Chapter 18

Validation of diffusion kurtosis imaging as an early stage biomarker of Parkinson's disease in animal models

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Abstract

Diffusion kurtosis imaging (DKI), which is a mathematical extension of diffusion tensor imaging (DTI), assesses non-gaussian water diffusion in the brain. DKI proved to be effective in diagnosis of different neurodegenerative disorders. Its sensitively detects microstructural changes in the brain induced by either protein accumulation, glial cell activation or neurodegeneration as observed in mouse models of Parkinson's disease. We used two experimental models of Parkinson's disease to validate the diagnostic utility of DKI in early and late stage of disease pathology. We present two DKI analysis methods: 1) tract based spatial statistics (TBSS), which is a hypothesis independent data driven approach intended to evaluate white matter changes; and 2) region of interest (ROI) based analysis based on hypothesis of ROIs relevant for Parkinson's disease, which is specifically used for gray matter changes. The main aim of this chapter is to provide detailed information of how to perform the DKI imaging acquisition and analysis in the mouse brain, which can be to some extent translated to humans.

Keywords (5-10 key words): Diffusion kurtosis imaging; Diffusion tensor imaging; Magnetic resonance imaging; Methamphetamine; Microstructural changes; Neurodegeneration; α-Synuclein; TNWT-61 Mice.

1. Introduction

Neurodegenerative disorders, such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD) and amyotrophic lateral sclerosis (ALS), represent a major challenge for patients, their families and healthcare providers due to longer life expectancy and aging of population. According to the epidemiological study published in Lancet Neurology in 2017, it has been reported that approximately 46 million people are living with Alzheimer's disease or other dementias, and more than 6 million people are affected by Parkinson's disease worldwide (1). It has been predicted that the number of the people affected with neurodegenerative disorders will double in next 20 years (2). The economic burden is high, e.g. in United States the annual cost of neurodegenerative disorders is around $ 800 billion (3), whereas in Europe it is around € 800 billion per year (https://ec.europa.eu/).

The currently available pharmacotherapies are symptomatic rather than curative, and they do not have a significant impact on the inevitable progression of the disease. Recent advances in understanding of the disease pathology will hopefully allow development of disease modifying treatment strategies, which may slow down or even halt the progression of the disease. However, such treatments will likely be effective only when the disease is diagnosed at an early stage (4). Unfortunately, so far, the neuroprotective agents promising at pre-clinical level failed to show efficacy in clinical studies (5). Early diagnosis should help identification of patient populations eligible for novel disease modifying therapy (6). Since there is no single clinical, biochemical or neuroimaging biomarker that allows exact diagnosis at early stage of the particular neurodegenerative disease, it makes clinical development of disease modifying therapies very challenging.

Although neuroimaging biomarkers are available, they do show some intrinsic limitations in early diagnosis or differentiating different neurodegenerative disorders. In the case of Alzheimer's disease post mortem analysis of brain tissue can only provide definitive diagnosis of AD, while clinical diagnosis of AD is usually made using volumetric magnetic resonance imaging (MRI), which detects the cortical atrophy in the brain (7). Although volumetric MRI can be used for tracking the progression of brain atrophy, it is of little significance for AD patients without atrophy, where it has low predictive value (8). Besides volumetric MRI, the FDG-PET (fluorodeoxyglucose positron emission tomography) imaging has been used for decades to assess the alterations in brain glucose metabolism in patients suffering mild cognitive impairment or AD, who show hypometabolism of glucose (9). Although hypometabolism of glucose has been reported in aging too, there are specific brain regions (e.g. temporal lobe, posterior cingulate cortex), which show very small changes with aging. These regions are severely affected in AD; therefore, the FDG-PET technique is able to capture the changes in this region sensitively (9). Recently detection of amyloid accumulation was made possible with PET imaging; however, there is a major limitation of this approach in understanding relationship of amyloid burden with cognition (9). Though neuroimaging techniques are becoming widely available, they do have limitations with respect to early diagnosis of AD.

In the case of PD, clinical diagnosis is fully based on clinical scoring of physical symptoms and psychological and cognitive status of the patient (10). Early diagnosis of PD is even more challenging because in the initial disease stage it is difficult to differentiate PD from other parkinsonian syndromes, such as multiple system atrophy or progressive supranuclear palsy. Though conventional structural and volumetric MRI approaches exist, they are not regularly used since they show changes only at the very late stage of the pathology (11). Imaging with a radiotracer such as single photon emission computed tomography using the dopamine transporter ligand [^{123}I] FP-CIT (DaTscan) was found to be effective in the early diagnosis of PD (12). However, physicians still have to rely on clinical diagnosis as it shows false a positive scan in some cases (13).

2. Diffusion weighted imaging techniques and their translational validity in Parkinson's disease

Human brain consists of 70% of water, therefore motion of water molecules within the brain can provide important insight into the tissue's microstructure. Diffusion of water is associated with structural organization of brain. There are several diffusion weighted imaging (DWI) MRI techniques. Diffusion tensor imaging (DTI) was developed to assess the motion of water molecules in tissue microstructure non-invasively (14, 15). DTI is sensitive for detecting the directional diffusion of water molecules. Most importantly, diffusion of water in the white matter is typified by strong directionality. In the white matter the water diffusion is not restricted parallel to axonal orientation, while it is largely restricted in the perpendicular direction. There are two main metrics in DTI, one is diffusivity, which measures the magnitude of water diffusion; the other is fractional anisotropy (FA) which is a measure of the directionality of diffusion. Diffusivity can provide a measure of axial or parallel diffusion (AD), radial or perpendicular diffusion (RD) or mean diffusion of AD and RD. Furthermore, FA provides information about directionality of water diffusion in living tissue (16).

Studies with DTI on neurodegenerative disorders have shown potential utility in early detection of white matter changes. Both clinical and pre-clinical studies on neurotoxin-based animal models of PD showed decrease in FA and increase in diffusivity metrics due to neuronal loss in the soma of substantia nigra (SN) ((14, 17-20). Olfactory dysfunction, which is one of the non-motor symptoms in PD, develops decades before clinical diagnosis and might be useful for early PD studies (21). Importantly, one DTI study found a decrease in FA in the olfactory system at an early stage of PD (22). Conversely, few studies have also reported increase in FA in SN (23, 24). A DTI imaging by Guimarães et al. on early PD, moderate PD and severe PD patients was able to find the significant DTI metric changes only in severe conditions, thus questioning the sensitivity of DTI in early diagnosis of PD (25). Of note, three meta-analyses have been published on DTI in PD patients, two of them asserting the results of studies with DTI are encouraging and it can be a promising biomarker for PD (19, 26), whereas the other one questioned the validity of DTI as an imaging biomarker in PD (27). This controversy may arise from sensitivity of DTI in white matter compared to gray matter. While gray matter allows a relatively isotropic water diffusion, white matter represents a highly

anisotropic environment. Thus, sensitivity of DTI in detecting gray matter microstructural changes is probably limited, while it allows a good assessment of the pathological changes in white matter. The major limitation of DTI lies in the fact that it considers the diffusion of water molecules in the brain as Gaussian, which infer the diffusion of water in the brain is free and unrestricted like water in a bucket. Considering the real structural complexity of the brain, it is merely an approximation and it can be expected that significant diffusivity changes may exist among tissue compartments (28; 29).

