Review

Preliminary examination of early neuroconnectivity features in the R6/1 mouse model of Huntington's disease by ultra-high field diffusion MRI

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Abstract

During the last decades, advances in the understanding of genetic, cellular, and microstructural alterations associated to Huntington's disease (HD) have improved the understanding of this progressive and fatal illness. However, events related to early neuropathological events, neuroinflammation, deterioration of neuronal connectivity and compensatory mechanisms still remain vastly unknown. Ultra-high field diffusion MRI (UHFD-MRI) techniques can contribute to a more comprehensive analysis of the early microstructural changes observed in HD. In addition, it is possible to evaluate if early imaging microstructural parameters might be linked to histological biomarkers. Moreover, qualitative studies analyzing histological complexity in brain areas susceptible to neurodegeneration could provide information on inflammatory events, compensatory increase of neuroconnectivity and mechanisms of brain repair and regeneration. The application of ultra-high field diffusion-MRI technology in animal models, particularly the R6/1 mice (a common preclinical mammalian model of HD), provide the opportunity to analyze alterations in a physiologically intact model of the disease. Although some disparities in volumetric changes across different brain structures between preclinical and clinical models has been documented, further application of different diffusion MRI techniques used in combination like diffusion tensor imaging, and neurite orientation dispersion and density imaging have proved effective in characterizing early parameters associated to alteration in water diffusion exchange within intracellular and extracellular compartments in brain white and grey matter. Thus, the combination of diffusion MRI imaging techniques and more complex neuropathological analysis could accelerate the discovery of new imaging biomarkers and the early diagnosis and neuromonitoring of patients affected with HD.

Key Words: brain repair; diffusion tensor imaging; Huntington's disease; neurite orientation dispersion and density imaging; neuroconnectivity; neuroinflammation; neuroplasticity; neuroregeneration; R6/1 mice; ultra-high field diffusion MRI

Introduction

Huntington's disease (HD), an autosomal dominant genetic neurodegenerative disease (NDD) with adult-onset, is characterized by cognitive impairment (known as chorea) during the early stages. As the disease progresses, these movements become more pronounced with progressive motor impairment, changes in personality, and a decline in cognitive abilities. A less common and more severe form of HD, known as the juvenile form, begins in childhood or adolescence. While there is some understanding of the genetic mutations associated with this disease, a region-specific polyglutamine expansion from the gene coding for the huntingtin protein that leads to aggregates of this mutant huntingtin (mHTT) in neurons, the specific mechanisms leading to a selective pattern of neuronal degeneration, remain vastly unknown. In this review article, we address some connectivity features associated with histological alterations to assess early structural biomarkers in HD.

Preclinical Models of Huntington's Disease

To understand the events taking place in a physiologically intact model that can simulate the human disease, a growing number of preclinical mammalian models have been developed. Although it is clear that the existing murine models do not fully represent HD pathology, they can be used as a fairly reliable approximation. Even further, the creation

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of complex models by the combination of transgenic HD mice and mice expressing fluorescent structural markers has advanced the detection of basic structural changes occurring during the presymptomatic stages of the disease (Gatto et al., 2015). Across the growing list of transgenic animals in the study of HD, the R6 mice represent one of the earliest and widely-used models. Since its establishment, this murine model was well-characterized by several neurological tests and molecular sets of markers. This transgenic model expresses exon 1 of the human HTT gene in a C57 background. Specifically, the transgene expression is driven by the human huntingtin promoter and resulting levels of transgene expression are around 31% of the mouse endogenous huntingtin. Considering the lifespan of murine model R6/2 with large CAG repeats (120 to 160 repeats), and an accelerated rate of disease, the development of the R6/1 mice line, with around 115 polyglutamine (CAG) repeats, more likely resembles the HD adult form (Garcia-Lara et al., 2018). Thus, this feature might make the R6/1 mice an accurate model to understand the molecular mechanisms of adult HD forms, as well as to evaluate new drugs or therapy in the HD research field.

Glia (astrocytes, microglia, and oligodendrocytes) in the early stages of the disease, have been identified in regions specifically related to neuronal degeneration in HD. Thus, the presence of focal reactive astrogliosis, in such selective regions, could be considered a normal biological response in an early altered microenvironment. In turn, as the disease progresses, the microenvironmental remodeling is followed by the release of cytokines accelerating even further HD progression (Rocha et al., 2016).

