Evidence of early cerebellar dysfunction in presymptomatic Parkinson’s disease: Data from quantitative anisotropy using magnetic resonance imaging, mitochondrial biochemistry and gene expression in PINK1 knock-out rat

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Introduction

Genetic models of Parkinson’s disease (PD) that recapitulate many of the neurobiological and behavioral aspects of disease progression are highly desired as we look to identify early biomarkers of PD long before the symptomatology. With the hope of identifying early biomarkers prior to any signs of PD we investigated the PINK1 (PTEN-induced putative kinase 1) knock-out (KO) rat. PINK1 is a mitochondrial protein kinase involved in protecting neurons from stress-induced mitochondrial dysfunction. Mutation in the PINK1 gene is a leading risk factor in familial PD. The behavioral onset of motor dysfunction in the PINK1 KO rat is well characterized with changes occurring by post-natal weeks 1-20. In this study, we combined analytical methods using a 3D segmented and annotated MRI rat atlas with quantitative anisotropy to identify sites of gray matter injury in both brain areas in type (WT) and PINK KO. These MRI studies were conducted between weeks 12-15 almost a month earlier than any signs of motor dysfunction.

Materials & Methods

Experimental Design

Wild type (WT) (n = 20) and PINK1 KO (n = 20) rats were obtained from Sage Labs. The imaging period and histopathological analysis were confined to a single set of 12-15 events in each behavioral. This protocol presents with no significant changes in motor activity or change in physiological levels in the rat or in controls.

Pink1 Knockout Phenotypic characterization of recessive gene knockout rat models of Parkinson’s disease

Main Results

Diffusion Tensor Imaging with Quantitative Anisotropy

Apparent Diffusion Coefficient (ADC) Map

Wild Type vs PINK1 KO

Diffusion Tensor Imaging

Diffusion Tensor Imaging can lead to significant image motion artifacts that are apparent only in the slices sampled by collinear slices. The imaging period was acquired with a diffusion-echo echo-planar imaging (EPI) pulse sequence having a planar imaging (EPI) pulse sequence having a planar direction of 0.313 x 0.313 mm and with in-plane resolution 15 x 15 x 15 mm. The imaging protocol was repeated twice for signal averaging. A 3D acquisition was performed prior to the analysis, following the elimination of acquisition points with motion artifacts. The remaining acquisition points were corrected for motion (in-plane and global deformation) susceptibility/variation using ANIMAL (animal Trust Center for Neuroimagining, London, UK).

Indices of Anisotropy (IA)

- Fractional Anisotropy (FA)
- Apparent Diffusion Coefficient (ADC)
- Lambda 1
- Lambda 2
- Lambda 3
- Radial Diffusion (RD)

mRNA levels of genes involved in Oxidative Stress and Mitochondrial Dysfunction

qPCR mRNA of Stress Related Genes

- NSE
- BAX
- BCL-2
- HSP 70

qPCR Mitochondrial DNAEncoded mRNA

- COX 1
- COX 2
- ND1
- ND5
- ND6

Molecular Markers of Oxidative Stress

ATP Levels

Glutathione Levels

Summary

- Using quantitative anisotropy we identified the cerebellum and the deep cerebellar nuclei as a potential area to be vulnerable to change in early Parkinson’s.
- In pilot studies (data not shown) the whole cerebellum was analyzed for markers of redox stress and proved to be significantly different from control cerebellum.
- In a subsequent study, gross segmentation of the brain of wild-type and PINK KO rats corroborated the early study but included mesencephalic dopaminergic system, forebrain and brainstem.
- Consistent with the previous results, cerebellum showed significant changes in ATP levels, GSH levels, oxidative damage and DNA methylation levels.
- Genes associated redox stress and mitochondrial dysfunction were altered.
- Similar changes were also observed in other brain regions including deep cerebellar nuclei and striatum.
- Some of these changes correlated with the changes in substantia nigra (SN) / VTA; however, the changes in cerebellum and cerebellar nuclei were more pronounced as compared to the SN / VTA.
- Interestingly, no changes were observed in prefrontal cortex and brainstem, particularly since the latter showed alterations in anisotropy.

Speculation

- A new methodology referred to as “in vivo neuromapping” (Kulkarni et al., Planen-2015) was used to identify the cerebellum as being vulnerable in the PINK1 KO model of Parkinson’s.
- The cerebellum has not been implicated in the pathophysiology of PD. No lesions, Lewy bodies or alpha synuclein aggregates.
- While there were also changes occurring in the mesencephalic DA system could the changes in the cerebellum be antecedent to any dopaminergic neuropathology?
- If so, could changes in the cerebellum contribute to the onset of PD?
- Could imaging of the cerebellum be used to identify biomarkers of presymptomatic PD?

We are presently using immunohistochemistry to explore the molecular changes occurring at the cerebellum and midbrain DA system in these PINK1KO rats.

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