To overcome this limitation, diffusion kurtosis imaging (DKI) which is a mathematical extension of DTI was developed. B value is a component that deciphers the strength and timing of the gradient which is used to generate diffusion images and in the case of DTI only 2 b values are used (16). However, DKI uses more than 2 b values to understand inherently non-Gaussian water diffusion in the neural tissue (28, 30). DKI considers the water diffusion to be non-restricted, and it measures the diffusional hindrance arising from microstructure such as tissue compartments, cell membrane and cell organelle. Hence, this technique is sensitive in detecting the restriction of water diffusion in both isotropic and anisotropic environments (16). DKI metrics are mean kurtosis (MK), axial kurtosis (AK) and radial kurtosis (RK). These provide ancillary information in addition to DTI metrics such as FA, MD, AD and RD and give detailed knowledge about the microstructural characteristics of the tissue. Increase in kurtosis values suggest higher tissue heterogeneity or substantial hindrance to the water diffusion. This might be due to protein accumulation, glial cell activation or other pathology in the brain (16, 28, 30, 31). While the validity of DTI is limited to white matter, the information from gray matter is largely controversial (32, 33). On the other hand, DKI is sensitive in both white matter as well as gray matter (30).

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In the case of Parkinson’s disease, it was Wang et al. who proposed the importance of DKI as a diagnostic imaging biomarker in Parkinson’s disease. Though in their studies he found increases in both kurtosis and FA in SN, MK showed the best diagnostic performance in ipsilateral SN (24). Similarly, Kamagata et al. found an increase in MK and FA in cingulate fibers. Moreover, the receiver operating characteristic (ROC) curve analysis revealed that sensitivity of MK was higher than FA in this particular region (34). To support the diagnostic utility of MK in early PD patients, Zhang et al. performed both DTI and DKI in SN and found increase in MK, which was correlated with Hoehn-Yahr (H-Y) staging and UPDRS III staging (35). UPDRS (Unified Parkinson’s Disease Rating Scale) evaluates the key areas of disability in PD divided in three subscales, while fourth subscale evaluates any complication to treatment. It is often used with Hoehn and Yahr disease rating scale (36). At UPDRS III staging the PD patients start feeling rigidity, tremor at rest and change of facial expression which was not present in previous stages. A recent study on DKI in PD patients which was a part of Swedish BioFINDER study also proved the sensitivity of DKI in PD patients (37).

Though there are several clinical studies of DKI with PD patients (24, 34, 37) currently, the exact origin of changes in kurtosis metrics are not yet understood. Because, histological evaluations of human brains are limited to post mortem studies, the DKI experiments using animal models of PD are

required to shed more light into the pathophysiological mechanisms responsible for changes in the kurtosis signal. If we validate DKI and explain the source of kurtosis signal in animal models, we will be able to contribute to early diagnosis of PD, when the neuroprotective treatment strategies will likely be useful.

3. Materials

The following text summarizes and explains in detail our methodological approaches and selection of materials and animal models used in our research. Where applicable, we aim to outline additional studies, identify knowledge gaps and point out potential pitfalls.

3.1 Laboratory animals and selection of a suitable model

Animal models are an important tool to study pathogenic mechanisms and therapeutic strategies and to develop imaging biomarkers in human diseases. They are crucial for translational science especially in the development of imaging biomarkers. A biomarker can be any quantifiable metric, which can detect a biological process or disease pathology and changes associated with the treatment. Therefore, it is ideal for any imaging biomarker to detect changes consistently in both animal models and humans. Though several animal models were found to be very similar to the human Parkinson's disease (38, 39), the majority of the compounds tested in these animal models fail to show efficacy in the human condition and thus provide low translational value (40). To reduce this rate of failure and better translate the changes behind imaging biomarker, a number of animal models mimicking the human disease pathology should be used to validate the proposed biomarker.

Parkinson's disease is characterized on the neuropathological level mainly by the loss of 50-70% of the dopaminergic neurons in SN pars compacta, presence of intracytoplasmic inclusions called 'Lewy bodies' mainly consisting of α-synuclein. Rodent models of PD-like phenotype match closely some aspects of human pathology, but they fail to reflect the condition in the complexity observed in humans. Therefore, in our research we decided to use two substantially different mouse models of PD – a transgenic one showing mainly accumulation of human α-synuclein with no neurodegeneration and a neurotoxin-based model featuring primarily loss of dopaminergic neurons.

Male mice were used in all studies. The mice were group housed in the Central Animal Facility of Masaryk University, Brno, Czech Republic, and maintained on a normal 12/12-hour light/dark cycle (lights on at 6 a.m.) with a constant relative humidity of 50-60% and temperature of 22±1ºC. Water and food were available ad libitum. Later they were transported to the animal house of the Institute of Scientific Instruments, Academy of Sciences of the Czech Republic, Brno, Czech Republic and maintained under the same conditions as in the previous location. All procedures were performed in accordance with EU Directive no. 2010/63/EU and approved by the Animal Care Committee of the Faculty of Medicine, Masaryk University, Czech Republic and the Czech Governmental Animal Care Committee, in compliance with the Czech Animal Protection Act No. 246/1992.

TNWT-61 mouse model

The murine Thy-1 promoter turned out to be particularly useful to drive high levels of expression of the human wild-type α-synuclein throughout the brain, a distribution similar to that observed in human PD. TNWT-61 model reproduces many features of sporadic PD, including progressive changes in dopamine release and striatal content, α-synuclein pathology, early motor and non-motor deficits suggesting that this model could be useful for the study of preclinical PD stages. Although TNWT-61 model exhibits progressive loss of tyrosine hydroxylase positive dopaminergic fibers in the striatum and thus worsening behavioral deficits characteristic of PD, the mice do not exhibit dopaminergic neuron loss in the SN. Surprisingly, neuroinflammation has been initially found only in striatum and subsequently in SN, whereas α-synuclein is overexpressed widely in the cortex, cerebellum, hippocampus, olfactory bulb, and brainstem. This makes TNWT-61 particularly well suited for studying the early stages of PD, before dopaminergic neuron loss occurs, and at the same time well suited for developing imaging biomarkers(38).

