Basic Science Abstracts

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Basic Science Abstracts

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Therapeutic exposures of CX-8998, a potent, selective and state dependent Cav3 channel antagonist in development for Essential Tremor and Parkinson's disease Tremor in Cav3 driven neurological models

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Objective: In this study, we utilized preclinical data to establish predicted human therapeutic exposures of the potent, selective and state-dependent Cav3 channel antagonist, CX-8998, currently in Phase 2 clinical studies for essential tremor and Parkinson's disease tremor.

Background: Cav3 is a mediator of subthreshold oscillations and excessive rhythmicity in pathophysiologic states found in tremor and is highly expressed in functional tremor network regions. Rodent models of tremor show enhanced Cav3 currents and burst-firing. Selective targeting of Cav3 channels has the potential to provide symptomatic benefit in conditions where Cav3 is an important component of disease pathophysiology.

Methods: We have investigated the potency and efficacy of CX-8998 and other Cav3 antagonists in rat preclinical models dependent on elevated Cav3 activity including harmaline induced tremor and the WAG/Rij genetic model of spontaneous absence seizures. Dose and time dependent effects of Cav3 antagonists on tremor and seizure were objectively quantified using measures of tremor power and electrocorticogram recordings.

Results: Cav3 antagonists suppressed both tremor and accumulated time in seizure in a dose and time dependent fashion. CX-8998 showed robust activity following single oral doses of 1-10 mg/kg with exposure response analysis indicating an effective plasma concentration threshold of 150-350 nM CX-8998 in rat. Adjusting for species differences in plasma protein binding predicts a human therapeutic plasma concentration range of 300-700 nM. Comparison of Cav3 antagonist potencies suggests that CX-8998 will result in substantially greater target engagement at achievable CNS concentrations compared to less potent and selective Cav3 antagonists zonisamide and ethosuximide.

Conclusions: Cav3 antagonists, including CX-8998, show robust efficacy in preclinical models dependent on Cav3 pathology including tremor and epilepsy. Evaluation of preclinical efficacy and pharmacology data for CX-8998 supports an achievable effective human plasma concentration range and suggest the potential for improved Cav3 target engagement compared to less potent and selective antagonists.

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A Preliminary Study of Cinnamomum verum on Anxiety, Locomotor Activity and Emotionality Behaviour in Wistar Albino Rats

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Objective: Introduction: Cinnamomum verum is a very common traditional Indian Spice known as Darchini. It is obtained from the inner bark of several species of the genus Cinnamomum; used in both sweet and savory foods. It is the best spice available in terms of its nutrition and health and contains unique healthy and healing property due to the presence of active components. Orally administrated C.verum extract reduces β-amyloid oligomerization and corrects cognitive impairment in Alzheimer's Disease Animal Models has been done by Anat Frydman et al., in 2011 but there are only a few studies done on its effect on stress and anxiety. This leads to a hypothesis to search for Cinnamon that may cause improvement in locomotor behavior or anxiety suppression.

Background: The present study was carried out to evaluate the anti-anxiety, locomotor activity and emotional behavior of C.verum, so as to validate its use as anti-anxiety, an anti-stressor drug in the Unani system of medicine.

Methods: Material and Methods: Female Wistar rats of weighing 150-180 gm were divided into 4 groups of 6 animals each. The animals in Group I were Control and Group II had acute noise stress (of 100dB intensity) for 30days, whereas animals in Group III gave aqueous extract of C.verum (200 mg/Kg/body weight) were dissolved in saline (1 ml) and administrated for 30 days orally and IV were given aqueous

extract of C.verum along with acute stress for 30days, respectively orally, once a day (duration of treatment has been stated with respective tests). A battery of tests viz. Open Field Behaviour Test and Elevated Plus Maze Test, were used to study the anti-anxiety, anti-stressor effect of C. verum. Statistical significance between the different groups was determined by one-way analysis of variance (ANOVA). If the groups showed a significant difference, Tukey's multiple comparison tests were done. The significance level was fixed at $P< 0.05$.

Results: In this study clearly infer that Cinnamom could alter the behavior pattern in rodents. In the open field, the Cinnamom treated animal significant decrease in its locomotor activity. However, in the present study, aggressive or escape behavior, such as jumping, digging, climbing, and gnawing was not exhibited by any of the animals during the open field test. The cinnamom treated group showed a marked decrease in immobilization and fecal bolus with the decrease in ambulation of the central and peripheral square when compared to the respective control groups indicated the fearlessness. Moreover, the cinnamom treated animals showed the marked decrease in rearing and grooming from the control as well as from the stress control animals.

Conclusions: C.verum may cause an improvement on anxiety, locomotor activity and emotionality behaviour in Wistar Albino rats. The improvement may be due to the metabolite of C. verum and the improvement can also be possible by the antioxidants generated during cinnamom metabolism.

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Neuroprotective role of Tinospora cordifolia in MPTP induced Parkinsonian mouse model

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Objective: To evaluate the neuroprotective effect of Tinospora cordifolia aqueous extract (TCAE) in Parkinsonian mice.

Background: Parkinson's disease, an age related neurodegenerative disorder, is characterized by progressive loss of dopaminergic neurons in substantia nigra pars compacta and projecting neurons terminals in striatum. Recently, several studies regarding Parkinson's disease have proven the role of oxidative stress in neurodegeneration and neuroinflammation.

Methods: Twenty four Swiss albino mice were divided into three groups; Control, MPTP and MPTP+TCAE. Two doses of MPTP (30 mg/kg body wt.) were given intraperitoneally at 16 h interval. Normal saline was given to Control group whereas MPTP+TCAE treatment group received 200 mg/kg body wt. of TCAE orally for 21 days. After completion of treatment, mice brains were isolated for performing biochemical assays, western blotting, and Immunohistochemical analysis.

Results: From the Immunohistochemistry and western blot analysis, it is evident that TCAE inhibits the MPTP-induced activation of NF-κB and its associated pro-inflammatory cytokines. Through, Real-time PCR analysis it was revealed that pro-inflammatory cytokines were found to be up-regulated in MPTP intoxicated mice while TCAE treatment significantly restored their levels. In addition, the expression level of IL-10 was found to be decreased in the diseased condition which was further restored by TCAE treatment. Tyrosine hydroxylase, an important enzyme which is used as marker in Parkinson's disease, its expression was found to be reduced in MPTP mice while on TCAE administration, its level was significantly restored.

Conclusions: Our result clearly indicates that Tinospora cordifolia provides neuroprotection against MPTP induced nigrostriatal dopaminergic neurodegeneration and shows potent anti-inflammatory activity.

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Cognitive effects of SIRT6 overexpression: Emerging role of astrocytes

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Objective: The aim of this work was to elucidate the effect of SIRT6 overexpression on cognition.

Background: Recent evidence show that astrocytes are important for higher brain function, and may be pivotal for learning and memory. Astroglial release of neurotrophic factors and cytokines alters the survival and function of neurons. Astrocytes also affect neuronal metabolism, a process essential for long-term

memory formation. The sirtuins, NAD+ dependent deacetylases, regulate longevity and metabolism. There is a growing body of evidence that SIRT6 affects longevity and metabolic profile.

Methods: Age-dependent effects of SIRT6 were examined using a battery of behavioral tests. Brains of aged mice were dyed using immunohistochemistry. The effect of SIRT6 overexpression in neurons or astrocytes on the susceptibility to neurotoxicity, was assessed by viability assays and the effect of conditioned media from wild-type and SIRT6 overexpressing astrocytes were examined.

Results: Aged SIRT6 overexpressing mice exhibit enhanced long–term spatial memory in comparison to their age-matched wild-type littermates. SIRT6 expression levels significantly increased in the hippocampus and frontal cortex. Staining these brain sections with neuronal marker NeuN or microglial marker Iba-1, found no significant difference between WT and mice overexpressing SIRT6. Staining with glial fibrillary acidic protein (GFAP), a marker for astrocytes, showed a significant increase in GFAP in mice overexpressing SIRT6. A significant increase in newly generated neurons, stained with doublecortin (DCX) was found in the hippocampus of SIRT6 overexpressing mice. In vitro, conditioned media of SIRT6 astrocytes protected neurons against 6-hydroxydopamine (6-OHDA) neurotoxic insults.

Conclusions: Aged SIRT6 overexpressing mice show enhanced cognitive capabilities, and an increased GFAP staining, a marker for astrocytes, in the hippocampus. These findings suggest that SIRT6 may not only enhance life span but also preserve life quality as it may delay age-related memory and cognitive decline. The effects of SIRT6 may be partially conveyed by astrocytes function.

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The possible neuroprotective potential of galantamine along with soya-lecithin and hydroxychloroquine against ICV-STZ induced cognitive dysfunction in rats

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Objective: The present study investigates the possible neuroprotective potential of galantamine with soya-lecithin and HCQ against intracerebroventricular streptozotocin (ICV-STZ) induced memory impairment in a rat model of sporadic dementia of Alzheimer's type

Background: Galantamine an acetylcholinesterase (AChEs) inhibitor used for the symptomatic treatment of Alzheimer's disease. Soya-lecithin is a good source of choline improves cognitive performance. Hydroxychloroquine (HCQ) an antimalarial drug with an anti-inflammatory property.

Methods: Animals received single bilateral ICV injections of STZ (3 mg/kg). Drugs galantamine (2 mg/kg), soya-lecithin (100 & 200 mg/kg), HCQ (25 & 50 mg/kg) and their combination was administered for a period of 21 days. Various neurobehavioral parameters, followed by biochemical (oxidative stress markers), AChEs level, molecular (TNF-α level), mitochondrial respiratory enzyme complexes (I-IV), neurotransmitter levels and histopathological (H&E staining) evaluations.