A hallmark of HD neuronal pathology is the presence of intracellular mHTT aggregates. In addition to the aggregates found in neurons, numerous studies have shown that inclusions are also present in cortical and striatal astrocytes (Khakh et al., 2017). And the presence of mHTT specifically in astrocytes and astrocyte dysfunction has been suggested to directly contribute to HD pathology (Khakh et al., 2017). The evaluation of the dynamic distribution of neuronal and glial populations has traditionally relied on immunohistochemistry. Histochemical methods have become one of the neuropathological gold standards in the evaluation of molecular and biochemical markers in NDDs. With this type of evaluation, we have shown the increase of astrocyte levels in the R6/1 mouse model at initial stages, striking in certain regions such as the hippocampus, as depicted in (**Figure 1A**) particularly associated with cognitive decline (Harris et al., 2019). On a cell level, critical structural properties can be further assessed by histomorphometry methods. As such, the use of qualitative-driven imaging techniques based on the level of cellular complexity (skeletonization and fractal dimension) can result in a richer evaluation of pathological features of NDDs. In that regard, our studies have shown that changes in cellular complexity add a significant level of microstructural information in HD. As an example, we described that astrocytic cells with elaborate branching processes (as assessed by their corresponding skeletonized structures, **Figure 1B**) had large fractal dimensions, particularly in complex deep grey matter (GM) structures, and revealed changes in branching complexity through time in HD mouse models (Gatto et al., 2021).

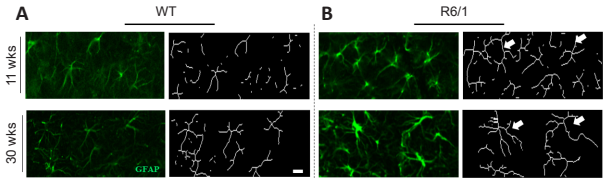

Figure 1 | **Histological evaluation of astrocyte density and complexity.** Astrocytes in the R6/1 HD model (a) and control WT (b) mouse brains. Representative images of 50 µm slices of mouse brains stained against GFAP (in green) to show astrocytic presence at the hilus of the hippocampus at 11 weeks (top) or 30 weeks of age (bottom). Corresponding skeletonized astrocytes are shown in black and white images next to their GFAP image. The images registered, were processed using Image J to obtain the skeletonized profiles of astrocytes (For methods, see Gatto et al., 2021). Notice the increase in cell complexity in the R6/1 mouse (arrows) compared to the WT mouse. Notice the increased branching in time (10 weeks *vs*. 30 weeks) that can be further processed and quantified by fractional dimension analysis. Scale bar: 10 µm. GFAP: Glial fibrillary acidic protein; R6/1: a transgenic mouse model of HD; WT: wild type mouse.

Imaging Technologies for Huntington's Disease

The association between molecular and cellular alterations with brain atrophy remains under investigation in many preclinical models of HD. To this end, brain atrophy through longitudinal *ex vivo* MRI studies in the R6/1 mouse line, as well as a histological assessment were performed by our group (Gatto et al., 2021). Although clinical changes in volumes in the caudate (Cau) and putamen (Pt) areas represent helpful early markers in the HD patient population (Domínguez et al., 2016), our measurements of the R6/1's striatum volumes (mouse brain structure equivalent to the Cau and Pt in humans) did not show a significant change compared to control mice, highlighting the importance of parameters derived from diffusion MRI (dMRI) (Gatto et al., 2021).

As such, the use of dMRI has been proven ideal to study microstructural events related to murine models of HD and the neuropathological exploration of intact models of HD (Gatto and Weissmann, 2019). The spatial evaluation and connectivity quantification between distant brain regions bring a new level of understanding of global neurodegenerative features in HD. The architecture of the axons in parallel bundles and their myelin shield facilitate the diffusion of water molecules along their main direction. If we apply diffusion gradients in at least six non-collinear directions, it is possible to calculate, for each pixel, a diffusion tensor and the average of fiber's direction, indicated by the tensor's main eigenvector and color-coded vectors, yielding cartography of the tracts' position as well as the direction. Moreover, using complex calculations from diffusivity parameters, diffusion tensor imaging (DTI) can estimate fiber organization, and quantify fractional anisotropy parameters. During the last decades, the steady development of ultra-high field diffusion MRI (UHF-dMRI) and coil design have improved the imaging resolution to capture architectural changes in brain tissue with higher detail (Gatto et al., 2018). Although such DTI studies were only limited to the study of white matter (WM) fractional anisotropy in the corpus callosum, *ex vivo* studies by our team on R6/1 brain samples conducted on a UHF-dMRI (16.7T) setup were able to evaluate higher macro details in brain tissue. Adjusting the characteristics of the magnetic field and gradient strengths, and with bioimaging techniques, we have monitored specific parameters during the early stages of the diseases (Gatto et al., 2021). In particular to the investigation of microstructural alterations, UHF-MRI represents an ideal method to quantify dynamic water redistribution across different tissue compartments, as described in the context of

HD and other NDDs (Garcia-Lara et al., 2018; Gao et al., 2020).