Methamphetamine (METH) mouse model

Methamphetamine is an addictive psychostimulant, which mainly acts as an indirect sympathomimetic. Several studies have reported that high doses of methamphetamine induce chronic dopamine nerve terminal damage in striatum and neuronal body loss in SN pars compacta (41, 42). It has been consistently reported that repeated administration of very high METH doses induces degeneration of dopaminergic nerve terminals followed by a decrease in dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels. It is also reported that it selectively induces loss of dopaminergic cell bodies in SN, sparing dopaminergic neurons of mesolimbic pathway (41, 43). The tenacious loss of dopaminergic nerve terminals is correlated with dopaminergic neuronal loss in SNc, which was evident from the Nissl staining and fluoro jade fluoresces (44). Importantly, methamphetamine selectively affects nigrostriatal dopaminergic neurons sparing the mesolimbic dopaminergic pathway simulating the conditions of PD. Additionally, striosomes of striatum compared to matrix are more susceptible to damaging effects of methamphetamine which is observed also in the case of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) administration (43). Methamphetamine model also shows motor symptoms similar to other neurotoxin-based animal models of PD (45). Therefore, it is suitable for assessment of neurodegenerative process as found in the late stage PD patients.

However, both models are far from perfect and more rodent models of PD should be used to adequately assess the validity of any neuroimaging biomarker. Ideally, the findings from one model featuring mainly pathological protein accumulation should be replicated in another model with analogous profile and more neurodegenerative models should be employed. Our decision to use these two models was based on their distinct pathological hallmarks, availability and previous experience with these models. We later performed a comprehensive study using rotenone model, which combines both neurodegenerative process and protein accumulation (data currently in preparation). However, it

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would be indeed very interesting to scan eventually all available models, such as MPTP, 6-hydroxydopamine, paraquat and others.

3.2 Imaging equipment

3.2.1 MRI system and coil setup

Based on our previous pilot experiments we used the MRI coil setup, which rendered the best signal-to-noise ratio and appropriate image quality. MRI measurement was performed on a high-field horizontal 94/30USR scanner (Bruker Biospin MRI, Ettlingen, Germany) equipped with a gradient system with strength up to 660 mT/m. MRI coil setup was composed of a 1H quadrature volume transmitter coil (inner diameter 86 mm) and a 1H four-channel surface mouse phased-array head coil as a receiver.

3.2.2 Data processing software

- Paravision 5.1 running in a console Bruker AVANCE III – acquisition commercial software. First of all, Paravision software was used to obtain MRI data for further data processing. Moreover, Paravision was used for conversion of voxel size of acquired data

Image analyses were carried out using several other tools:

- MATLAB R2010a (The MathWorks Inc., Natick, MA, USA) software – In house Matlab code programmed was used for conversion of MRI DICOM Bruker data format to NIFTI data format
- ExploreDTI v4.8.4 Software – ExploreDTI was used for calculation of the diffusion maps and diffusivity directions together with parametric maps.
- ImageJ software (NIH, Bethesda, MD, USA) - free software was used for various brain regions delineation according to the mouse brain atlas (Paxinos and Franklin, ~~2001~~)
- FSL (FMRIB's Software Library) software (FMRIB, Oxford, UK) – FSL software was used for eddy current correction and movement artifacts tract-based spatial statistics analysis together with brain extraction and tract-based spatial statistics

3.2.3 Animal monitoring and respiratory gating instrumentation

Respiratory gating together with respiratory rate monitoring was used to reduce motion artefacts. In cases where the respiratory curve would not be stable, the respiratory gating would not work properly and the DKI sequence would take longer time. MRI compatible equipment made by Small Animal Instruments (Model 1030, SA Instruments, Inc., Stony Brook, NY, USA) was used during all MRI procedures. Respiratory rate was monitored by small pneumatic pillow sensor and temperature was maintained with a small rectal thermistor probe.

3.2.4 Animal bed with warming and fixation system

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[JR13] megjegyzést írt: Ami, please, cite again - 48

The necessity of a proper animal heating system and appropriate fixation system secured normalization of animal vital functions together with reduction of motion artefacts. An animal bed with a water heating system was used to maintain stable animal body temperature. Tooth-bar and ear-bars were required to fix a the head of the animal.

[P14] megjegyzést írt: restraint? immobilization?

[JR15R14] megjegyzést írt: Not really, the animal was anesthetized, so it was not immobilized (nor restrained). It was actually fixed. We are open to accept another term, but "fixed" was the best we could think of.

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3.3 Animal anesthesia system

We used easily controllable continuous inhalation isoflurane anesthesia. The selection of anesthetic agent was based on long-term experience in our lab (46) and the evidence that isoflurane seems to be suitable for prolonged MRI scanning compared to other anesthetics such as propofol, dexmedetomidine, or ketamine (47). Moreover, isoflurane can be used repeatedly and provides short post anesthesia animal recovery. Importantly, the constant isoflurane concentration was maintained at level 1.5-2% to prevent anesthetic agent fluctuation and to secure regular respiratory curve. Isoflurane anesthetic system consisted of an isoflurane vaporizer (G.A.S. Ltd., Keighley, UK), flowmeter with medical grade air carrier gas and induction chamber with scavenger unit with a carbon filter (UNO Actisorb Anaesthetic Gas Filter). Mask for the anesthesia was integrated into the animal bed and tubing.

4. Methods

4.1 Preparation of animal for scanning

Animal was anesthetized using a mix of medical air and 0.5% isoflurane at flow rate 1 l/min increasing every minute in the induction chamber until reaching 3% concentration of isoflurane to provide slow anesthesia onset and to prevent stress. In general, stress can alter physiological parameters, deepen interindividual variability and consequently worsen data quality. The mouse was then placed on the animal bed horizontally and teeth were hooked by a tooth bar. Head was immobilized by placing ear bars gently to the ears to maintain motion restriction for brain imaging. Both tooth and ear bars were fixed also on the animal bed. The nose was gently placed into the anesthetic mask for continuous delivery of isoflurane. Pneumatic pillow sensor was gently placed under the abdomen and the respiratory curve was controlled to get a good signal. The sufficient level of anesthesia was controlled according the animal respiration rate. The optimal respiration rate was maintained in the range of 50 – 60 breaths per minute. Continuous inhalation anesthesia was used during whole MRI protocol to prevent motion artefact and immoderate stress during MRI scanning. The isoflurane was maintained at 1.5-2% level. The thermistor probe was placed intrarectally to control the temperature. The temperature was maintained at around 37°C to stabilize basal vital functions of animal and to avoid hypothermic stress. Moreover, maintaining temperature of animals was essential for all experiments because temperature instability or fluctuation could potentially alter water spin density and consequently provide false results or results containing errors (48-50). We

switched on the water heating system 1 hour before starting measurements. With this procedure, we were able to maintain stable temperature of each animal. The animal did not lose its body heat by being placed on an unheated animal bed. Eye ointment (Vidisic gel) was applied on mouse eyes to prevent corneal ulceration. Finally, the quadrature coil was fixed over the head of animal and the animal was placed in the middle of magnetic field.