Results: ICV-STZ administration significantly impaired cognitive performance indicated by MWM test, increased oxidative stress (raised lipid peroxidation, nitrite concentration, reduced glutathione, catalase activity), AChEs level, increased TNF-α level, decrease neurotransmitter levels, mitochondrial dysfunction and histopathological alterations as compared to sham treatment. Chronic treatment with galantamine (2 mg/kg), soya-lecithin (100 & 200 mg/kg), HCQ (25 & 50 mg/kg) significantly improved cognitive performance in MWM test, reduced AChEs activity, neuroinflammation, oxidative damage, TNF-α level, restored mitochondrial respiratory enzyme complex (I-IV) activities and histopathological alterations as compared to ICV-STZ treated animals. Further, combinations of soya-lecithin (100 & 200 mg/kg) and HCQ (25 & 50 mg/kg) with galantamine (2 mg/kg) and soya-lecithin (100 & 200 mg/kg) and HCQ (25 & 50 mg/kg) combination suggests the modulation of the neuroprotective potential as compared to their effect alone in ICV-STZ treated animals. Further, the present study suggests the combination potential of soya-lecithin (100 & 200 mg/kg) and HCQ (25 & 50 mg/kg) with galantamine (2 mg/kg) and it was found that galantamine (2 mg/kg) significantly modulate the neuroprotective potential of soya-lecithin (100 & 200 mg/kg) and HCQ (25 & 50 mg/kg) combination in ICV-STZ treated rats as compared to their effect alone.

Conclusions: The present study suggests that co-administration of galantamine with soya-lecithin and HCQ significantly improves cognitive performance in ICV-STZ treated rats as compared to their effect alone.

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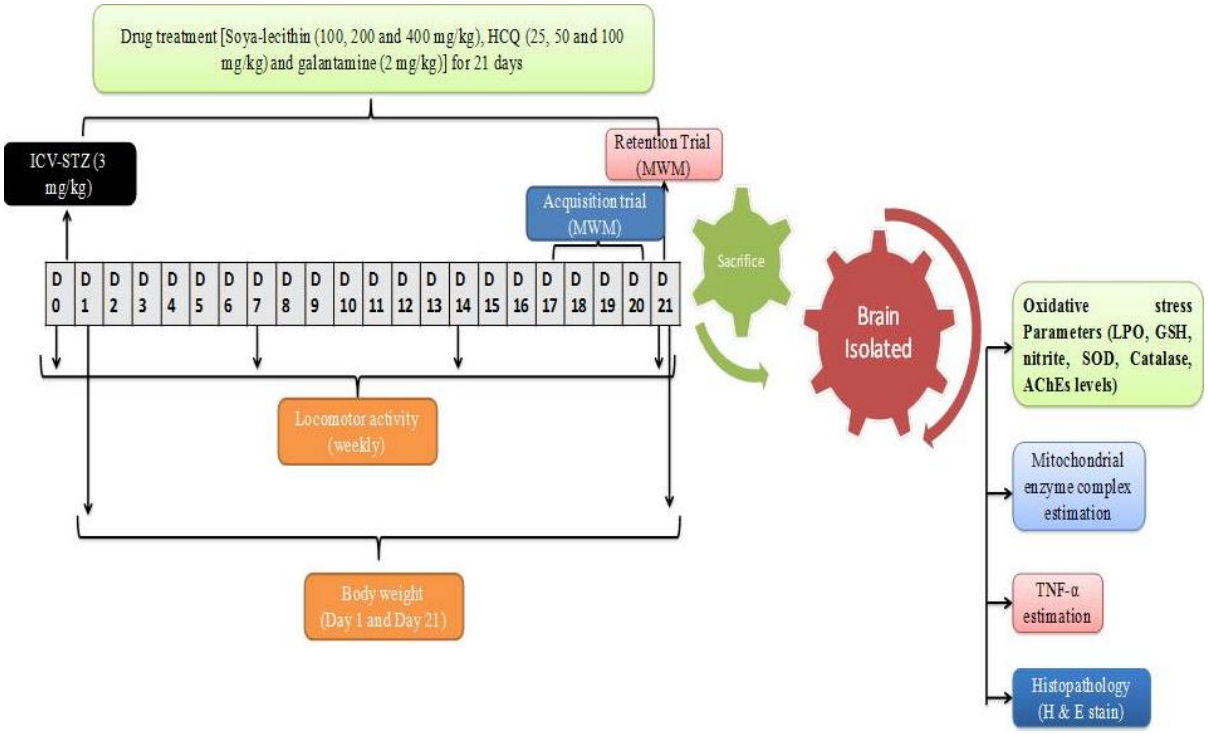

FIG. 1 (64) Experimental protocol design (ICV-STZ: intracerebroventricular streptozotocin; MWM; Morris water maze; IAL: initial acquired latency; RL retention latency; LPO: lipid peroxidase; GSH: reduced glutathione; SOD: superoxide dismutase; AChEs: acetylcholinesterase; TNF: Tumor necrosis factor; H&E: hematoxylin & eosin stain).

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Dielectric Properties of Blood Plasma Predict Cognitive Impairment in an Amyloid-Beta-Induced Rat Model of Alzheimer's Dementia

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Objective: This study presents a novel method of predicting the behavioral pattern in male Sprague-Dawley rats with impaired learning and memory, based on the measurement of the dielectric properties (dielectric constant and conductivity) of blood plasma at microwave frequencies at different time points.

Background: Alzheimer's disease (AD) is a chronic debilitating neurodegenerative disease for which there is no cure and no single diagnostic test. Now referred to "Alzheimer's dementia" or "dementia due to Alzheimer's", AD describes those in the dementia stage of the continuum.

Methods: Thirty (30) rats received 10µl of amyloid-beta bilaterally injected directly into their dorsal hippocampus to mimic lesions similar to those observed in patients with AD. Ten (10) control rats received 10µl of saline. Spatial learning and memory tests were conducted using the Morris Water Maze (MWM) prior to and following the bilateral intra-hippocampal injections. Animals were sacrificed on day 3, 7, 10 and 14 following the intra-hippocampal injection. Blood plasma was collected for determination of protein activity using a microwave technique. The microwave measurements were performed by the rectangular cavity perturbation method in the S-band of microwave frequency with the blood plasma collected from all rats.

Results: The amyloid-beta lesioned rats showed greater memory deficits when compared to saline injected rats. We found a change in the dielectric properties of the amyloid-beta samples but not the controls at the extended time point.

Conclusions: This study provides a new method of predicting cognitive impairment associated with AD.

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Abnormal signaling along the non-canonical molecular cascade GRK6/β-arrestin2/Akt/GSK-3β in the putamen is associated with tardive dyskinesia following chronic haloperidol exposure in a primate model

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Objective: To investigate the neurochemical basis of tardive dyskinesia (TD) in an experimental primate model.

Background: TD is a potentially irreversible motor complication occurring in one third of subjects during long-term exposure to centrally acting dopamine D2/3 receptor antagonists such as antipsychotic drugs. The induction and maintenance mechanisms remain elusive. We previously documented an upregulation of striatal D3 (not D2) receptors specific to TD-expressing monkeys in direct medium spiny neurons. Multiple kinase pathways are modulated by antipsychotic drugs, but the distinct impact of conventional vs. atypical drugs and their involvement in TD are unknown.

Methods: Under animal research ethics approval, we chronically exposed capuchins to haloperidol (N=11) or clozapine (N=6). Six unmedicated animals served as controls. Using Western blots, total and phosphorylated protein kinase levels (normalized over GAPDH or tubulin protein levels) were measured in the putamen in cAMP-dependent and -independent pathways associated with dopamine D2/3 receptor signaling. An immunofluorescence technique was used to reveal the differential expression of phospho[Ser473]Akt (pAkt) in D3+ cells.

Results: Five haloperidol-treated animals developed typical TD, and no TD was observed in the clozapine group. Total protein kinase levels were not altered by any treatment. While haloperidol enhanced phospho[Thr202/Tyr204]-p44/42 ERK1/2 levels by 3.4 fold, no difference was observed between TD-expressing and TD-free monkeys. Phospho[Thr34]-DARPP-32 levels were elevated 1.7 fold only in TD animals. Haloperidol specifically reduced putamen GRK6 (64%), β-arrestin2 (57%), and phospho[Ser9]GSK-3β (69%) levels in TD animals. Levels of pAkt were generally reduced (46%) by haloperidol, and were higher in TD-expressing (+47%) relative to TD-free animals. Further, pAkt/D3 colocalization was increased (40%) in the putamen of TD monkeys compared to TD-free animals. Phosphorylated protein kinases levels in the clozapine group were similar to controls, but GRK6 protein levels were upregulated 1.7 fold.

Conclusions: Our results suggest that failure to up-regulate striatal GRK6 and inactivate GSK-3β signaling may contribute to striatal D3 receptor upregulation and development of experimental TD. If confirmed, these changes may offer novel avenues for preventing or palliating TD. Supported by the Canadian Institutes for Health Research.

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Intrastriatal Fyn silencing as a gene therapy strategy to manage levodopa induced dyskinesia in a mice model of Parkinson's disease

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Objective: To genetically abrogate Fyn expression to reduce levodopa induced dyskinesia (LID) in the 6-OHDA mice model of Parkinson's disease (PD), using intrastriatal-injected lentiviral (LV) particles carrying a micro-RNA against Fyn (miR-Fyn).

Background: The management of LID in one of the greatest challenges in PD research because to date there are no available pharmacological alternative therapies to PD with full clinical efficacy. We have recently proposed Fyn as a novel target to control LID (Sanz-Blasco & Bordone et al. 2017). Fyn is a Src

tyrosine kinase located at the postsynaptic density zone that regulates the N-methyl-D-aspartate (NMDA) receptor by phosphorylation of the NR2B subunit at Tyr-1472 in response to dopamine D1 receptor stimulation.

