Phenotypic severity of primary distorsion dystonia is uncovered with diffusion tensor imaging in brains of mouse models

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Introduction

Primary torsion dystonia (PTD) is an autosomal-dominant inherited neurodegenerative disease and manifests as abnormal, involuntary twisting movements reflecting selective dysfunction of CNS motor circuits (1). Transgenic mouse models for PTD have focused exclusively on the pathophysiology of the GAG deletion in the torsinA gene while none of the models did address molecular and cellular changes caused by human wild-type torsinA overexpression (hWT). However, overexpression of the hWT torsinA protein might also be a detrimental factor for the neuronal cell. TorsinA is a peripheral membrane protein and is involved in trafficking of membranes and organelles and vesicle transport (2). This means not solely morphological alterations but also brain function is abnormal in PTD. Elucidation of this issue requires the application of in vivo imaging techniques complementary to immunohistochemical staining and neurochemical analysis. We approached this with in vivo diffusion tensor experiments (DTI). Based on the restricted manner in which water diffuses, DTI enables to uncover the microstructural architecture by analyzing a quantitative parameter e.g. the fractional anisotropy (FA).

Material and methods

In this study we used hWT torsinA mice (n=5), hΔGAG torsinA mice (n=5) and age matched (5 months) nontransgenic littermates (n=5). All mice were anaesthetized using isoflurane (Forene®) and refined monitoring systems maintained the physiological parameters within strict boundaries. All MR experiments were performed on a 7Tesla Bruker Pharmascan (Ettlingen, Germany). The imaging protocol included a conventional multi-slice spin echo imaging sequence modified by adding a Stejskal-Tanner diffusion sensitizing gradient pairs. The sequence was modified for a multi-shot echo planar acquisition with diffusion sensitizing gradients along 30 different directions. Images were collected to cover the whole brain (15 axial 1mm slices). Total acquisition time for each subject was 40 minutes and in plane resolution after reconstruction was (136 * 136) μm^2. On a pixel-by-pixel basis, the FA was derived using software written in Matlab (MathWorks, Natick, MA, USA). Anatomy-based ROI analysis was performed on selected grey matter structures and white matter tracts. Color coded maps were displayed simultaneously and contain two types of information obtained from DTI; image intensity reflects diffusion anisotropy and fiber orientation is represented by color.

Results and Discussion

The (right) figure underneath shows that ROI based calculation of FA in structures of the motor circuit have significant increased FA in the hΔGAG torsinA mice for the corpus striatum (cs), cerebellum (cb) and motor cortex (mt). An increase in FA was already demonstrated for the putamen in human patients (3). The increased FA corresponds with microscopical findings showing that nigral dopaminergic neurons were larger and more closely spaced hΔGAG torsinA mice. The striatal pathology seems more restricted to the more posterior part at Bregma +0.5mm and -0.5mm. Disruption of striatal connection is associated with loss of dopaminergic nigrostriatal neurons as reflected by the decreased dopamine levels demonstrated by complementory neurochemical analysis (data not shown). FA for the hWT torsinA mice is perfectly normal within the striatum but shows an increasing trend in the cerebellum and motor cortex which matches the observed motor behaviour abnormalities.

How the changes in FA parallel the pathological neural changes is of primary concern for further studies and identification of tracts using the color maps and delineating fibre bundles that trace the trajectory of the vectors of water diffusion could help. A new scanning protocol with a sufficiently high resolution for fibre tracking has been developed and will be used use to focus on specific pathways of dopamine and/or serotonin which have been postulated as being involved by the obtained neurochemical data.

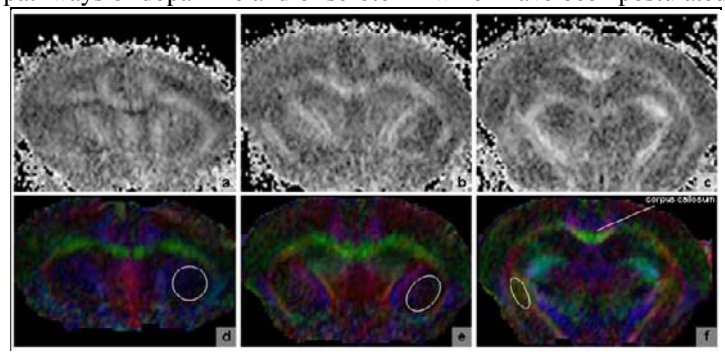
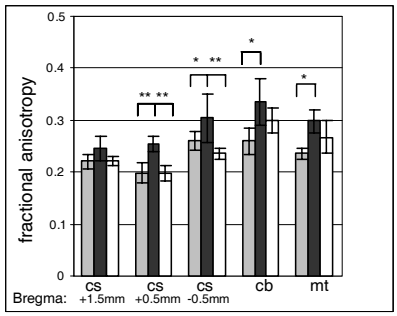


Illustration of a hΔGAG torsinA mouse brain map for 3 consecutive slices representing FA values (a-c) and color coded maps (d-f) representing the fiber orientation and thus axon preservation in major white matter structures such as the corpus callosum. ROI based analysis of the striatum in consecutive slices is illustrated on the color coded maps.



*Mean FA values of the striatum of hΔGAG torsinA mice ■ are compared with mean FA values of hWTtorsinA □ and of nontransgenics ▩ The same was done for an ROI in the cerebellum and the motor cortex (Mann-Whitney: * p<0.05, ** p>0.01).*

1.Ozelius,Nat.Gen.1997
2.Neuwald, Genome Res. 1999
3.Rostasy, Neurobiol Dis. 2003)