4.2 MRI data acquisition protocol

4.2.1 Localizer

Pilot scan was acquired to check brain position in the center of the coil.

- Method: Fast low-angle shot (FLASH) sequence was used to obtain axial, coronal, and sagittal brain images to localize the brain position and to correct brain position inside the magnetic field.
- Parameters: field of view (FOV) - 30 × 30 mm, 128 × 128 acquisition matrix, three orthogonal slices of 1 mm thickness, echo time (TE) was 3 ms, and repetition time (TR) was 200 ms with FA 30.
- Total acquisition time – 25 s.

4.2.2 Anatomical images

T2-weighted brain scans were acquired to obtain anatomical images and localize Bregma 0 slice. The Bregma 0 slice was characterized by anatomical structures - corpus callosum, merging anterior commissures – anterior parts and striatum according to the Paxinos Mouse Brain Atlas. The Bregma 0 was always positioned as 8th slice from 15 adjacent slices to maintain the same brain slices positioning in all experiments. Moreover, this positioning allowed also acquiring all ROI, specifically SN, hippocampus, sensorimotor cortex, striatum and thalamus. The slice thickness was 0.5 mm to obtain a high-resolution image. The brain scans were obtained to be used as a reference for the diffusion kurtosis imaging (DKI).

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[ED20] megjegyzést írt: Paxinos, G. & Watson, C. *The rat brain in stereotaxic coordinates.* (Elsevier, 2007)

- Method: 2D rapid acquisition with relaxation enhancement (2D RARE) sequence was used.
- Parameters: FOV - 24 × 24 mm, 256 × 256 acquisition matrix, 15 adjacent slices of 0.5 mm slice thickness, RARE factor of 8, TR was 2500 ms with 4 averages.
- Total acquisition time – 6 min.

4.2.3 DKI acquisition

Diffusion-weighted images were acquired. The DKI protocol included the acquisition of six b-values (b=0, 500, 1000, 1500, 2000, and 2500 s/mm^2) along with 30 non-collinear directions, δ=4 ms, Δ=11 ms, with seven averages used for b=0 acquisition and four averages for each other b-value.

- Method: spin echo-echo planner imaging (SE-EPI) was used.

- Parameters: FOV - 24 × 24 mm, 98 × 128 acquisition matrix, 15 adjacent slices of 0.5 mm slice thickness, TE was 25 ms using 300 kHz bandwidth, TR was ~5 s depending on respiratory rate.
- Total acquisition time – 1 h 40 min.

4.2.4 Respiratory gating procedure

It was necessary to configure segmented EPI acquisition triggered to the respiration cycle. Three images were acquired in the same time of the breath cycle to prevent imaging artefacts. Nevertheless, due to this fact the scanning time depended on respiratory rate. Therefore, range of 50 – 60 breaths per minute was maintained which seemed to be optimal.

4.2.5 End of MRI measurement

The animal was removed from the magnetic field immediately after DKI sequence was finished. The animal was placed on a heating pad with the oxygen mask in case it was having breathing difficulty. Otherwise, it was returned to the cage and checked for recovery. Afterwards, Nutra-Gel Diet™(Bio-Serv) was provided to the animal to supply water and nutrition after scanning. Animals were always transport back to the animal facility after complete recovery.

4.3 Processing of data

The acquisition matrix of DKI images were 98 X 128 which was reconstructed to 256 X 256 with the help of Paravision 5.1 software. The raw MRI data comes in Bruker format therefore it was converted to NIfTI format, which can be handled by MRI data analyzing software. A locally modified, freely available MatLab script was used for conversion and the size of the voxels was enlarged. Signal to noise ratio was calculated and compared for b-values maps to check data quality. Diffusion data were corrected for eddy currents and movement artifacts to the first non-diffusion weighted image with FSL (51). ExploreDTI v4.8.4. Software (52) calculated the diffusion maps using b-values and diffusivity directions. With six b-values we had the possibility to calculate parametric maps in different ways. For DTI parameters, data were processed and calculated on a voxel- by-voxel basis with six b-values to produce parametric maps (MD, AD, RD, FA, MK, AK, and RK), and with two b-values to produce DTI-derived parametric maps (MD, AD, RD and FA) in a “conventional” manner. From all the possible fitting methods, the robust estimation of tensors by outlier rejection (RESTORE) proved to be the best choice for our data set. Once both DKI and DTI parametric maps are prepared, the ROI and TBSS analysis procedure differs. Please check the respective section for further analysis.

4.5 Results and interpretation of ROI-based analysis

ROI based analysis is hypothesis driven as we select the regions based on the presence of pathology in that particular region. ROI is specific for detecting microstructural changes in gray matter, while

TBSS analysis is specific for white matter changes. In our studies, the selected regions were those generally affected in the Parkinson's disease. We have assessed several slices of the brain allowing evaluation of the same ROIs repeatedly and expressed as an average. Specifically, the regions were: SN, striatum, sensorimotor cortex, hippocampus, and thalamus.

After getting the maps for different diffusion tensor and diffusion kurtosis metrics, the ROI selection on b = 0 images was drawn manually according to the mouse brain atlas (53) with the help of FA maps using Image J software for various brain regions. The ROIs were delineated on FA map, because it gives better contrast and visualization for differentiating the brain regions.

Once the ROI was drawn on the FA map, it was saved, and the same ROI was transferred on another metrics to get all diffusivity and kurtosis variables.

DKI results obtained with TNWT-61 mouse model and METH treated animals are summarized in the Table 1. The primary aim of this line of research was to compare the sensitivity of DKI in two different mouse models of PD as described earlier. The changes in the kurtosis, diffusivity and fractional anisotropy values were compared to wild type or vehicle treated mice.