Methods: Four miR-Fyn sequences under the control of synapsin promoter were designed, cloned in LV vectors and tested in vitro for silencing efficiency. We selected the one with the highest silencing proficiency and used Western blot to validate Fyn knockdown in vivo. Finally, we induced degeneration of the nigrostriatal dopaminergic pathway by injecting 6-OHDA into the medial forebrain bundle, and selected the animals with a remarked deficit of the contralateral forepaw in the cylinder test. Then, mice were challenged with L-DOPA to induce LID before or after the intrastriatal injection of miR-Fyn or a control sequence. LID were registered every 3 days for 2 weeks. Postmortem dopaminergic denervation was carefully determined by tyrosine hydroxylase (TH) immunodetection to ensure an equal level of degeneration between groups. We also determined the level of FosB-ΔFosB, a well-accepted marker of LID, and Fyn silencing by western blot.

Results: The selected miR-Fyn reduced Fyn protein by ~50% in neuronal cell line and by ~25% in the entire striatum. Considering the neuronal specificity of the transcript and the time expression of the miR-Fyn within the striatum, this was enough to significantly downregulate LID in a paradigm of dyskinesia and correlated with FosB-ΔFosB expression.

Conclusions: Our data demonstrate that Fyn is a potential target to control LID and set the grounds for its putative therapeutic use in PD.

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Interaction between mGluR5 and NR2B is increased in 6-OHDA parkinsonian rats with L-dopa-induced dyskinesiaInteraction between mGluR5 and NR2B is increased in 6-OHDA parkinsonian rats with L-dopa-induced dyskinesia

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Objective: In this study, we verified whether the interaction of mGluR5 and NR2B exerts an important effect on the development of LID.

Background: L-dopa is the most effective drug for relieving the motor symptoms of Parkinson’s disease (PD). However, the appearance of L-dopa-induced dyskinesia (LID) compromised the use of the drug. Evidence has shown that dysfunction of the glutamatergic system plays a key role in the development of LID and that the use of metabotropic glutamate receptor 5 (mGluR5) antagonists can significantly improve dyskinesia. However, the mechanisms underlying this alleviation are not well understood. In addition, the interaction of glutamate receptors in the striatum appears to be critical for the development of LID.

Methods: We analyzed data from 120 6-OHDA-lesioned SD rats. As the scores assigned for AIMs and parkinsonian disability are non-parametric, the data was analyzed using a Kruskal-Wallis test followed by Dunn’s test for multiple comparisons when comparing data over multiple days or with Mann–Whitney U test. The western blot and Q-PCR data were normally distributed and were analyzed using one-way ANOVA followed by LSD post hoc comparisons when appropriate, as indicated in the figure legends.

Results: In the present study, we found that interaction between mGluR5 and the N-methyl-D-aspartate (NMDA) receptor subunit NR2B was increased in 6-hydroxydopamine-lesioned parkinsonian rats. Disrupting the mGluR5-NR2B interaction via antagonist or Tat peptide administration led to a decrease in synaptic mGluR5-NR2B trafficking. This decreased mGluR5-NR2B interaction was accompanied by a significant reduction in the severity of LID. In addition to the reduced interaction, ERK1/2 phosphorylation levels, PKC protein expression levels, and L-dopa-induced c-fos and prodynorphin (pdyn) mRNA levels in the lesioned striatum were reduced; these molecules have been associated with LID. Significant correlations were observed between abnormal involuntary movement (AIM) scores and the levels of the NR2B-mGluR5 interaction, PKC protein expression and ERK1/2 phosphorylation.

Conclusions: This study supports the hypothesis that the interaction of mGluR5 and NR2B plays an important role in the development of LID, inhibition of the mGluR5-NR2B interaction may contribute to the MTEP- and MK801-induced down-regulation of ALO AIM scores and aid in recovery from LID.

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Dopaminergic impairments following repeated exposure to stress on the neurotoxicity of lambda-cyhalothrin through inflammatory cytokines in rats

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Objective: Impact of psychological stressors in modulating the toxicity of environmental chemicals has been suggested, however the mechanism this is not clear.

Background: Repeated stress exaggerates the toxicity of environmental chemicals/drugs including metals and pesticides have been reported in number of experimental studies although the correct mechanism associated with is not clearly understood. The present study has been carried out to investigate the consequence of immobilization stress (IMS), a psychological stressor on the neurobehavioral toxicity of lambda-cyhalothrin (LCT), a new generation type-II synthetic pyrethroid with imminent uses to control insects, pest and vector born disease in public health programmes.

Methods: Rats were encountered with IMS (placed in plastic restrainer for 15 min/day; one session/day) for 28 days or exposed with LCT (3.0 mg/kg body weight, p.o.) for 3 days (on days 26, 27 and 28) or pre-exposed to IMS for 28 days followed by LCT treatment for 3 days. Plasma corticosterone, blood brain barrier (BBB) permeability and inflammatory cytokines in corpus striatum were estimated by standard procedure. Further, effect of dopamine signaling associated with motor functions was also assessed by standard protocol.

Results: Increased the levels of plasma corticosterone and alteration of blood brain barrier permeability was found in rats pre-exposure to IMS followed by LCT treatment as compared to IMS or LCT alone. Additionally, decreased the expression of dopamine signaling and neuroinflammatory cytokines in corpus striatum have also been observed in these rats as compared to rats exposed to either IMS or treated with LCT alone. These changes coupled with motor dysfunctions. Marginal changes was observed in dopamine signaling and inflammatory cytokines in corpus striatum including plasma corticosterone levels and blood brain barrier permeability associated with motor functions in rats exposed to either IMS or treated with LCT alone as compared to controls.

Conclusions: The results revealed that repeated stress considerably modulates the neurotoxicity of LCT through neuroinflammatory cytokines associated with dopaminergic singling which regulate the movement disorders.

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Effects of PGE inhibition on Striatal Neuroinflammation in 6-OHDA lesion

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Objective: In this study, we investigated the role of prostaglandin E2 inhibition, pro-inflammatory cytokine concentration and microglial activation in a Parkinsonian rat

Background: Neuro-inflammation plays a role in the microenvironment disturbance in Parkinson's disease (PD). Cytokine and non-cytokine induced pathways affect glial cell activation following injury.

Prostaglandin E2 has been implicated in the non-cytokine response to inflammation. This raises the question of whether manipulation of the inflammatory response pathways could lead to therapeutic interventions for PD

Methods: Male Sprague Dawley rats were lesioned stereotaxically with 6-OHDA. Bromelain which inhibits PGE2 was used to treat a subset of the rats, pre-lesion, 24 and 72hrs post- lesion. Behavioural assessments using the Open field, cylinder and step tests were carried out. Systemic and striatal concentration of pro-inflammatory cytokines and the quantification of cd11b/cd86 as a measure of glial cell activation were assessed

Results: 6-OHDA injection resulted in marked motor impairment which was alleviated by pre-lesion bromelain treatment. Bromelain treatment also resulted in the suppression of both systemic and striatal pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) as well as changes indicative of suppression of gliosis

Conclusions: Bromelain treatment reveals interconnectivity between the cytokine and non-cytokine mediated neuro-inflammatory pathways suggesting that PGE2 inhibition plays a role in protecting against dopamine neurodegeneration and may therefore be considered as part of a therapeutic strategy used to attenuate PD disease progression.

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Studying genes involved in abnormalities of the basal ganglia and iron homeostasis using gene co-expression network analysis

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Objective: To unravel the disease mechanisms underlying neurodegeneration with brain iron accumulation (NBIA) and to identify potential novel disease genes.

Background: NBIA is clinically and genetically a heterogeneous group of movement disorders characterized by iron accumulation in the basal ganglia. The questions remains why and how iron accumulates so focally in the basal ganglia as only two of the 10 NBIA genes are directly involved in iron metabolism. It is also not known if the iron accumulation contributes to disease. Additionally, a large proportion of the NBIA cases remain genetically undiagnosed.

Methods: 75 HPO annotated genes involved in abnormality of the basal ganglia and 28 HPO annotated genes related to abnormality of iron metabolism were used to generate gene co-expression networks using GeneNetwork*. A PANTHER overrepresentation test for Gene Ontology (GO) slim biological process was performed using Fisher's Exact with FDR multiple test correction.

Results: Constructing a gene co-expression network specific for abnormalities of the basal ganglia that included 2 NBIA genes, we identified DNA metabolic process and DNA repair as top hits. Metabolic processes were also significantly overrepresented including nitrogen compound and nucleobase-containing compound processes as well as primary metabolic processes. Of the 141 genes correlating with abnormality of the basal ganglia and 127 genes associated with abnormality of iron metabolism, 2 genes and 4 pseudogenes were shared between the two networks and highlight those as novel NBIA candidate genes.

Conclusions: Gene co-expression analysis exposed genes with a yet unrecognized role in abnormality in basal ganglia and in abnormality of iron homeostasis. DNA damage and DNA repair might play a role in diseases caused due to abnormalities of the basal ganglia including NBIAs.

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Dissecting molecular signatures of X-linked dystonia-parkinsonism (XDP) through integrative genomics studies

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Objective: To determine the causal locus and functional mechanism associated with XDP using integrative genomics methods in patient-derived and genome-edited induced pluripotent stem cell (iPSC) models.

Background: XDP is a neurodegenerative movement disorder with variable clinical presentation. The genetic cause has been attributed to seven disease specific changes (DSCs) observed in all cases on Xq13.1. None of the DSCs have annotated functions and previous studies have suggested the region is recalcitrant to recombination. The causal mechanism has thus remained elusive.

Methods: We established an international consortium for XDP and aggregated specimens from 792 individuals (403 XDP males, 23 heterozygous carrier females, 352 controls, and 14 non-manifesting carriers). We developed fibroblast lines (n=45) and an iPSC resource from 12 patients and their unaffected relatives, including differentiation to four neuronal lineages. We then integrated genome and transcriptome assembly using six technologies (Illumina whole-genome sequencing [WGS]; 10X Genomics assembly; DNA capture sequencing [CapSeq]; RNA CapSeq; Pacific Biosciences long-read RNA CapSeq; total RNAseq).

