Diffusion kurtosis imaging detects microstructural alterations in brain of α-synuclein overexpressing transgenic mouse model of Parkinson's disease: a pilot study

Peter Latta[1], Amit Khairnar[1], Eva Drazanova[2], Jana Kucerova[1], Anas Arab[1], Birgit Hutter-Paier[3], Daniel Havas[3], Manfred Windisch[4], Zenon Starcuk Jr. [2], Boguslaw Tomanek[1,5], and Irena Rektorova[1]

[1]*Central European Institute of Technology, Masaryk University, Brno, Czech Republic,* [2]*Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno, Czech Republic,* [3]*QPS Austria GmbH, Graz, Austria,* [4]*NeuroScios GmbH, Graz, Austria,* [5]*University of Alberta, Edmonton, Alberta, Canada*

Purpose: The development of "neuroprotective" treatments is a central challenge for future Parkinson's Disease (PD) therapy. To evaluate efficacy and to monitor disease-modifying effects, reliable surrogate markers for early PD diagnosis and progression are needed when patients might still be eligible for neuroprotective treatment[1]. Diffusion kurtosis imaging (DKI) is an extension of conventional diffusion tensor imaging (DTI) and it incorporates non-Gaussian water diffusion behavior in neural tissues. DKI allows non-invasive *in vivo* assessment of tissue microstructure by mapping water proton motion within the tissue microenvironment[2]. The aim of our study was to evaluate whether diffusion imaging as assessed by DKI metrics provides a sensitive tool for differentiating between transgenic mice over-expressing human α-synuclein (TNWT-61) and wild-type (WT) littermates.

Methods: 9 month old male transgenic mice over-expressing human wild-type α-synuclein under the murine Thy-1 promoter (TNWT-61) (n=7) and WT (n=7) were provided by QPS Austria Neuropharmacology. DKI data were obtained with a Bruker Avance 9.4T MRI system equipped with a gradient system with strength up to 660 mT/m. Mice were anaesthetized using isoflurane inhalation (1.5–2%) and monitored to maintain constant physiological parameters. Fast low angle shot (FLASH) scout images were used to localize the mouse brain. For the DKI acquisition, diffusion-weighted images were acquired with two-shot spin-echo planar imaging (SE-EPI). Respiratory gating was used to prevent motion artifacts. The generalized auto calibrating partially parallel acquisitions (GRAPPA) with acceleration factor 2 was used to improve image quality and diminish susceptibility-caused artifacts. The DKI protocol included six b-values (b=0, 500, 1000, 1500, 2000, and 2500 s/mm^2 (with δ=4 ms and Δ=11 ms) and 30 non-collinear directions. The SE-EPI sequence was applied with the following parameters: FOV=24×24mm, acquisition matrix=98×128, echo time TE=25ms, 300 kHz bandwidth and TR ~5 sec depending on respiratory rate, for a total acquisition time of approximately 1 hour 40 min. The DKI data were processed and calculated on a voxel-by-voxel basis to produce parametric maps (MD, AD, RD, FA, MK, AK, RK) using software called a Diffusion Kurtosis Estimator[3]. The conventional DTI parametric maps were calculated from a subset of DW images using two b-values (b=0 and 1000 s/mm^2). Averaged diffusion, fractional anisotropy, and kurtosis were obtained from multiple regions: SN (1 slice), striatum (average of 5 slices), sensorimotor cortex (average of 5 slices), hippocampus (average of 3 slices) and thalamus (average of 2 slices). The region of interest (ROI) selection on b=0 images was drawn manually according to the Paxinos Mouse Brain Atlas[4] with the help of fractional anisotropy maps using ImageJ® software for the various brain regions as shown in the **Fig. 1**.

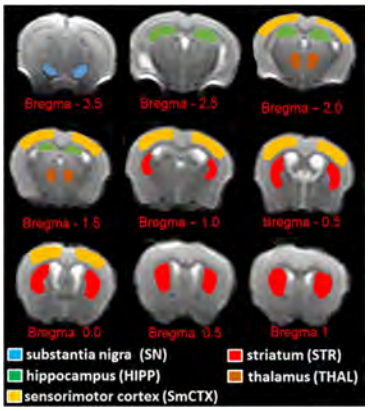

Fig. 1. Illustration of the region of interest (ROI) delineation.