		Mean kurtosis					Axial kurtosis					Radial kurtosis				
Model	Time-point	SN	STR	HIPP	CTX	THAL	SN	STR	HIPP	CTX	THAL	SN	STR	HIPP	CTX	THAL
METH	5 days	↓	↓		↓							↓				
	1 month	(↑)	↑	↑	↑											
TNWT-61	3 months		↑			↑							↑			
	6 months	↑	↑			↑							↑			↑
	9 months	↑	↑		↑	↑		↑		↑	↑					↑
	14 months	↑	↑	↑	↑	↑				↑	↑				↑	↑

↑ increase
↓ decrease
no change

		Mean diffusivity					Axial diffusivity					Radial diffusivity					Fractional anisotropy				
Model	Time-point	SN	STR	HIPP	CTX	THAL	SN	STR	HIPP	CTX	THAL	SN	STR	HIPP	CTX	THAL	SN	STR	HIPP	CTX	THAL
METH	5 days									↑		↑					(↓)				
	1 month		↓	↓								↓					↑	↑	↑	↑	
TNWT-61	3 months		↓																		
	6 months												↓			↓	↑		↑		
	9 months															↓					
	14 months				↓	↓				↓	↓					↓					

Table 1: Summary of DKI results (Khairnar et al., ~~2015a, 2015b, 2016~~, Arab et al., ~~2019~~)

Legend: The table shows an overview of our previously published data. The symbols (↑) and (↓) mean higher or lower value close to significant level, i.e. a trend. The numbers of animals were as follows. METH study: at 5 days METH (n = 11) and SAL (n = 5) and at 1 month, METH (n = 9) and SAL (n = 6). TNWT-61 study: 3 and 6 months (n = 15 per control and transgenic group), 9 months (n = 7 per control and transgenic group), 14 months (n = 9 transgenic and n = 12 control mice).

From the Table 1 it can be clearly seen that MK is the most sensitive read out as compared to other DKI and DTI metrics. We found significant changes in the MK already in 3-months old TNWT-61 mice in striatum and thalamus, while the zenith of the PD-like phenotype is considered 9 months of age (54). Importantly, none of the DTI parameters was able to detect these early pathological changes

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[JR22R21] megjegyzést írt: All issues are addressed in the Legend, the sex of mice (males) is stated in 3.1 Animals.

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affecting gray matter, which strengthens the importance of DKI in early detection of PD. The observed increase in MK might be due to increased structural complexity or tissue heterogeneity in the respective regions due to α-synuclein accumulation. It also is consistent with previous reports suggesting Lewy body inclusions or α-synuclein accumulation might have influence on change in MK (56). Interestingly, even though the TNWT-61 mice start to show wide-spread α-synuclein accumulation starting at the age of 10 days, at 3-months almost all the regions we considered for ROI analysis are reported to show α-synuclein accumulation (38, 57). Surprisingly, although SN, hippocampus and sensorimotor cortex are reported to show similar or even higher expression of α-synuclein accumulation as compare to striatum and thalamus, we observed increase in kurtosis only in striatum and thalamus in 3-months old mice. Based on these results, we can conclude that MK is sensitive in detecting α-synuclein accumulation-induced microstructural changes rather than α-synuclein accumulation *per se* (55, 57).

In contrast to the α-synuclein overexpressing transgenic model, in the case of METH model at the early 5-days' time-point we found decrease in MK in SN, striatum and sensorimotor-cortex. Decrease in MK generally indicates a decrease in hindrance to the diffusion of water molecules. There is a logical explanation of the opposite effect we have observed in TNWT-61 and METH treated mice due to the different nature of the two models. METH model is a neurodegenerative model, while TNWT-61 model is of prodromal type in which degenerative changes occur only at very late stage (38). Neurodegenerative changes induced by METH cause lower hindrance to diffusion of water in the brain due to reduction in structural complexity or tissue heterogeneity (16). Therefore, we can say that the decrease in MK observed in the METH model might be due to degeneration of dopaminergic neurons. Conversely, in the case of TNWT-61 mice either the α-synuclein accumulation or α-synuclein accumulation induced changes have likely increased hindrance to diffusion of water due to increase in structural complexity or tissue heterogeneity.

Taken together, we can conclude that MK is sensitive to detect microstructural changes induced by α-synuclein accumulation as well degenerative changes induced by METH and is having the sensitivity and diagnostic utility in both early as well as late stage of disease pathology.

Importantly at 1-month time-point in METH model we found increase in MK in striatum, hippocampus and cortex which is opposite to the results we obtained with MK at 5-days. This discrepancy can be explained by recovery of dopaminergic nerve terminals by axonal sprouting or protein accumulation in METH treated mice. It has been reported in the preclinical studies there exists a recovery phenomenon for the striatal and SN dopaminergic system (58). A recent study of DKI in a rat stress model also reported increase in MK in amygdala of stressed rats which was found to be due to an increase in neurite density. This might be the reason we observed increase in MK after 1 month of METH administration due to generation of neurites as recovery phenomenon might have increased non-gaussian diffusion in striatum and hippocampus.

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A study by Fornai et al. reported that administration of METH to mice causes the formation of intracellular inclusions similar to Lewy bodies in PD which mainly consist of α-synuclein (59). Hence, a possibility of accumulation of α-synuclein in METH treated mice cannot be ruled out. The common thing we have found in both the METH and TNWT-61 mouse models is at the late stage of disease pathology both the models exhibit increase in MK in SN, striatum and sensorimotor cortex which may be due to increased structural heterogeneity and hence hindrance to diffusion of water molecules (possibly due to α-synuclein accumulation).

It is important to emphasize that we did not find any consistent significant changes of FA in the two models. However, several preclinical and clinical studies with DTI have reported significance of FA metric in early diagnosis of PD (14, 22). In the METH model at 1-month time-point we have found increase in FA in SN, striatum hippocampus and cortex which agrees with a study performed by Van camp et al. with 6-hydroxydopamine lesioned rats (23), while there is a report showing a decrease of FA in SN due to degeneration of dopaminergic neurons (17). This discrepancy in DTI results might be due to intrinsic limitation of DTI, as it considers the diffusion of water molecule as gaussian meaning there is no hindrance to diffusion of water molecules in the brain, whereas in actuality it is non-gaussian and is hindered by cell constituents and cell membrane. Therefore, DTI seems to be sensitive to detecting white matter changes, while DKI is sensitive in detecting gray matter changes. Furthermore, three meta-analyses on DTI in PD have been published and there is so far no consensus on the importance of DTI in early diagnosis of PD (19, 26, 27). By contrast, DKI by considering non-gaussian diffusion of water molecule which is close to the situation in the living tissue, is sensitive in detecting both gray as well as white matter microstructural changes.