Results: Genomic analyses detected all 7 DSCs and 47 additional XDP-associated variants. Haplotype reconstruction further revealed five recombination events and eight derivatives of the most common haplotype in this cohort, suggesting that recombinations occur regularly in XDP, and narrowing the critical locus to a region that includes TAF1 exclusively. Transcriptome assembly and expression analyses discovered a signature associated with XDP in TAF1 that involved aberrant splicing and intron retention (IR) that terminated in proximity to an intron 32 SVA insertion and negatively correlated with TAF1 expression in XDP probands. The hexameric repeat in this SVA was found to be polymorphic in probands and unstable in culture, ranging from 35-52 repeats, and inversely correlated with age at onset of XDP (r2=0.5). Remarkably, CRISPR/Cas9 excision of the SVA rescued the aberrant splicing signatures and normalized overall TAF1 expression in XDP cells [figure1]. Transcriptome profiling across diverse neuronal lineages is ongoing; analyses to date suggests convergence on multiple neurodevelopmental and neurodegenerative pathways and co-expression networks.

Conclusions: These studies suggest a novel transcriptional signature associated with an SVA insertion in XDP. Disease onset correlated with SVA repeat length, and aberrant transcriptional signatures could be rescued using CRISPR/Cas9 editing, suggesting a potential therapeutic target.

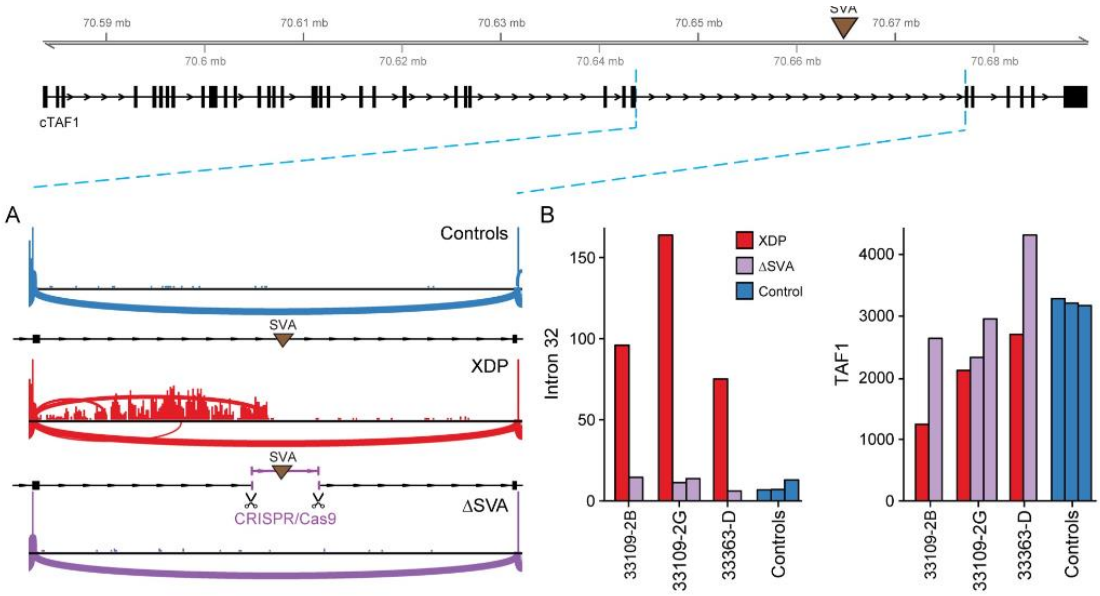

FIG. 1 (126) Transcnptome assembly and expression analyses identify aberrant splicing and intron retention in TAF1. Terminating in proximity to an intron 32 SVA insertion and negatively correlated with TAF1 expression in XOP. CRISPR/Cas9 excision of the SVA rescued the aberrant splicing signatures and normalized overall TAF1 expression.

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Epigenetic silencing in the humanized mouse model of Friedreich ataxia

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Objective: To investigate if DNA hypermethylation of the abnormal FXN gene in Friedreich ataxia is present in disease-relevant tissues and if it is tissue-, repeat-, and age-dependent.

Background: Friedreich ataxia is a progressive sensory ataxia caused by a GAA trinucleotide repeat expansion in intron 1 of the FXN gene. The expanded repeat produces repressive heterochromatin that results in transcriptional silencing of the FXN gene. Clinical manifestations, such as ataxia and cardiomyopathy are positively correlated with the size of the expanded repeat. Despite the silencing, the FXN promoter and surrounding CpG island (CGI) remain unmethylated. However, the FXN CGI shore is hypermethylated in Friedreich ataxia, which is a known cause of transcriptional silencing.

Methods: DNA from various tissues from the humanized mouse model of Friedreich ataxia, both young (1 mo) and old (12 mo) containing 8, 200, and 450 GAA repeats, were analyzed for DNA methylation using bisulfite deep sequencing.

Results: Hypermethylation of the CGI shore was noted in all disease-relevant tissues in both the GAA-200 and GAA-450 mice. Methylation was dependent on the repeat length. Strikingly, FXN CGI shore methylation decreased over time, and this was most pronounced in the heart of GAA-200 mice.

Conclusions: FXN DNA methylation reduces throughout life in the presence of a short expanded allele, and may explain the lower prevalence and severity of cardiomyopathy in Friedreich ataxia patients with short repeats.

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Compound-heterozygous mutations in VPS13D are a novel cause of spastic ataxia and lead to mitochondrial dysfunction

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Objective: To identify the genetic cause in two sisters with spastic ataxia and to functionally characterize mitochondrial function in patient-derived cells.

Background: Spastic ataxia is a clinically and genetically highly heterogeneous disease, the genetic basis of which is largely unknown.

Methods: We performed exome sequencing in the two affected sisters and their healthy parents. Detected variants were filtered for rare, protein-changing variants shared by the sisters. The most promising candidate gene, VPS13D, has previously been linked to mitochondrial dysfunction in Drosophila. Therefore, we investigated mitochondrial morphology (form factor) and function (ATP synthesis) in patient-derived fibroblasts. The effect of the nonsense variant on VPS13D transcription was investigated by quantitative PCR, cDNA sequencing, and cycloheximide treatment in patient-derived fibroblast lines.

Results: The disease onset in both sisters was in the third decade of life. The 35-year-old sister presented with oculomotor abnormalities including saccadic pursuit and square wave jerks, appendicular and gait ataxia, spasticity and reduced deep sensory functions. Brain MRI was normal. The 29-year-old sister was wheelchair-bound due to combined spastic ataxia, and additionally presented dysarthria. Exome sequencing revealed compound-heterozygous variants in VPS13D [c.5409C>A [Tyr1803Ter] and c.12629C>T [Ala4210Val]) in both patients. Sanger sequencing of cDNA showed lower expression of the nonsense mutated allele compared to the missense mutated allele, resulting in a ~50% reduction in the total mRNA level of VPS13D in the patient compared to non-mutation carriers. This reduction was caused by nonsense mediated mRNA decay as demonstrated by cycloheximide treatment in cultured cells. Patient´s fibroblast cells showed more roundly mitochondria and disrupted mitochondrial interconnectivity in comparison to controls with elongated organelles and complex network. These structural mitochondrial changes were accompanied by a reduced ATP production rate.

Conclusions: Our study demonstrated that compound-heterozygous variants in VPS13D cause a movement disorder along the ataxia-spasticity spectrum and make VPS13D the fourth VPS13 paralog

involved in neurological disorders. Analyses of patient-derived fibroblasts suggest that mutations in this new ataxia/spasticity gene impact on mitochondrial structure and function. Of note, mitochondrial dysfunction has been associated with other ataxias and spastic paraplegias.

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The Ultrastructural study of the effects of different types of chronic motor deficit on the morphology of limbic, extrapyramidal and neocortical regions of rat brain

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Objective: To study the effect of chronic restraint stress and moderate motor deficit on the ultrastructure of central and lateral amygdalar nuclei, hippocampal CA1 and CA3, caudate nucleus and neocortical motor area of rat brain

Background: The ability of chronically limited motor activity to produce alterations on the functioning of the central nervous system has been studied extensively [1-2]. However, many aspects need further elucidation. Relatively numerous studies have examined the impact of motor deficit on behavior and brain chemistry [3-4], while the effect of motor limitations on brain structure have described only in a few studies [5-7]. Besides, the biggest part of investigations has been focused on chronic restraint stress, while the consequences of moderate forms of motor deficit are not well known [8-10]. Various pathological conditions alter fine structure of the brain. The advances of molecular medicine demand the knowledge of such alterations. Transmission electron microscope is the powerful technique allowed the elucidation of the structure of neuron at nanometer resolution.

Methods: Chronic Motor Deficit - Adult Wistar rats were separately subjected to 40 d restriction of motor activity for 8 hours/day in wire grids of 10"×9" fitted with a Perspex plate of 9"×6.5". In blood plasma of these rats corticosteron level (measured with ELISA kit) was elevated. Mild Motor Deficit – Young adult Wistar rats were separately subjected to 40 d restriction of motor activity in mesh cage of 15"×12" for 4 h /day. The construction of cage provided the possibility to increase the values in X and the values in Y in parallel with the increase of the size of animal. In these rats plasma corticosterone level was normal.

Results: - Both motor deficits affect the ultrastructure of limbic and extrapyramidal regions. - Restraint stress produces more significant pathological changes. They are particularly numerous in the central amygdalar nucleus, then - in other limbic areas. - Moderate motor deficit produces mainly superficial alterations that are mostly concentrated in caudate nucleus. - In both cases motor cortex retains almost normal structure.