Researchers (Rattray et al., 2013) found a progressive decline in both motor and non-motor related behavioral tasks in presymptomatic R6/1 mice (11 weeks of age). Measuring regional brain volumes, the authors found significant GM atrophy by 17 weeks of age. Moreover, age-related brain volume loss was validated using a semi-automated morphometry assessment, mHTT inclusions were found to be widely distributed throughout the R6/1 brain tissue. Despite R6/1 mice exhibiting substantial brain atrophy, visualized through MRI, and having a robust pathological phenotype, changes in GM neuronal density and cortical neuronal counts did not show a strong correlation between the two phenomena (Rattray et al., 2013). Although it remains unclear what the underlying mechanisms driving brain volume loss and behavioral disturbances are, mechanisms of retrograde axonal injury (dying-back) have been proposed as a plausible explanation for the early loss in neuronal connectivity.

Intrinsic tensor properties in DTI techniques, at large, are not able to fully detect all the information associated with GM changes (Gatto et al., 2020). Nevertheless, some of the early neuroinflammatory and infiltrative cellular elements associated with the neurodegenerative process increase the tissue complexity features, and amenable to be captured by non-Gaussian dMRI techniques (Gatto et al., 2019). The ability of dMRI to detect water diffusion directionality has been one of the pillars in the development of imaging techniques that can grossly represent WM tracts. Fiber reconstruction from brain structures centered in vulnerable brain structures has demonstrated disruption of WM tracts, i.e., connectivity, during early HD stages in R6/1 mice (Gatto et al., 2021). Also, this technique provides the means to assess the macro-scale architecture of brain connectivity, characterized as the anatomical link between different brain regions. While network-based analyses conducted so far offered mixed results which are not easily interpreted in the light of clinical outcomes, current diffusion MRI studies seem to be the initial point towards the characterization of a reduction in the network of the HD patients' connectome. Thus, this technique can easily assess the disruption of long-range WM tracts and the disconnection and reconnection of main hubs and nodes from a default network mode.

Genetic defects determine a progressive failure of the cellular structural integrity and potentially trigger brain connectivity failure in the context of HD. The inflammatory response could determine, partly, the loss of axonal connectivity. In particular, astrocytes also promote synapse formation, influence synapse stability, and help refine neural connectivity; neuronal structural plasticity and spine dynamics are also regulated by astrocytes (Fields et al., 2015). The tractography analysis of superficial and deep grey matter (GM) structures in the R6/1 brain revealed a pattern of local and broader alterations of connectivity (**Figure 2A**). Starting in early presymptomatic stages, a progressive and intrinsic reduction of connectivity within GM structures (1–2 mm fiber length) seems to be the scenario of complex microstructural changes, analyzed traditionally in WM fibers (up to 30 mm). Interestingly, our connectomics analysis seems to evidence the importance of considering not only the overall number of axons in these interconnecting fascicles (strength of these connections) but also the number of interconnecting nodes, which showed a marked increase in our studies (**Figure 2B**). Such topographic rearrangements in susceptible regions of the HD brain (particularly noticed in larger WM fibers of up to 50 mm) showed changes in the number and redistribution of such connections during the presymptomatic and symptomatic stages of the disease (**Figure 2C**). Altogether, the integration of these imaging results not only point towards the dynamic adaptative nature (neuroregenerative and neuroplastic properties) of the brain but also give new hints to characterize this phenomenon and its potential clinical application using early neuroimaging biomarkers. As an example, combined biochemical and imaging studies such as MRI spectroscopy have shown chemical changes in the basal ganglia regions (putamen) of HD patients, thus, potentially useful in clinical practice (Padowski et al., 2014; van den Bogaard et al., 2014).

