Regional Cerebral Metabolic Activity in Genetic Mouse Model of Parkinson's Disease: an NMR Investigation for Biomarkers

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TARGETED AUDIENCE: Researchers and clinicians interested in biomarkers and brain energy metabolism in Parkinson's disease.

INTRODUCTION: Parkinson's disease (PD) is the second most common neurodegenerative disorders affecting about 1-3% in people over 60 years of age with no biomarker for the clinical detection of the disease. Pitx3 is a homeobox domain transcription factor which is responsible for the embryonic development of the substantia nigra (SN). Pitx3 knockout (Pitx3$^{-/-}$) mice, which exhibit malformation of SN leading to loss of dopamine innervations in striatum, is a good model to study chronic PD[1]. The objectives of the current study are to identify biomarker for the early detection of PD using *in vivo* ^{1}H MRS, and understand the impact of loss of dopaminergic neurons in the SN on the cerebral metabolism and neurotransmission of glutamatergic and GABAergic neurons in different brain regions by using ^{1}H-[^{13}C]-NMR spectroscopy in conjunction with infusion of [1,6-$^{13}C_2$]glucose.

MATERIALS AND METHODS: Two groups of one year old male mice; Group A: Wild Type (WT, n=5); Group B: Pitx3$^{-/-}$ (n=5) were used for the study. For ^{1}H MRS study, mice were anesthetized with isoflurane (1-2%) mixed in air and head positioned under surface coil (i.d. 25 mm). The probe assembly was placed in a 14.1T Vertical bore magnet interfaced with 600 MHz NMR spectrometer (Bruker, Avance II). ^{1}H MRS was performed in the striatum using STEAM pulse sequence (voxel size: 2×2×2 mm^3, TE/TR: 3/3000 ms, no of average: 768). Intensity ratio of metabolite to creatine was quantified using LCModel[2]. For metabolic studies, overnight fasted mice were infused with [1,6-$^{13}C_2$]glucose (i.v.) for 10 min[3]. At the end of the experiment, head was frozen *in situ* in liquid N_2 and metabolites were extracted from frozen cerebral cortex and striatum[4]. The concentration and ^{13}C enrichment of amino acids were measured from ^{1}H-[^{13}C]-NMR spectra of tissue extracts. The cerebral metabolic rate of glucose oxidation (CMR_{Glc}) by glutamatergic and GABAergic neurons was calculated from the amino acids labeling from [1,6-$^{13}C_2$]glucose[6].

RESULTS AND DISCUSSION: *In vivo* ^{1}H MRS suggested elevated intensity of Glu and GABA in the striatal region of Pitx3$^{-/-}$ mice (Fig. 1A). LCModel analysis showed significantly ($p<0.0005$) higher level of GABA and Glu to creatine ratio in the region (Fig. 1B). These results were confirmed using *ex vivo* ^{1}H-[^{13}C]-NMR spectroscopy which indicated an increase in GABA level (WT 4.9±0.7; Pitx3$^{-/-}$ 6.7±0.9 µmol/g, $p=0.015$) in the striatum of Pitx3$^{-/-}$ mice. ^{13}C Labeling of Glu_{C4} (WT 1.86±0.14; Pitx3$^{-/-}$ 1.49±0.15 µmol/g, $p=0.0015$), $GABA_{C2}$ (WT 0.37±0.08; Pitx3$^{-/-}$ 0.24±0.04 µmol/g, $p=0.0063$) and Gln_{C4} (WT 0.27±0.06; Pitx3$^{-/-}$ 0.17 ±0.08 µmol/g, $p=0.026$) from [1,6-$^{13}C_2$]glucose was found to be decreased significantly in the striatum of Pitx3$^{-/-}$ mice suggesting reduced glucose oxidation by glutamatergic and GABAergic neurons (Fig. 2B) and neurotransmitter cycling. Glucose hypometabolism was also observed in the cerebral cortex region (Fig. 2A). Neuronal metabolism is coupled with neurotransmitter cycling between neurons and astrocytes[7]. Therefore, our finding of reduced CMR_{Glc} indicates decreased neurotransmitter cycling between neurons and astroglia in Pitx3$^{-/-}$ mice. The finding of decreased labeling of Gln_{C4} is consistent with reduced synaptic transmission in the striatum and cerebral cortex of Pitx3$^{-/-}$ mice. Reduced cerebral glucose oxidation together with elevated GABA levels in the striatum could be used as a biomarker for the early diagnosis of PD in humans.

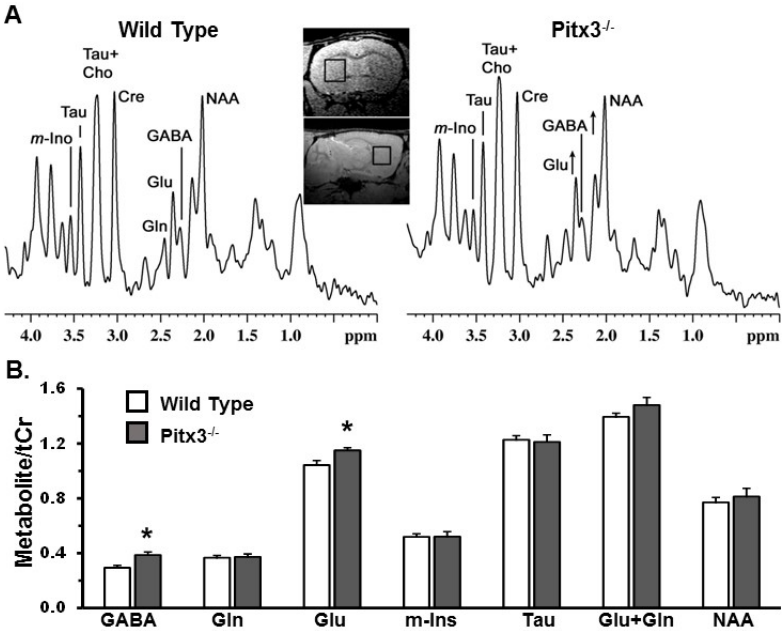

Fig 1 (A) *In vivo* ^{1}H MR spectra, **(B)** concentration of metabolites in striatum of WT and Pitx3$^{-/-}$ mice showing elevated Glu and GABA. *p<0.05

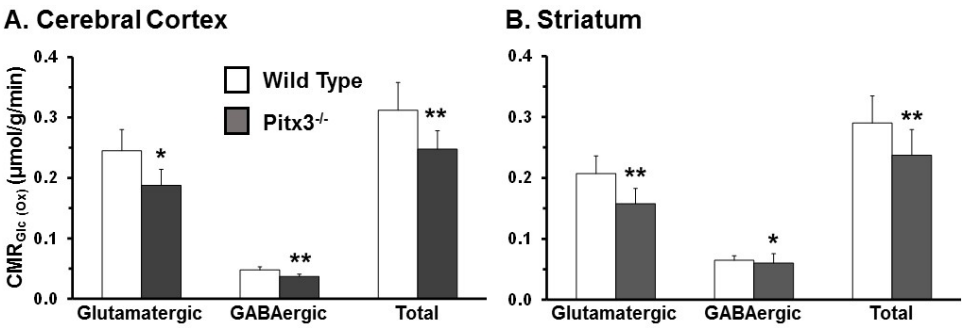

Fig. 2 $CMR_{Glc(Ox)}$ in **(A)** Cerebral cortex, **(B)** Striatum of WT and Pitx3$^{-/-}$ mice. *p<0.05, **p<0.01

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