Conclusions: Moderate motor deficit does not alter significantly the fine structure of brain. More substantial alterations should produce stress, which accompanies severe forms of motor deficit.

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Safinamide inhibition of in vivo glutamate release in a rat model of Parkinson's Disease

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Objective: To evaluate whether safinamide inhibits in vivo glutamate (Glu) release in a rodent model of Parkinson's disease (PD), i.e. the 6-hydroxydopamine (6-OHDA) hemilesioned rat.

Background: Safinamide is a novel drug endowed with a dual mechanism of action, i.e. blockade of MAO-B and inhibition of glutamate release (Caccia et al., Neurology 67, Suppl 2, S17-S23, 2006), approved as add-on to levodopa therapy in Parkinson's disease (PD). In naïve rats, safinamide, likely via use-dependent sodium channel blockade, inhibited in vivo Glu release from stimulated nerve terminals in the subthalamic nucleus (STN) and its projection areas, globus pallidus (GP) and substantia nigra reticulata (SNr), but not dorsolateral striatum (DLS). The effective free brain concentration range was close to the affinity value for sodium channels (Morari et al., J Pharmacol Exp Ther 364, 198-206, 2018) and overlapped that estimated in PD patients at the therapeutic dose of 100 mg.

Methods: One microdialysis probe was implanted in the DA-depleted DLS or GP, SNr and STN of 6-OHDA hemilesioned rats. Rats were treated with safinamide (15 mg/kg i.p.) and, 30 min later, reverse dialysis of veratridine (10 microM) was performed for 30 min in each area. Control rats were treated with saline. Glu was measured by HPLC coupled with fluorometric detection.

Results: In control rats, veratridine caused a transient, about 2-fold rise of Glu levels in all nuclei examined. Safinamide differentially inhibited the veratridine-evoked Glu release with almost complete inhibition in STN and GP but no effect in DLS and SNr. Safinamide did not affect spontaneous GLU efflux.

Conclusions: This study provides the first evidence that safinamide inhibits the stimulus-evoked Glu release in the DA-depleted rat basal ganglia, namely the GP and STN areas in which increased glutamatergic activity is known to produce PD motor complications. This suggests that the therapeutic effects of the drug on PD symptoms may, in part, be mediated by a reduction in Glu levels in GP and STN. The different response to safinamide in DA depleted SNr, also involved in the control of motor symptoms, needs to be further investigated. These data support the notion that the dual mechanism of action of safinamide is relevant in PD.

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Abrogation of Rotenone Induced Behavior, Biochemical and Mitochondrial Deficits in Mice by Curcumin and Ginkgo Biloba: New Combinational Therapy Approach for Parkinson's Disease

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Objective: This study was designed with the objective to find out the possible synergistic effect of Curcumin (Cur) and Methanolic extract of Ginkgo biloba (EGB) Leaf in Parkinson's disease (PD). Cur and EGB were explored for their neuroprotective effect in rotenone-induced neurodegeneration in mice model of Parkinson's disease.

Background: Both Cur and EGB, are natural polyphenolic, highly potent bioactive compounds. Cur has been used for centuries in traditional medicines in India. Curcumin showed several biological and pharmacological activities including potent antioxidant, cardiovascular disease, anticancer, anti-

inflammatory effects and neuroprotective effect. Ginkgo biloba is one of the most widely used herbal remedies in the world to treat the symptoms of early-stage Alzheimer's disease, vascular dementia, peripheral claudication, and tinnitus of vascular origin.

Methods: Rotenone (1mg/kg/ i.p.) was used to induced PD in mice. Chronic rotenone administration for 21 days produced significant impairment in the behaviour pattern (learning, memory, motor coordination), decrease oxidative function (superoxide dismutase, catalase and reduced glutathione level) and mitochondrial function (Complex-I, Complex-II, Complex-III) as compared to normal control group of mice.

Results: Simultaneous treatment with Cur and EGB combination at different dose level (50, 100 and 200 mg/kg, p.o) for 21 days significantly improved behaviour parameters ($P < 0.001$), oxidative damage ($P < 0.001$) and mitochondrial enzyme complex activities (< 0.05, $P < 0.01$, $P < 0.001$) as compared to negative control (rotenone-treated) group. The results of present study showed that the Cur and EGB combination restored the motor deficit function and enhance oxidative and mitochondrial functions.

Conclusions: The outcome of present investigation confirms that the intraperitoneal administration of rotenone in mice induces neurobehavioral and biochemical changes mimicking those observed in Parkinson's disease. Treatment of mice with combination of Cur and EGB produced significant neuroprotection probably mediated through its antioxidant activity and provides a strong justification for the therapeutic potential of these compounds in management of PD.

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Delivery of AAV alpha synuclein to a mouse and rat provides a useful model for the assessment of anti-parkinsonian efficacy of chronic inhibition of LRRK2

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Objective: 1. To create an in vivo model system to be used to assess the effect of chronically inhibiting LRRK2 pharmacologically. 2. To confirm previous reports that chronic administration of LRRK2 inhibitors produces an increase in size and number of type II pneumocytes in the lung. 3. To identify the therapeutic index between efficacious doses and doses that produce measurable effects in lung.

Background: The LRRK2 kinase is one of the most studied potential disease modifying targets for the treatment of Parkinson's disease (PD). Previous work demonstrated that chronic administration of the LRRK2 inhibitor, PFE-475, AAV alpha synuclein treated rats has beneficial effects on the PD symptomology, supporting the hypothesis that LRRK2 inhibitors are effective PD therapeutics (Daher et al., 2015). In mice, chronic in diet administration of the LRRK2 inhibitor MLI2 produces a mild lung phenotype (Fell et al., 2016).

Methods: Various strains of rats and mice were treated with AAV alpla synuclein. Once strains had been identified that were responsive to the treatment we assessed the effect of chronic administration of the LRRK2 inhibitor, PFE-360, which has previously been tested in animal models. In life endpoints included assessment of behavior using a fine motor kinematic analysis and imaging for DAT levels. Ex vivo endpoints included measuring DA levels by HPLC and TH by stereology. Lungs were collected for histopathological analysis.

Results: We found that there are significant strain differences in both rat and mouse, but that there are individual strains that respond to the AAV alpha synuclein treatment with reliable and robust parkinsonian effects, including a behavioral effect. Interestingly, we found no evidence that LRRK2 overexpressing rats or mice had an exaggerated phenotype relative to WT controls. We have reproduced the effect in lung following chronic dosing of MLI2 and have found a similar effect with a structurally distinct LRRK2 inhibitor, PFE-360. Using MLI2, we also demonstrate that the effect repeatedly reverses within a week of withdrawal of

drug administration. To assess to potential efficacy of a LRRK2 inhibitor, C57 mice were administered AAV alpha synuclein and then chronically treated with the LRRK2 inhibitor PFE-360. Behavioral measures were taken using the fine motor kinematic assay; TH and DA were also measured.

Conclusions: There are substantial strain and species differences in the response of rodents to AAV alpha synuclien treatment. Chronic in diet administration of a LRRK2 inhibitor produces a mild effect in the mouse lung and that effect is reversible within one week of drug withdrawal. Chronic administration of a LRRK2 inhibitor protects mice against AAV alpha synuclein mediated parkinsonian deficits.

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Ginsenoside Rb1 protects dopaminergic neurons through Foxm1-Nurr1 pathway in mice model of Parkinson's disease

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Objective: In the present work, we investigated the protective effects of ginsenoside Rb1 in dopaminergic neurons through Foxm1-Nurr1 pathway in mice model of Parkinson's disease.

Background: Parkinson's disease(PD) is the second most common neurodegenerative disease which is caused by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta(SNpc). The underlying mechanisms of the PD has not been fully understood. Foxm1 is a transcription factor of proliferation. Foxm1 could bound to the promoter region of the Nurr1 which is important in development and maintenance of dopaminergic neurons.

Methods: C57BL/6J mice were used to administerd MPTP(30mg/kg) toxin to establish the PD mice model and treated with Rb1(5 mg/kg, 10 mg/kg, 20 mg/kg). "Pole test" was used to test the motor coordination of mice. The dopaminergic cells in the SNpc region were measured with tyrosine hydroxylase-positive immunofluorescent staining. TUNEL was applied to measure the apoptosis of cells in SNpc region. Protein and mRNA expression of Foxm1 and Nurr1 were determined with western blot and Reversed Transcript Polymerase Chain Reaction.

Results: Rb1 treatment ameliorated the behaviour injury and dopaminergic cells loss induced by MPTP in a dose-dependent manner in mice model of PD. The cell apoptosis of the SNpc region was reduced with the Rb1 compared with the MPTP group. Protein and mRNA expression of Foxm1 and Nurr1 was induced with Rb1 treatment compared with the MPTP group.

Conclusions: Our results provide the evidence that Rb1 has neuroprotective effects via Foxm1-Nurr1 pathway in mice model of Parkinson's disease.

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Neuromodulatory properties of flavonoids from Persea americana Peel: Role in CNS redox homeostasis and neurobehavioral markers

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Objective: Isolating flavonoids from Peel of Persea americana fruits & testing their effects on CNS redox homeostasis culminating in neurobehavioural amelioration among rats.

Background: Cellular stress is a main "Threat to homeostasis" & a set of related physiological & behavioral adaptive responses. Stress in CNS in conjugation with peripheral organs triggers dysregulation of cellular balance leading to psychiatric, endocrine, metabolic & autoimmune diseases. Allopathic therapies though effective have a plethora of adverse effects; hence there is a dire need for natural adoptogens which

increase the resistance to aversive inputs & result in totiprotection against stress. Persea americana mill (Avocado, Lauraceae) is a nutritious supplementary fruit tree, of which pulp has an excellent culinary usage globally. However, antioxidant properties of peel are markedly higher than that of the pulp as evidenced with in vitro chemical systems. Despite this, the peel are not considered for the use either in culinary or medical preparations.