In PD, there are different post translational modifications in α-synuclein protein, and it has been reported that the α-synuclein, which has been found postmortem in the brains of PD patients, consists of phosphorylated and proteinase K resistant α-synuclein. Similarly, TNWT-61 mice are reported to show large punctate of proteinase k resistant α-synuclein starting from 1 month of age in thalamus, whereas in the SN it showed variable sizes of proteinase k resistant α-synuclein aggregates in 5 months-old mice (60). This is consistent with our findings in 3-months old TNWT-61 mice, where we observed an increase in MK in thalamus but not in SN. Conversely, in 6-month-old mice we found an increase in MK in both thalamus as well as SN and might be due to presence of proteinase k resistant α-synuclein. These results may have clinical importance as MK seems to be able to detect regional differences in proteinase k resistant α-synuclein induced microstructural changes and a correlation of proteinase K resistant aggregates with Lewy body inclusions was reported, underpinning the importance of these aggregates in synucleopathies (61).

Several studies have reported the increase in MK might be due to an increase in structural complexity or heterogeneity due to presence of protein accumulation or glial cell activation (7, 62-65). In our DKI study with TNWT-61 mice we also found glial cell activation, so we cannot rule out the changes in kurtosis values due to glial cell activation (60). Watson et al. reported that TNWT-61 show strong

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microglial cell activation in striatum as early as 1 month of age, whereas in SN it starts to show at the age of 5-6 months with very similar microglial morphology to that observed in PD patients (60). There are reports suggesting that the membrane barrier to diffusion of water is altered in the presence of activated microglia. This might be the reason we found significant increase in MK in striatum but not in SN at 3-months' time-point, whereas at 6-month time-point we were able to see an increase in MK in both SN as well as striatum. We can conclude that MK is probably sensitive also to detecting regional changes with glial cell activation.

There is a debate over the beginning of PD pathology development either from striatum containing dopaminergic nerve terminals or from SN consisting of dopaminergic cell bodies (66). In our DKI studies with TNWT-61 model we found changes in MK first in the striatum and later in SN, which correlates with the presence of proteinase k resistant α-synuclein and microglial cell activation first in the striatum and later in SN. This suggests that the α-synuclein accumulation-induced changes start in dopaminergic nerve terminals and later the pathology develops in dopaminergic cell bodies as detected by MK.

Interestingly, the most affected region in TNWT-61 mice at all time-points was the thalamus. It might be due to low ratio of endogenous to human α-synuclein protein compared to other brain regions at approximately (1:6.5) (38), which justifies the changes induced by human α-synuclein. Our DKI study with 14-month-old TNWT-61 mice was the first to report significant negative correlation between α-synuclein accumulation and decrease in diffusivity in thalamus and a trend towards positive correlation of α-synuclein accumulation with an increase in kurtosis (55). In contrast there was no DKI changes in METH model at both time-points in the thalamus. The contradictory results might be due to differences in the induction of PD pathology. TNWT-61 mouse model shows mainly overexpression of α-synuclein without neurodegenerative changes, while METH induces neurodegeneration first and shows presence of α-synuclein after neurodegenerative changes.

4.6 Results and interpretation of tract-based spatial statistics (TBSS) data

On parametric maps brain extraction was performed using Brain Extraction Toolkit from FSL (67). The default mode is not suitable for mouse brain analysis, since only partial mouse brain was measured. For that reason center of gravity was determined, and all maps were checked and corrected manually if it was necessary. A non-hypothesis driven method, a whole-brain analysis of the white matter tracts, tract-based spatial statistics (TBSS), was chosen to investigate microstructural alterations. TBSS (68) script was modified to fit it to the mouse brain (69). All of the 3D FA volumes were registered together non-linearly, and using the registration matrices, the best registration target was chosen with a free-search method. Then a study-specific volume was used as a template for the final transformations. The mean FA map was calculated and a skeleton, the center of the main white matter tracts, was created at the threshold at FA = 0.2. Each mouse brain's aligned FA data was projected on to the skeleton. The process was done for all of the parametric maps. Non-parametric

tests were used for statistical analysis: (1) the general linear model (GLM) design with permutation test (10,000 permutation) and (2) cluster-based thresholding were used with the predefined threshold (t = 2.3) to compare groups. The altered white matter tracts were identified according to the mouse brain atlas (53).

TBSS data interpretation: Tract based spatial statistics is the analysis technique to measure the DKI changes in white matter. At early stage of both TNWT-61 and METH model, TBSS analysis detected no white matter changes. This is an interesting phenomenon, given the substantially different nature of the two models. Conversely, at later time points such as 6, 9 and 14 months-old TNWT-61 mice and 1-month time point of METH model we detected significant white matter changes by TBSS analysis. This may indicate that the α-synuclein accumulation or neurodegenerative changes are likely to start in gray matter and later spread to white matter. Most importantly in the TBSS analysis both models showed alterations in similar regions. Specifically, we observed changes in tracts coming from or towards the thalamus such as the mammillothalamic tract and lateral thalamic nuclei. This was not the case in gray matter in which only the TNWT-61 mice showed changes in thalamus (54, 55, 70). The thalamus plays a crucial role in the passage of motor information to the sensory and motor cortex. It is strongly connected to the cerebellum and hippocampus. Impairment in the thalamic network may create difficulties in recalling motor skills and fine tuning of motor movements (71). It has been reported that thalamic noradrenaline deficiency might be involved in genesis of motor and non-motor symptoms (72). So α-synuclein pathology in the thalamus and sensitive detection of α-synuclein accumulation induced changes by DKI may have clinical importance in diagnosis of PD.

The white matter changes previously reported in PD patients were a decrease in MK and FA in cingulate fibres (34, 73). In contrast, our studies found increases in kurtosis and FA in the cingulate fibre in both models (45; 55; 70). This contradiction might be due to the intrinsic limitations of animal models being unable to adequately mimic the complex pathology present in human PD.

With TBSS analysis we have found a trend of an increase in kurtosis and a decrease in diffusivity in the anterior and posterior commissure, cingulate fibres, mammillothalamic tract and thalamic nucleus. Hence, it seems that wherever there is a protein accumulation or glial cell activation we also observe an increase in kurtosis and a decrease in diffusivity due to increased hindrance to diffusion of water molecules. However, this is a hypothesis that remains to be proven.

Taken together, TBSS results further confirm that DKI is sensitive in detecting both gray matter as well as white matter changes induced by PD-like pathology and this imaging technique does have potential to diagnose PD patients both at early stage as well as late stage of disease.

5. Notes

5.1 Stability of body temperature

Maintaining body temperatures of animals was essential for all experiments, because any temperature instability or fluctuation could potentially alter water spin density and consequently provide false

results or results containing errors (48-50) To ensure optimal conditions, we always switched on the water heating system in the animal bed 1 hour before we started scanning. This allowed to maintain a stable temperature of each animal, because the mice did not lose body heat on being anesthetized and placed on a cold bed. Moreover, also in the winter months an air heating system was required for the beginning of measurements. When using an air heating system, a paper blanket was placed over the animal's body to avoid acral body burns (mainly ears and tail).

