications [4, 6], the current study found only a trend toward volume loss in the Cb.

As fMRI signals have been shown to correlate with neuronal activity [7, 8], local field potentials (LFPs) were examined and compared to rCBV findings. LFPs were recorded in horizontal brain slices containing Hc, lateral EC, and TC of 6-week R6/2 mice and their WT littermates. R6/2 mice had significantly higher frequencies of LFPs in all four regions, including increased duration of LFPs in the lateral EC. Picrotoxin (a GABA_A receptor blocker) induced a significant increase in LFP frequencies in all four regions, as well as an increase in LFP durations in the lateral EC, in R6/2 mice but not WT. Picrotoxin induced synchronized discharges resembling seizure-like events in only the R6/2 mice. To examine the link between hyperexcitability of slices and seizures *in vivo*, audiogenic seizure (AGS) sensitivity was tested by exposing animals to a high-intensity siren. WT did not exhibit any **seizures** with scores higher than level 2 on the Racine scale [9]. However, all 6-week R6/2 mice exhibited seizures which scored at level 3 or above. The epileptic propensity was not tested in the 14 week-old group, when significant volume changes occur.

Glucose utilization was analyzed *via* measurement of *in vivo* [¹⁴C] 2DG uptake [10] as an alternative mode to examine energy metabolism in 6-week R6/2 mice and their WT littermates. Whole-brain axial slices were prepared and 2DG uptake was analyzed in Hc, EC, St, TC, PMC, PFC, Cb, and Th. R6/2 mice had significantly decreased glucose uptake in St, PMC, and PFC. In conclusion, R6/2 mice demonstrated altered neurometabolic coupling, as the St, PMC, and PFC exhibited increased rCBV values but decreased glucose uptake.

Discussion and Significance

Individuals carrying the HD mutation present structural and functional neurological alterations well before overt motor symptoms arise. Thus, therapies designed to prevent or delay neurodegeneration should be administered during preclinical stages of HD. Neuroimaging techniques such as MRI, fMRI, and diffusion tensor imaging, can detect changes within the brains of these individuals up to decades prior to a clinical diagnosis. Unfortunately, previous imaging studies employing these techniques have yielded variable results [11]. Therefore, to consistently detect preclinical changes in HD and track the effects

of treatment, further studies are required and reliable biomarkers must be identified. The current publication by Cepeda-Prado et al. uses novel imaging methods to examine structural and functional changes in an HD animal model and may aid in finding a reliable candidate biomarker to evaluate preclinical HD progression. To track the progression of HD and the effects of preventative therapies, longitudinal studies are required. However, such experiments are extremely complicated, expensive, and slow to conduct in patient populations and therefore preclinical studies in animal models are necessary. Previously, due to technical limitations, MRI and fMRI were not widely used on mice. However, with the methodology implemented in this study, such experiments are now possible. Although, Cepeda-Prado et al. did not uncover any significant structural alterations in **early symptomatic** animals, structural alterations were detected by 14 weeks of age. In contrast, functional alterations were detected much earlier. This outcome supports previous conclusions that functional neurological changes arise prior to any detectable structural changes and therefore can serve as earlier biomarkers [11]. The authors suggest that rCBV maps may function as potential biomarkers of HD progression as rCBV alterations occur as early as 6 weeks (early symptomatic), are progressive, and become greater by 14 weeks (late symptomatic stage). rCBV can be measured noninvasively in HD patients and should be further investigated as a possible biomarker. However, caution must be used when extrapolating these results to human patients as these measures can be differentially affected. For example, regional cerebral blood flow (a measure related to rCBV) is actually decreased in the PFC and St in preclinical HD mutation carriers [12], which is opposite to the result found in mice in the current report but may be related to early cell loss as seen in HD patients but not in the mice.

The R6/2 HD mouse model presents with an extremely rapidly progressing phenotype and is often considered to model juvenile onset HD [5, 13]. Similar to the increased predisposition of R6/2 mice to seizure activity, as described previously and confirmed in this publication, infants and children with HD often present epileptic seizures. Therefore, despite the R6/2 being a very useful model for assessing HD alterations because of its aggressive phenotype, it may not always be the optimal model to use when searching for a reliable biomarker for adult onset HD. Future studies should examine rCBV maps and neurometabolic coupling in slower progressing full-length transgenic or knock-in models of HD.