Methods: Persea fruits were peeled & the skin was dried, pulverized &extracted with solvent gradient system to achieve maximum flavonoid fraction. The Persea americana peel flavonoids (PAPF) rich fraction was dried under pressure and refrigerated. Adult Male wistar rats (12 wk old) were supplemented with PAPF (50mg/ kg bw/d, p.o., 28days). Rats were monitored weekly for change in body weight, psychological stress & locomotor behavior using open field test. Terminally, brain regions were assessed biochemically for neuronal redox status among mitochondria and cytosol.

Results: PAPF did not affect the growth of rats however they demonstrated a marked reduction in normal stress behavior & locomotor function in open field test. From the first week of PAPF supplements, rats showed improved natural exploratory activity in terms of 'rearing' and boxes/lines crossed in OFT. Additionally, rats receiving PAPF spent more time in the central area than the periphery suggesting improved locomotion. Further, there was a significant reduction in the oxidative markers like hydroperoxides, lipid peroxides & increased glutathione levels among cytosol and mitochondria of the brain regions from PAPF groups. Additionally, GST activity levels were also modulated with PAPF supplements.

Conclusions: Our data strongly suggest a neuroameliorative propensity of flavonoids from Persea americana fruit peel as evidenced from the improved stress/locomotion and CNS redox status. Future studies are designed to elucidate neuro-molecular markers involved.

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Neuromodulatory propensity of Ginko biloba to offset rotenone induced behaviour and biochemical alteration

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Objective: The aim of present study was to evaluate neuromodulatory propensity of Gingko Biloba in rotenone induced Mice Model of Parkinson Disease in terms of improving behavioural and biochemical aspects.

Background: Gingko Biloba is an ancient Chinese tree which have substantial experimental evidence for its neuroprotective potential and their memory enhancement activity. Various reports on potential of Gingko Biloba apllication in neurological disorder has been evaluated in many in-vitro and in-vivo preclinical studies.

Methods: Animals were exposed to rotenone (1mg/kg) for a duration of three weeks showed remarkable diminished in behavioural paradigm (memory, learning and locomotor activity) oxidative protection (decreased activity of superoxide dismutase , catalase and reduced glutathione level , lipid peroxidation and nitrate estimation) and mitochondrial enzyme activity (complex-II succinate dehydrogenase (SDH), complex-III MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-H-tetrazolium bromide) as compared to normal control group of mice.

Results: Oral administration of Gingko biloba at three different doses (50,100 and 200 mg/kg p.o.) were able to rescue animals significantly by improving behavior parameters ($P < 0.001$) oxidative damage ($P<0.001$) and mitochondrial enzyme complex activities (<0.05, $P <0.01$, $P < 0.001$) as compared to rotenone induced group (negative control). It is observed that extract of Gingko Biloba act as a neuromodulator by restoring motor deficits and increased the activities of antioxidant and mitochondrial enzymes activity.

Conclusions: The outcome of present study proves neuroprotective role of Gingko Biloba against rotenone induced Parkinson's in mice and offers a strong explanation for the therapeutic prospective of Gingko biloba in the management of PD .

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Could curcumin be the preventive key of parkinson's disease?

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Objective: The present study evaluates the possible protective effect of curcumin on rats exposed to aluminum which is a major risk factor of parkinson's disease.

Background: Aluminum is the most abundant metal on the earth crust, it can access to the body via gostrointestinal and lung tissue, it crosses the blood-brain barrier and forms deposits in brain regions such as substantia nigra of patients affected by Parkinson's disease and in other cerebral areas of different neurodegenerative diseases, some previous studies demonstrated that aluminum induced changes in many neurotransmitter levels including dopaminergic system. Medicinal plants are very variable but some of them is used as spice such us curcumin witch is extracted from the rhizome of the plant 'Curcuma longa'.Curcumin exhibit a variety of biological and pharmacological activities such as namely antioxidant potential.

Methods: Experiments were carried out on wistar rats exposed to aluminum chlorid(0,3%) in drinking water during 4months since intra-uterine age, the aluminum intoxicated group received concomitantly curcumin by oral gavage (30mg/kg B.W.) for the same duration as mentioned previously, we evaluated the locomotor activity of rats using 'open field test' and using the immunohistochemistry procedure, with tyrosine hydroxylase antibody (TH: the key enzyme of dopamine synthesis) we evaluated the TH immunoreactivity in the substantia nigra.

Results: Our results showed, a significant decrease of TH immunoreactivity in the substantia nigra in chronic aluminum intoxicated rats,this decreased of TH immunolabelling was remedied with daily curcumin administration.Concerning the locomotor performance, aluminum induced a significant decrease of locomotor activity in aluminum intoxicated group.In the aluminum intoxicated-curcumin treated group, the locomotor activity increased significatively in comparison with aluminum intoxicated group.

Conclusions: Curcumin might have a neuroprotective effect against aluminum-induced alterations on dopamine neurotransmission and locomotor activity witch are the major signs of Parkinson's disease , thus curcumin might be the preventive key of Parkinson's disease.

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Neuroprotective effects of coffee ingredients against rotenone-induced neurodegeneration in parkinsonian model

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Objective: In this study, we examined neuroprotective effects of coffee ingredients, caffeic acid (CA) and chlorogenic acid (CGA), against rotenone-induced neurodegeneration using primary cultured cells and parkinsonian mice.

Background: Epidemiological analyses showed that daily drinking coffee and teas decreases the risk of Parkinson's disease to 40-50%. Among coffee ingredients, CA has catechol structure and its ester with quinic acid, CGA exerts antioxidative properties. Rotenone as a pesticide, mitochondrial complex I inhibitor, has been used to reproduce neurodegeneration of enteric nervous system that precedes nigral dopaminergic neuronal loss.

Methods: Male C57BL/6J mice were chronically subcutaneously administered with low dose rotenone (2.5 mg/kg/day) for 4 weeks using an osmotic mini pump. The animals were daily orally treated with CA or CGA for 1 week before rotenone exposure and during 4-week rotenone infusion, totally for 5 weeks. Brain and intestinal sections were immunostained by antibodies for tyrosine hydroxylase (TH), β-tubulin III and GFAP. Primary cultured enteric neurons and glial cells were prepared from the intestine of Sprague-Dawley

rat embryos at 15 days gestation. Cells were treated with CA or CGA for 24 h in advance, and then exposed to rotenone for 48 h.

Results: Chronic subcutaneous administration of rotenone reduced the number of TH-positive cells in the substantia nigra and immunoreactivities of β-tubulin III and GFAP in the intestinal myenteric plexus of mice. Daily administrations of CA or CGA inhibited rotenone-induced damage of not only nigral dopaminergic neurons but also enteric neurons and glial cells. In primary cultured cells, low dose rotenone promoted dose-dependent reduction of β-tubulin III-positive enteric neurons in the neuron-glia cocultured system but not in the enriched enteric neuronal culture. Furthermore, treatment with CA or CGA markedly prevented rotenone-induced cell loss of enteric neurons and astrocyte-like glial cells.

Conclusions: These results suggest that intake of coffee ingredients CA and CGA prevents rotenone-induced neurodegeneration in both of the brain and myenteric plexus.

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Chlorogenic acid exerts anti-neuroinflammatory effect by suppressing NF-κB pathway in Parkinsonian mice model

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Objective: The objective of this study was to investigate the anti-neuroinflammatory effect of chlorogenic acid (CGA) in the 1-methyl-4-phenyl-1,2, 3, 6- tetrahydropyridine (MPTP) model of Parkinson's disease (PD).

Background: Parkinson's disease, a second most wide-spread age-related chronic movement disorder, which shows damaged dopaminergic (DA) neurons and aggregates of α-synuclein protein within the substantia nigra pars compacta (SNpc). Studies in animals and human from last few years have suggested that the degeneration of DA nigrostriatal pathway is mainly influenced by inflammation-induced oxidative stress and neurotoxicity due to pro-inflammatory cytokine production.

Methods: 30 mice were divided into 5 different groups, viz., control, MPTP-treated group and rest three groups were given 25 mg/kg, 50 mg/kg and 100mg/kg body weight (wt.) of CGA, respectively after MPTP intoxication. After completion of treatment, experimental mice were subjected to behavioral analysis to check motor impairment and further their brains were isolated and used to check the expression of inflammatory markers by Immunohistochemical staining and real-time PCR analysis.

Results: Immunohistochemical studies have shown the reduced activation of NF-κB, leading to decline in the production of pro-inflammatory cytokines such as IL-1β and enhanced production of anti-inflammatory cytokines such as IL-10 in CGA medicated parkinsonian mice model. Real-time PCR analysis has shown the reduced gene expression of pro-inflammatory markers (TNF-α, IL-1β) due to CGA treatment after MPTP intoxication. Enhanced TH immunoreactivity was seen after CGA treatment in MPTP-intoxicated mice. Motor deficits were also found to be upgraded through rota-rod, narrow beam walking test and hanging test. Supplementation of CGA has protected the DA neurons from degeneration in SNpc and nerve terminals in the striatum from the MPTP insult.

Conclusions: Altogether, our study indicates CGA attributed to its anti-neuroinflammatory effect against MPTP-induced DA neurodegeneration by down regulating NF-κB activity and thus can be used as a therapeutic drug for the prevention and management of PD, in which 50mg/kg body wt. CGA was seen to have most prominent effect.

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Role of metformin in diabetic aging female rat brain: a future therapy for neurodegenerative diseases

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Objective: The objective of this study was to investigate effects of metformin on glucose transporter (GLUT1, GLUT3) expression, intracellular calcium levels, expression of synaptic molecules synaptophysin and synapsin I, biomarkers of oxidative stress such as antioxidant capacity (FRAP), malondialdehyde (MDA), reduced glutathione (GSH), protein carbonyl (PCO), reactive oxygen species (ROS) and neurolipofuscin in diabetic aging brain of female rats.