5.2 Standard handling procedures

Standard environmental conditions, housing and handling of animals is an important aspect of any animal experiment. Environmental enrichment may be useful, but not a necessity in group-housed mice. Ideally, all animals should be handled regularly to lower their stress reaction to human contact. Generally, the stress can impair the whole measurement procedure due to instability of animal physiological functions (e.g. irregular breathing or fluctuating temperature) together with higher interindividual variability of results, prolonged anesthesia induction, necessity of higher dosing of anesthetic, or injury of animal due to unpredicted behavior. We place each animal in the preparation room at least 45 min before starting the measurement to habituate. Moreover, we also used red induction chamber for inducing isoflurane anesthesia which seemed to be more effective than using a transparent induction chamber.

5.3 Good fixation of animal and small instruments during measurement

We used a tape to fix every mobile component, such as head surface coil, thermistor probe, anesthesia tubing and MRI compatible ERT module used for measuring the respiration rate from a small pneumatic pillow and temperature. The tape was also used for fixation of animal by gently pressing it on to the pneumatic pillow. We used to take an approximately 10 cm long strip of tape and stick an approximately 3 cm long strip of tape in the middle of long strip to acquire non-adhesive part and to avoid the animal hair depilation. The pitfall of using tape occurred only in the case of using an air heating system because the warm air induced the tape glue instability. We solved it by double taping.

5.4 Specific neuropathological process of animal model of PD

As discussed previously, each animal model mimics certain features of PD-like neuropathology, but none of them is perfect. We have performed a longitudinal DKI study using low-dose intragastric rotenone administration to model PD-like phenotype (74). The dataset is currently in preparation, but we faced a problem in data interpretation, because we observed the majority of significant results in the grey matter before the mice reached the zenith of the rotenone-induced PD-like behavioral phenotype. It seems, that at early stage of the pathology, increased MK detects pathological protein accumulation. Interestingly, at later stage, the increase of MK was lost, likely due to more pronounced neurodegeneration. Taken together, it is possible, that one pathological phenomenon is able to mask

another (i.e. neurodegeneration may mask protein accumulation). This may potentially limit the usefulness of DKI in the diagnosis of PD, but on the other hand it may become a useful tool in tracking the progression of the neuropathology.

5.5 Motion artefacts

Motion artefacts seem to be crucial in DKI imaging because this sequence is highly sensitive to motion. We experienced very good results with combination of head/ear bars fixation together with respiratory gating as a prevention of motion artefacts and no necessity of ECG gating. Afterwards, diffusion data were also corrected for eddy currents and movement artifacts with FSL software.

5.6 Statistical considerations

Robust statistical analysis requires some kind of correction for multiple comparisons, which represents a golden standard in the majority of preclinical studies. The level of statistical significance was set at $p < 0.05$ in all studies; however, we opted not to correct the statistical results. This kind of studies are exploratory and we analyzed all DKI variables in as many relevant ROIs as possible. This renders a very high number of comparisons. So, the risk of false positive findings is considerable, but after a stringent correction (e.g. Bonferroni), we lose all significant findings. Importantly, our aim was to reveal the most useful metrics and ideally find a pattern and replicate the findings in more animal models. In our later studies, we decided to supplement the data with 95% confidence intervals for mean differences in all cases, which appears to be the most transparent way. Clinical relevance of our data will need to be confirmed in future clinical trials.

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6. DKI imaging in PD patients and its comparison with animal models

~~Recently~~ Clinically DKI imaging has gained an attention as a useful diagnostic tool for early diagnosis of neurodegenerative disorders. ~~In the case of clinical studies with PD patients also it has shown its importance as an early imaging biomarker.~~ The first study with DKI imaging on 30 PD patients compared with 30 age matched healthy controls was reported by Wang et al.~~,~~ in 2011 which was ~~compared with 30 age matched healthy controls~~. They found ~~increase in~~higher MK in caudate and putamen and ~~increase in~~higher MK and FA in SN in PD patients. Importantly the MK in ipsilateral SN has showed the best diagnostic performance with sensitivity of 0.92 and specificity 0.87. Surprisingly, the traditional ~~D~~diffusion tensor metrics including FA ha~~s~~ve showed little importance in diagnosis of PD in this study (Wang et al., 2011). As explained before the increase in tissue heterogeneity induces increase in MK (Jensen et al., 2005) ~~, in the present study~~and it was hypothesized that L~~l~~ewy body inclusions in the SN might be responsible ~~to show increase in~~for higher MK values in SN~~.~~ (Gianelli et al., 2011). ~~-~~Similar to Wang et al., 2011, a study involving around 72 untreated PD patients ~~found~~ reported also higher ~~increase in~~ MK in SN with very high sensitivity of 0.944 and specificity 0.917 ~~higher than what was observed by Wang and his colleagues~~ (Zhang G et

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al., 2015). In this study ~~they performed the~~a correlation with H-Y staging and UPDRS-III scores was performed, indicating ~~and found~~ positive correlation with ~~increase in~~ MK in SN again ~~proving~~ further supporting the diagnostic importance of MK in PD. Later ~~the~~ a similar ~~group~~ author collective published another 2 studies ~~on~~ using the DKI imaging in PD patients, which report ~~and found increase in~~ higher MK in SN especially in patients showing striatal silent lacunar infarction, ~~(SSLI)~~ which might be caused by h~~H~~yperhomocysteinemia and ~~changes in~~ MK values had positive correlation with disease severity (Zhang G et al., 2017, 2018). In contrast to all these ~~studies~~clinical trials, a recent DKI study on 26 PD patients found significantly lower MK values bilaterally ~~decrease in MK~~ in SN in both early and advanced PD patients compared to healthy controls (Guan J et al., 2019). The reason ~~behind~~ of discrepancy ~~in the results~~ might be due to differences in H and Y staging of the PD patients, difference in scanning protocols and processing of the data ~~may have given inconsistent results~~ (Guan J et al., 2019). ~~All~~ However, all these studies combined do suggest~~ed~~ MK may help clinicians in early diagnosis of PD patients and in detection of severity of PD ~~patients~~.