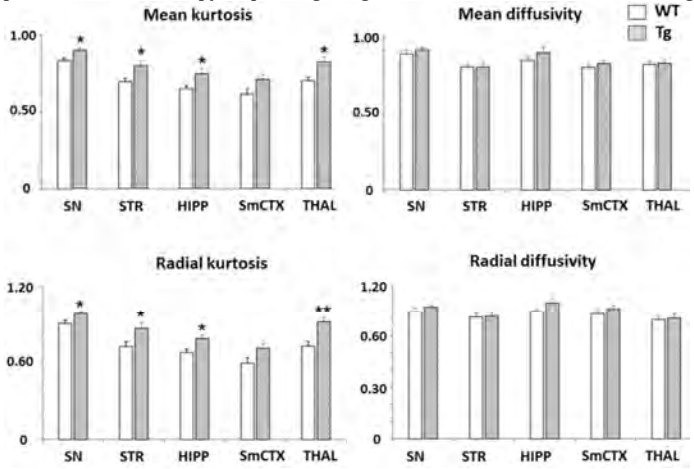

Fig. 2. Comparison of DKI and DTI derived parameters for the wild-type mice (WT) and wild-type (WT) mice. The ROI selection is described in Fig.2. Kurtosis and fractional anisotropy are dimensionless units, diffusivity values are given in x10^{-3} mm^2/s. Data are expressed as mean values +SEM, n=5 for WT and n=6 for Tg, *p < 0.05, **p < 0.01.

Behavioral study: The following tests were performed in order to evaluate motor deficits: Challenging Beam Traversal Test, Pole test, Grid test[5]and Beam Walk test[6].

Results: TNWT-61 transgenic mice showed significant differences in DKI parameters as compared to the WT littermates. Specifically, the MK and RK values were significantly elevated in the TNWT-61 group in substantia nigra (p=0.021 and 0.048, respectively), striatum (p=0.036 and 0.047), hippocampus (p=0.048 and 0.032), and thalamus (p=0.025 and 0.009); see **Fig 2**. TNWT-61 transgenic mice showed no significant differences with conventional DTI parameters (MD, AD, RD and FA) as compared to the WT littermates. We have evaluated the motor performance in TNWT-61 (Tg, n=7) and wild-type (WT, n=7) mice and confirmed significant impairments in the TNWT-61 model.

Discussion: This current study demonstrate, for the first time in a PD mouse model, a significant increase in DKI parameters (MK and RK) but not in the conventional DTI parameters (FA, MD, AD, RD) in the SN, striatum, thalamus, and hippocampus of TNWT-61 mice as compared to the WT littermates. We also found characteristic motor coordination deficits in the 9 month old TNWT-61 mice which is in line with previous studies and supports the dysfunction of the nigrostriatal system. Our findings accord with the hypothesis that the pathology induced by α-synuclein accumulation may result in both behavioral and microstructural gray matter changes that induce a decrease in free diffusion of water and, hence are expressed by an increase in kurtosis metrics. However, the DKI changes in TNWT-61 mice may not be specific for the α-synuclein accumulation and could also reflect gray matter changes caused by other brain pathologies reported previously in this PD model, such as iron accumulation or glial cell activation[7,8]. In this pilot study we also addressed the issue of sensitivity of the two DWI (DTI and DKI) methods that have been available and explored for diagnostic purposes in human PD studies with so far inconclusive results. DKI provided an added advantage over DTI by providing information about non-Gaussian diffusion of water and better characterization of microstructural changes in gray matter.

Conclusion: Our results demonstrate that unlike DTI, DKI is sensitive enough to detect gray matter pathology in the TNWT-61 model of PD, and hence it may improve diagnosis of PD. This is an important step forward, showing that DKI provides more diagnostically relevant data than routine DTI thus it could be advised to be applied despite a longer acquisition protocol.

References: 1) Stocchi F et al. Mov Disord. 2013;28:3-7. 2) Jensen JH et al. NMR in biomedicine. 2010;23:698-710 3) Tabesh A et al. Magn Reson Med. 2011;65:823-36. 4) Paxinos G et al. 2nd ed. San Diego: Academic Press; 2001. 5) Fleming SM et al. J Neurosci. 2004;24:9434-40 6) Suidan GL et al. PloS one. 2013;8:e59032 7) Chesselet MF et al. Neurotherapeutics. 2012;9:297-314 8) Wang JJ et al. Radiology. 2011;261:210-7

Acknowledgement: This work was supported by the projects - CZ.1.07/2.3.00/30.0009, CZ.1.05/1.1.00/02.0068, MEYS CR (LO1212), CZ62760225, CZ.1.05/2.1.00/01.0017 and RVO:68081731.