Background: The emerging view is that diabetic brain features many symptoms that are best described as accelerated brain aging. Metformin is the most frequently used oral anti-diabetic drug, which apart from hypoglycaemic activity, improves serum lipid profiles, positively influences the process of haemostasis, and possesses anti-inflammatory properties.

Methods: Young (3 months) adult (12 months) and aged (24 months) rats will be diabetic by using alloxan monohydrate. Metformin was administered i.p. at a dose of 200 mg/kg/day for 30 days to both control and diabetic aging rats. A detailed study was carried on expression of glucose transporter, calcium levels, biomarkers of oxidative stress. Morris water maze with expression of synaptic molecules synaptophysin and synapsin I and ultrastructural studies of brain region by magnetic resonance imaging.

Results: Present study shows that there was a similar pattern of increased intracellular calcium levels, neurolipofuscin, MDA, PCO, and ROS levels, and a decrease in levels of FRAP , GSH and (GLUT1, GLUT3) expression in brain of both aging and diabetes. On the other hand, metformin treated groups exhibited significant reduction in helped to reverse the age related changes studied, to normal levels. Metformin treatments improved attention and memory functions with enhanced the levels of synaptic molecules synaptophysin and synapsin I. Our data showed that exogenous administration of Metformin brought these changes to near normalcy in diabetic aging female rats.

Conclusions: The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders including metabolic syndrome and neurodegenerative diseases.

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Differential glial and neuronal response in Lipopolysaccharide induced Parkinson's disease model: Modulation with Apocyanin and Curcumin

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Objective: To understand the differential susceptibility of neuronal and glial populations to lipopolysaccharide (LPS) mediated insult in an animal model of PD. Also, to test the efficacy of apocyanin, a NADPH oxidase inhibitor and curcumin in modulating glial and neuronal response in LPS induced PD model

Background: Converging lines of evidence suggest that glia associated neuroinflammatory processes may account for the progressive death of dopaminergic neurons in Parkinson's disease (PD). Recent studies have also highlighted that glial and neuronal cultures behave differently to a toxic insult and suggests a protective role of glial cells in case of neurotoxicity. Also, Apocyanin, an established microglial NADPH oxidase inhibitor and curcumin has been proved to have beneficial effects in modulating anti-inflammatory as well as anti-oxidative effects in case of lipopolysaccharide induced PD model.

Methods: LPS (5µg/kg b.wt) was injected intranigral using a digital stereotaxic apparatus. Apocyanin (10mg/kg/ day) and curcumin (40mg/kg b.wt) was administered beginning form day 1 of LPS injection intraperitoneally daily for a period of 21 days. At the end of experiment glial and neuronal population was isolated from mid brain region of rat brain.

Results: Following LPS injection significant augmentation in the gene as well as protein expression of transcription factor NFκB as well as proinflammatory cytokines (TNF-α, IL-1α, IL-1β), NADPH oxidase subunits gp91PHOX and gp21PHOX were observed in the glial as well as neuronal fractions thus suggesting

the prevalence of inflammatory responses and activation of NADPH oxidase complex. With this significantly compromised glutathione system as well as other antioxidant enzymes (SOD, Catalase), total ROS production were observed in neuronal and glial fraction. It was analysed that inflammatory and oxidative stress markers in case of glial population was severely altered in comparison to neuronal population. With apocyanin and curcumin treatment marked improvement in inflammatory as well as oxidative stress markers were observed in case of neuronal and glial population.

Conclusions: This can be concluded that apocyanin and curcumin both play an important role in modulating glial cell functions thus revealing its potential anti-inflammatory role along with its NADPH oxidase inhibiting property. Study also suggests a protective role of glial cells by upregulating their transcriptional factors and oxidative stress markers in order to protect neurons against LPS insult. Therefore, its neuroprotective role could be further evaluated in other toxicological conditions.

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The Small Molecule Alpha-Synuclein Misfolding Inhibitor, NPT200-11, Produces Multiple Benefits in an Animal Model of Parkinson's Disease

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Objective: To evaluate effects of NPT200-11 on Parkinson's disease (PD)-relevant outcomes including alpha-synuclein pathology, neurodegenerative markers and motor performance in transgenic mouse models of PD.

Background: Accumulation of alpha-synuclein (ASYN) in neuronal and other CNS cell types may contribute to the underlying pathology of synucleinopathies including Parkinson's disease (PD), dementia with Lewy bodies (DLB) and Multiple Systems Atrophy (MSA). In support of this hypothesis for PD, ASYN immunopositive aggregates are a prominent pathological feature of PD, and mutations and gene multiplications of human wild type (WT) ASYN cause rare familial autosomal-dominant forms of PD. Moreover, aberrant and progressive accumulation of alpha-synuclein (ASYN) pathology correlates with motor and non-motor dysfunction in PD patients. Targeted therapeutics that interfere with the ASYN pathogenic pathway could prevent, stop or slow the neurodegenerative processes in PD and other synucleinopathies.

Methods: NPT200-11, a new generation ASYN misfolding inhibitor, was evaluated in studies with pharmacokinetic, behavioral, neuropathological and in vivo imaging endpoints utilizing wildtype and multiple alpha-synuclein transgenic mouse models of PD.

Results: The bioavailability and brain uptake of NPT200-11 were confirmed in mouse pharmacokinetic studies. Efficacy evaluations in the Line 61 ASYN transgenic mouse model of PD demonstrated benefits of 3 months of 5 mg/kg NPT200-11 treatment on ASYN brain levels, multiple motor endpoints, and downstream neuropathology (including preserved striatal DAT – a potentially translatable finding for clinical development). Longitudinal and repeated in vivo retinal imaging of GFP-tagged ASYN in PDNG78 ASYN transgenic mice over the course of a 3 month study revealed time-dependent reductions in retinal ASYN pathology in 5 mg/kg NPT200-11-treated transgenic mice.

Conclusions: Taken together, these studies provide pre-clinical proof of pharmacological activity and support continued development of NPT200-11 as a disease-modifying treatment for Parkinson's disease.

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Lycopene ameliorates haloperidol induced orofacial dyskinesia in rats: Possible neurotransmitters and neuroinflammation modulation

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Objective: The study was undertakento investigate the role of striatal neurotransmitters and neuroinflammatory cytokines in the neuroprotective effect of lycopene against haloperidol induced orofacial dyskinesia in Wistar rats.

Background: Tardive Dyskinesia is a severe side effect of chronic neuroleptic treatment consisting of abnormal involuntary movements, characterized by orofacial dyskinesia. Lycopene, a potent antioxidant and anti-inflammatory agent has been reported to provide neuroprotection in animal models of other movement disorders.

Methods: Rats were administered with haloperidol (1 mg/kg, i.p for 21 days) to induce orofacial dyskinesia. Lycopene (5 and 10 mg/kg, p.o) was given daily 1 hour before haloperidol treatment for 21 days. Behavioral observations (vacuous chewing movements, tongue protrusions, facial jerking, rotarod activity, grip strength, narrow beam walking) were made on 0th, 7th, 14th, 21st day after haloperidol treatment. On 22nd day, animals were sacrificed and striatum was used for estimation of biochemical parameters (malondialdehyde, nitrite and endogenous enzyme (GSH), proinflammatory cytokines [Tumor necrosis factor, Interleukin 1β, Interleukin 6] and neurotransmitters level (dopamine, serotonin, nor epinephrine, 5-Hydroxyindole acetic acid (5-HIAA), Homovanillic acid, 3,4-dihydroxyphenylacetic acid).

Results: Haloperidol treatment for 21 days significantly impaired muscle co-ordination, motor activity and grip strength with enhanced orofacial dyskinetic movements. In addition, haloperidol treatment significantly increases free radical generation (increases MDA and nitrite levels, decreasing GSH levels) and level of pro-inflammatory cytokines (Tumor necrosis factor, Interleukin 1β, Interleukin 6) whereas decrease the level of striatal neurotransmitters (dopamine, serotonin, nor epinephrine, 5-Hydroxyindole acetic acid (5-HIAA), Homovanillic acid, 3,4- dihydroxyphenylacetic acid). Lycopene (5 and 10 mg/ kg, p.o) treatment along with haloperidol significantly attenuated impairment in behavioral, biochemical, neurochemical and neuroinflammatory markers.

Conclusions: The present study highlight the neuroprotective effect of lycopene and is attributed to antioxidant property, modulation of of neuro-inflammatory cytokines and striatal neurotranmitters level.

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Rotigotine protects dopaminergic neurons by targeting serotonin 1A receptors on astrocytes

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Objective: In this study, we examined neuroprotective effects of rotigotine and involvement of serotonin 1A (5-HT1A) receptors on astrocytes in neuroprotective action of the drug using primary cultured cells and parkinsonian mice.

Background: We previously reported that stimulation of 5-HT1A receptors on astrocytes promoted astrocyte proliferation and upregulated an antioxidative molecule metallothionein (MT) to act as a neuroprotectant in parkinsonian mice. Rotigotine, a dopamine receptor agonist, also possesses a 5-HT1A agonistic property.

Methods: Primary cultured neurons and astrocytes were prepared from the mesencephalon and striata of Sprague-Dawley rat embryos at 15 days of gestation. Cultured astrocytes were treated with rotigotine (1 μM) and/or a 5-HT1A antagonist WAY100635 (10 nM) for 6 h or 24 h to evaluate nuclear translocation of Nrf2 or MT expression respectively. Mesencephalic neurons were treated with conditioned media from rotigotine- and/or WAY100635-treated astrocytes for 24 h followed by 6-hydroxydopamine (6-OHDA). Changes in the number of dopaminergic neurons were examined. The hemi-parkinsonian mice unilaterally lesioned by intrastriatal injections of 6-OHDA were treated with rotigotine (0.125, 0.25 or 0.5 mg/kg, s.c.) for 7 days, and immunohistochemical analyses were performed using brain slices.