Pre-clinical studies with DKI are scarce and require widespread investigation to detect the molecular mechanism behind MK changes in SN. It is ~~difficult~~ impossible to recapitulate the human PD pathology in animals; therefore, ~~it is difficult to find~~ all the features of human pathology in one animal model do not exist. For this reason, we ~~gone~~ used two models featuring different sets of PD-like hallmarks as explained earlier~~with transgenic mice overexpressing alpha synuclein which is one of the important hallmarks of PD and may show prodromal phase of PD, while another one METH model which shows neurodegenerative changes in SN~~. In TNWT-61 model ~~similar to clinical studies~~ we ~~also found~~ observed higher ~~increase in~~ MK in SN which was related to presence of proteinase k resistant alpha synuclein and microglial cell activation which might have increased the structural heterogeneity in SN~~.~~ (Khairnar et al., 2015a, 2015b, 2016). This result is in accordance with clinical evidence. ~~While~~ Conversely, in ~~our~~ the METH model ~~initially~~ we found ~~decrease in~~lower MK in SN, ~~which~~ was related to acutely-induced neurodegenerative changes, whereas in the late stage we found ~~increase in~~higher MK likely due to accumulation of alpha synuclein or other pathological proteins developed ~~after neurodegeneration~~later (Arab A et al., 2018).

~~If we talk about~~Considering the white matter changes in PD patients it was Kamagata and his colleagues in 2013 reported changes in white matter in PD patients using DKI imaging. In a DKI study with 17 PD patients, they reported ~~decrease in~~lower MK and FA in anterior cingulum. MK has showed the best diagnostic performance in anterior cingulum with sensitivity of 0.87 and specificity 0.94. In a later study using 12 PD patients. ~~by~~ the same group ~~on 12 PD patients~~ reported lower MK values in posterior corona radiata and superior longitudinal fasciculus ~~(SLF)~~ with TBSS analysis~~;~~, however, no FA changes were observed. ~~Most i~~Importantly these regions ~~are white matter regions which~~ show~~s~~ presence of crossing fibres and in DTI imaging FA and MD metrics get influenced by presence of crossing fibres. Therefore, we can ~~say~~ conclude that MK ~~is having~~has a ~~the~~ potential to detect changes in both gray and white matter. The reason behind ~~decrease in~~lower MK values

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observed in white matter is quite difficult to explain. It might have occurred due to neuronal loss or deposition of axonal ~~l~~Lewy neurites.

~~However~~Conversely, in our pre-clinical studies with DKI imaging in both animal models we found increase in kurtosis and decrease in diffusivity in anterior and posterior commissure, cingulate fibres medial longitudinal fasciculus, mammillothalamic tract and thalamic nucleus which might be related to glial cell activation or deposition of alpha synuclein aggregates. The existence of aggregates of alpha synuclein in the white matter and its potential ~~repurcussions~~repercussions has not yet been studied in the TNWT-61 mouse model. So far, there is only one study which described the presence of axonal alpha synuclein aggregates in TNWT-61 mice, especially C-terminal fragments in parallel to human Lewy body disease patients, and found axonal transport deficits in these mice (Games et al. 2013). Thus, further pre-clinical studies are needed to determine the mechanism behind changes in kurtosis and diffusivity metrics in the white matter.

Few of the ~~recent~~ available DKI studies reported changes in putamen and thalamus with DKI imaging in PD patients. DKI study with 105 PD patients found ~~increase in~~higher MD and ~~decrease in~~lower MK, which ~~was~~ were correlated with severe motor and cognitive symptoms, ~~. W~~while in the thalamus ~~increase in~~higher MD and ~~decrease~~ lower FA ~~was~~ were correlated negatively with severity of PD symptoms (Surova Y et al., 2016). Later, the similar ~~group~~ author collective performed a longitudinal DKI imaging study~~ies~~ with 76 PD patients in which gray matter analysis was done by ROI based ~~analysis~~ approach and white matter by TBSS analysis. The~~is~~ 2 year follow up study reported ~~decrease in~~ lowerFA in the putamen of PD patients as compared to healthy control (Surova Y et al., 2018). Similar to this study, other DKI ~~study~~ trial with 35 clinically confirmed PD patients also reported ~~decrease in the~~lower FA in the putamen. Along with this, the authors~~y~~ also reported ~~increase in~~higher radial kurtosis in SN and globus pallidus and ~~decrease in~~lower mean and axial kurtosis in red nucleus and thalamus (Bingbing G et al., 2020).

In our pre-clinical studies with TNWT-61 and METH model we also found changes in kurtosis and diffusivity in striatum and thalamus. In TNWT-61 mice we ~~found~~ observed significantly ~~increase in~~higher MK in striatum and thalamus, which might be related to microglial cell activation and presence of proteinase k resistant alpha synuclein, respectively. ~~Whereas~~ Conversely, in METH mouse model we found ~~decrease in~~lower MK at the initial time point and ~~increase in~~higher MK at the later time point. As discussed before, the ~~decrease in~~lower MK values might be related to neurodegenerative changes induced by METH administration, which has decreased the structural heterogeneity, while at later time point we found ~~increase in~~higher MK likely due to neuronal sprouting as a recovery process which may have increased the tissue heterogeneity.

Overall, we can ~~say~~ conclude, that our pre-clinical studies may help to decipher the underlying pathological processes responsible for ~~increase in~~ MK values. We hypothesize, that higher MK is exerted by ~~due to~~ accumulation of alpha synuclein, mainly proteinase k resistant alpha synuclein and microglial cell activation; whereas ~~the decrease in~~lower MK suggests the neurodegenerative changes.

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7. Conclusion

The present chapter discusses the importance of DKI imaging in diagnosis of neurodegenerative disorders, particularly Parkinson's disease. In DKI imaging, MK was found to be the most sensitive readout to detect both early as well as late stage of disease pathology. This was confirmed by studies in two different animal models of PD with completely different neuropathological hallmarks. Our DKI imaging studies provided indirect evidence of mechanisms underlying changes in kurtosis and diffusivity metrics observed in PD patients such as Lewy body deposits containing α-synuclein and glial cell activation may cause increase in kurtosis while neurodegenerative changes lead to decrease in kurtosis.

We have performed two types of analysis of DKI data, region of interest (ROI) based analysis, which is a hypothesis driven analysis sensitive to detect gray matter changes and a tract based spatial statistics (TBSS) analysis, which is a data driven approach used for detection of white matter changes. ROI based analysis seems to have importance in early diagnosis of α-synuclein accumulation induced changes while TBSS analysis started to show changes in the later stage indicating gray matter is affected first and later the changes start in white matter.

We found that MK by measuring the hindrance to diffusion of water molecule to be sensitive in detecting microstructural changes in both gray as well as white matter. Hence, we suggest that MK may serve as an early biomarker for detection of PD and it can be explored in other neurodegenerative disorders.

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Statement of interest

None to declare.

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