Previous research in HD mouse models, including in the R6/2, has shown biphasic behavioral and electrophysiological alterations. For example, early in the progression of the phenotype, animals displayed increased N-methyl-D-aspartate (NMDA) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propionic acid (AMPA) receptor-mediated currents in striatal medium-sized spiny neurons. However, later in the progression of the phenotype, animals showed a decrease in these responses [14–16]. HD patients demonstrate progressive motor symptoms characterized by chorea during early stages of HD and akinesia during later stages. Interestingly, in the current publication, biphasic changes were not observed as rCBV values were increased in the St, TC, PMC, and PFC at 6 weeks and continued to increase up to 14 weeks. LFPs, seizure activity, and glucose utilization were only examined at 6 weeks of age and therefore longitudinal changes were not traced. As one of the main findings of this publication is that R6/2 animals have altered neurometabolic coupling based on the

mismatch between decreased glucose consumption and increased rCBV values at 6 weeks, it would have been very useful to also examine glucose utilization during the late symptomatic stage and determine if neurometabolic coupling is similarly altered in late stage HD. In particular, R6/2 mice develop a diabetic phenotype which may preclude adequate glucose utilization. In addition, neurometabolic changes can be at odds during interictal and ictal activity. Indeed, it has been shown that an epileptogenic area is hypometabolic during the interictal phase but hypermetabolic during the ictal period. It would be interesting to precipitate audiogenic seizures in the course of fMRI studies in R6/2 mice and examine if the neurometabolic uncoupling still occurs.

Finally, the mechanisms of seizure generation could be different depending on the **protocol** used. In the present study electrophysiological recordings and seizure propensity *in vitro* were examined in limbic areas, while the *in vivo* experiments used the audiogenic type of epilepsy. As the R6/2 model appears to display reflex and not limbic epilepsy, it would be important to determine changes in other regions such as the inferior colliculus and the frontal cortex in addition to recordings from limbic areas. One caveat in the Cepeda-Prado et al. study is the CAG repeat length of the mice. While the original R6/2 mouse had ~150–160 CAG repeats, they mice used in these experiments average 251 CAG repeats. In these mice (*just took out a few words to shorten*), as repeat length increases to over 200 because of “anticipation” by breeding down the male line, the phenotype becomes significantly less aggressive [17–19]. Seizure susceptibility in the R6/2 decreases with increased CAG repeat length, especially as repeat length nears 300 [17]. It remains unclear how this change in repeat length will affect these metabolic measures.

Conclusions and Future Perspectives

Cepeda-Prado et al. conclude that rCBV alterations in St, TC, PMC, and PFC can be detected in early symptomatic R6/2 mice. Therefore, rCBV measurements may serve as an invaluable biomarker for detecting preclinical alterations of HD and evaluating effects of therapies in preclinical HD mutation carriers. Although further studies of rCBV in full-length transgenic and knock-in HD animal models, as well as in HD patients are necessary, the current findings provide significant evidence and support for the use of functional changes to track HD phenotypic progression. HD progresses slowly, therefore identifying only one biomarker which can be used to monitor the patient throughout the entire span of the disease may not be optimal. Further longitudinal studies will be required to identify multiple markers to track HD progression during different stages of the disease. For example, additional experiments examining glucose consumption and neurometabolic coupling at multiple time points in HD may be a useful starting point.

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Executive Summary

Summary

- HD brains undergo degeneration in the striatum, as well as **in** a number of extra-striatal regions including the cortex, hippocampus, and hypothalamus.
- fMRI is an important tool in identifying functional/metabolic changes that may appear prior to any detectable structural changes.

Methods and Results

- R6/2 mice exhibit increased CBV in the St, TC, PMC, and PFC **at** as early as 6 weeks of age, as well in the Hc and Th by 14 weeks of age.
- Although no structural changes are observed at 6 weeks, Hc, St, and Neo undergo significant volume loss in the R6/2 by 14 weeks.
- R6/2 mice have increased frequency of LFPs in the TC, lateral EC, Sub, and DG at 6 weeks, as well as synchronized discharges in response to GABA_A receptor blockade resembling seizure activity. This is consistent with increased susceptibility to seizures in these animals.
- Glucose uptake is reduced in the St, PMC, and PFCs (3 of 4 regions showing increased rCBV) in 6-week R6/2 mice.
- Early symptomatic R6/2 mice have altered hemodynamic coupling (*moved*).

Discussion and Significance

- rCBV may serve as a biomarker for detecting preclinical alterations of HD and evaluating effects of preventative therapies.
- Functional alterations can be detected prior to structural ones during the progression of HD.

Conclusion and future perspective

- Further longitudinal studies will be required to identify multiple biomarkers of HD progression.
- Future experiments in full-length **transgenic** and knock-in HD models at multiple time points of HD progression are necessary.