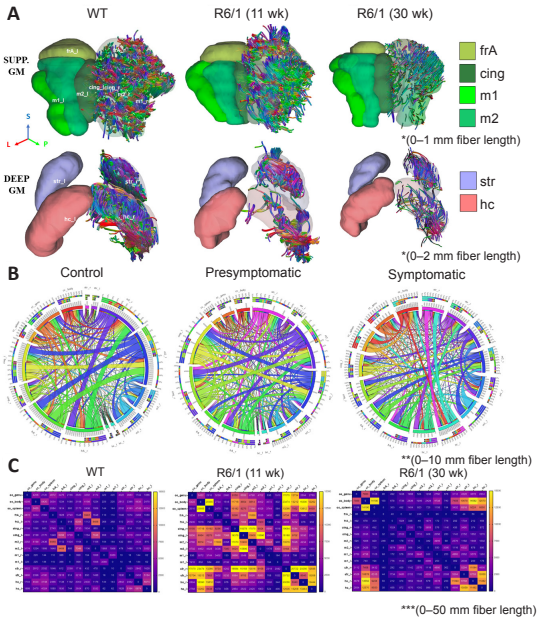

Figure 2 | Evaluation of early changes in neuronal connectivity in cortical grey matter regions of the R6/1 mouse.
(A) Evaluation of intrinsic fiber tracts in wild-type (WT) mouse brain (left), R6/1 mouse at 11 weeks (presymptomatic, middle), and R6/1 mouse at 30 weeks (symptomatic stage, right). Note an increasing number of tracts in the presymptomatic stage. (B) Circos connectivity diagrams representing local fiber tracts in each segmented GM region of interest (ROIs). Note that the thickness of each ribbon, which evaluates the strength of the association between each ROI, decreases as the disease progresses. However, the number of nodes seems to compensate for the loss in connectivity during the early stages of the disease. (C) Connectivity network matrix measurements are represented by heat maps accounting for the number of fibers connecting at regional brain network levels. Note the increasing amount of connectivity in cortical regions, particularly at the early presymptomatic stage of the disease and the redistribution at later (symptomatic) stages in the R6/1 mice. The information displayed is preliminary (n = 1). Further studies, including more animals and statistical analysis, are intended in future work. Method: A multi-shell diffusion scheme was used for tractography reconstructions, with b values of 1000, 2500, and 5000 s/mm^2. Twenty gradient directions were used. The in-plane resolution was 0.15 mm. The b-table was checked by an automatic quality control routine to ensure its accuracy. The diffusion tensor was calculated, and a deterministic fiber tracking algorithm was used. A seeding region was placed at the whole brain. The ROI was placed in the striatum region with a volume size of 5 mm^3. The anisotropy threshold was 0.0960938. The angular threshold was 90 degrees. The step size was randomly selected from 0.5 to 1.5 voxels. The fiber trajectories were smoothed by averaging the propagation direction with a percentage of the previous direction. The percentage was randomly selected from 0% to 95%. Tracks with a length shorter than 0 or longer than 20 mm were discarded. Manually segmented ROIs were used for brain parcellation, and the connectivity matrix was calculated by using the count of the connecting tracks. The connectivity matrix and graph theoretical analysis was conducted using DSI Studio (http://dsi-studio.labsolver.org). Colored vector showing directions S (superior, blue); L (lateral, red); P (posterior, green). cing: Cingular cortex; frAc: fractional accessory area; hc: hippocampus; l_: left; m1: primary motor area; m2: supplementary motor area; R6/1, a transgenic mouse model of Huntington's disease; r_: right; str: striatum; Supp: superficial; ts: threshold; wk: week; WT: wild type.

The combination of imaging and animal models is ideal to test new therapeutic approaches. As an example, studies testing therapeutic agents in HD, such as Tetrabenazine and Dantrolene, alleviated the motor deficits and reduced the striatal neuronal loss in a yeast artificial chromosome transgenic mouse model of HD (YAC128 mice) (Wang et al., 2010; Chen et al., 2011). Molecular compounds have been tried to promote neurogenesis in the R6 mice models (Gil and Rego, 2009). Experiments with subcutaneous administration of fibroblast growth factor 2 showed cell proliferation in the subventricular zone, enhancing cell migration and replenishing medium spiny neurons in the striatum (Jin et al., 2005). A study by Cho et al. (2007) found similar effects in the subependymal subventricular zone with an intraventricular administration of adenoviral brain-derived neurotrophic factor and restoration of motor function in the R6/2 mice. In the case of the R6/1 mice, experimental therapeutic approaches to enhance neuro connectivity have been limited and need further testing (Li et al., 2005).

Conclusions

In this review, we demonstrated that information gathered from UHFD-dMRI and imaging diffusion techniques applied to preclinical animal models of HD can be a useful tool to unveil alterations in axonal connectivity, immunoinflammatory, and regenerative events (Liang et al., 2016). Future work will focus on the implementation of more accurate diffusion techniques to continue the novel characterization of the brain that will potentially lead to early bioimaging markers in HD.

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