Results: Rotigotine treatment induced nuclear translocation of Nrf2 and upregulated MT in astrocytes. Pretreatment with conditioned media from rotigotine-treated astrocytes significantly decreased 6-OHDA-induced dopaminergic neurotoxicity. These effects were completely blocked by 5-HT1A antagonist.

Conclusions: These results suggest that rotigotine exerts neuroprotective effects against dopaminergic neurodegeneration by targeting 5-HT1A receptors on astrocytes.

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Neuroprotective Effects of A Combination of Curcumin and Mucuna Prurience in Rotenone Induced Mice Model of Parkinson's Disease

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Objective: In the current study we have evaluated possible synergistic neuroprotective effect of two Poly-Phenols from Curcumin and Mucuna Prurience. The combine treatment of Curcumin and Methanolic Extract of Mucuna prurience seed was explored for their possible neuroprotective role against rotenone induced behaviourial, mitochondrial and oxidative dysfunction in mice model of Parkinson's disease.

Background: Curcumin and extract of Mucuna prurience are natural polyphenolic, highly effective bioactive compounds. Mucuna prurience is popular Indian medicinal plant which has long been used in ayurvedic system of medicine. They revealed numerous biological and pharmacological activities with effective antioxidant, cardiovascular disease, anticancer, anti-inflammatory effects, antidiabetic, anticholestrolemic, aphrodisiac and neuroprotective properties in cell cultures and different animal models.

Methods: Chronic 21 days administration of rotenone (1 mg/kg i.p.) significantly reduced behavioral parameter (Memory, learning and locomotor activity), antioxidant status (Decreased activity of lipid peroxidation, superoxide dismutase, catalase and reduced glutathione level) and mitochondrial Complex-II-Succinate Dehydrogenase (SDH), Complex III- MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-H-tetrazolium bromide) enzymes activities as compared to normal group of mice.

Results: Combination of Curcumin and extract Mucuna prurience treatment for 21 days showed significant ($P<0.001$) improvement in behavior parameter, oxidative damage and mitochondrial enzyme complex activities as compared to rotenone-treated (negative control) group. The result of present investigation shows that Curcumin and extract of Mucuna prurience in combination restored the motor deficiency and increases the antioxidant and neuroprotective activities.

Conclusions: The finding of present study demonstrates that the role of both Curcumin and extract of Mucuna prurience potential against rotenone-induced Parkinson's in mice and could serve as therapeutic strategies for the management and prevention of Parkinson's Disease.

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Investigation on the neural effects by differing the parameters of electrical stimulation for the relief of parkinsonian symptoms

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Objective: To identify a dominant parameter of galvanic vestibular stimulation (GVS) which affects the neural responses for the relief of parkinsonian symptoms

Background: Typical parkinsonian symptoms are akinesia, rigidity, tremor and postural instability. According to multiple studies, these symptoms of Parkinson's disease (PD) might be relieved by galvanic

vestibular stimulation (GVS). However, it is still elusive which parameter in GVS is a dominant factor to influence the neurological mechanism during the relief. Here, we investigated the neuronal effects by strength and rate of stimulation, which are the core factors to generate GVS.

Methods: Twenty three neurons in the vestibular nucleus (VN) from fifteen guinea pigs (490-594g) were stimulated by GVS and their responses were filtered and recorded (Plexon, US). GVS was designed as three types; 100μA DC with a 60-second resting period (type I), 100μA DC with a 120-second resting period (type II), and 200μA DC with a 60-second resting period (type III). The effect by strength or rate was induced by increasing the DC amplitude or the interval of resting period, respectively. Using the firing rates by each type of stimulations, three responding slopes were calculated. The inner angles between the slopes from type I and II indicated the effect by rate while that between the slopes from type I and III was for the effect by strength.

Results: The inner angles indicated that either modified rate or strength of stimulation affected its relevant neuronal responses, 52.2% and 65.2% by the rate and the strength, respectively. Based on the statistical test, however, the effect by the rate was little ($p=0.34$). On the other hand, that by the strength induced a significant change in the neuronal responses ($p=0.04$).

Conclusions: The strength influenced on the pattern of the neuronal response more than the rate.

References: This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (2010-0020163 & NRF-2016R1D1A1B03930657).

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Emulating the natural timing of dopamine receptor activation in Parkinson's Disease

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Objective: To emulate physiological dopamine receptor activation, in order to restore temporal patterns of dopamine-like signalling and normalise function in the striatum in Parkinson's disease.

Background: L-Dopa is the mainstay therapy for treating Parkinson's disease, however, its duration of effectiveness without side effects is limited to 5-10 years in most people. Much focus has been on smoothing L-Dopa blood levels to presumably stabilise slow fluctuations in brain dopamine levels thought to become prominent as the disease progresses. This approach is successful in select groups of people. However, we hypothesised that improved treatment requires natural patterns of dopamine receptor activation, involving short, phasic events on a background of low tonic levels of dopamine activity. Such phasic activity has been shown to reinforce motor circuits, with a critical temporal conjunction between cortical activity and dopamine release. Thus, inappropriate timing of the dopamine signal following prolonged L-Dopa treatment could reinforce unwanted movements and lead to dyskinesias. We aimed to design technology to emulate the natural dopamine release pattern and evaluate its efficacy in animal models.

Methods: We developed a liposome-based system to carry dopamine receptor agonists systemically to therapeutic target areas. Coupled with a brain non-invasive controller system, we applied electrophysiology, electrochemistry and behavioural approaches to measure agonist release and evaluate cellular effects in target areas in the rat. We have also developed a sheep neurotoxin model, that will allow scale-up to an animal with a similar brain architecture to humans.

Results: We have obtained proof-of-concept of excitatory and neuromodulator effects of phasic receptor activation in striatal spiny neurons recorded in vitro. We have also demonstrated in vivo proof-of-concept of apomorphine release in urethane-anaesthetised rats. Finally, we obtained preliminary data on dopamine-agonist release in behaving animals.

Conclusions: Our preliminary work established proof-of-concept that our system releases dopamine agonists on behaviourally-relevant timescales. Ongoing work will scale up this system to a large animal model of Parkinson's disease and determine efficacy at restoring normal movement.

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Yerba Mate (Ilex paraguaiensis) protects dopaminergic neurons degeneration and improve their maturation in culture

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Objective: To evaluate the effect of Mate leaves extracts on the survival and maturation of dopaminergic neurons in vitro.

Background: Parkinson's disease (PD) is caused by the progressive degeneration of dopaminergic neurons from the SNpc which are particularly susceptible to degenerate by causes still unknown. Some mechanisms such as neuronal activity, calcium homeostasis and oxidative stress are involved in neuronal death. Stimulants, like nicotine and caffeine have been pointed as neuroprotective agents for dopaminergic neurons and their consumption is inversely associated with the development of PD. Similar results have been shown with the intake of "yerba mate" in the Argentinean population (Gatto et al 2015). Yerba mate is a popular beverage in some South American countries and it has been reported to have stimulant effect over the central nervous system.

Methods: We used a validated in vitro model, involving the degeneration of dopaminergic neurons obtained from mouse embryos. On these cultures we tested different doses of an extract of yerba mate obtained from dried leaves. Cells were fixed and immunostained with an anti-TH antibody to determine the number and morphology of dopaminergic neurons. We have also analyzed and quantified by HPLC the main components of the yerba mate extract and tested them separately in the same model.

Results: We found that yerba mate extract induces a strong neuroprotective effect on dopaminergic neurons as well as a dramatic increase in their dendritic development. We identified that two of the components highly present in yerba mate, theobromine and chlorogenic acid, showed neuroprotective effect but weaker than the whole extract. In parallel we have seen that the neuroprotective effect of caffeine (another important compound present in the extract) is modest, in comparison with the whole extract, but similar in percentage to that described before.

Conclusions: We have demonstrated a neuroprotective effect of Yerba mate over dopaminergic neurons in vitro, providing a biological support to the previous population-based study. Our data suggest that this effect is not due solely to caffeine contents, therefore, we propose yerba mate as a novel natural compound with putative strong neuroprotective activity, which provide new insights to understand the mechanism of neuronal survival.

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The anti-dyskinetic effect of the clinically-available 5-HT3 receptor antagonist granisetron in the 6-OHDA-lesioned rat model of Parkinson's disease

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Objective: To determine the effect of the serotonin 3 (5-HT3) receptor antagonist granisetron, a clinically-available anti-emetic, on L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia.

Background: Dopamine replacement with L-DOPA is the most effective symptomatic treatment for Parkinson's disease (PD), however, long-term therapy leads to dyskinesia. 5-HT3 receptor blockade reduces dopamine levels in the striatum, which suggests that 5-HT3 receptor antagonists could mitigate the aberrant release of dopamine that occurs in dyskinesia, thereby diminishing the severity of dyskinesia in PD. In the present study, we hypothesised that granisetron would alleviate dyskinesia in the 6-hydroxydopamine (6-OHDA)-lesioned rat.

Methods: Following 6-OHDA lesion of the right medial forebrain bundle, rats underwent the cylinder test to assess the degree of parkinsonism. Severely parkinsonian rats were selected for priming with L-DOPA

to exhibit stable and reproducible abnormal involuntary movements (AIMs). On experimental days, granisetron (0.0001, 0.001, 0.01, 0.1 or 1 mg/kg) or vehicle was administered in combination with L-DOPA, and duration and amplitude of axial, limbs and oro-lingual (ALO) AIMs severity were evaluated. Following a 2-day washout period, preference for the un-lesioned forelimb was measured by the cylinder test to assess the effect of granisetron on L-DOPA anti-parkinsonian action.

Results: In combination with L-DOPA, granisetron 0.01 mg/kg significantly diminished both duration and amplitude of ALO AIMs by 46% and 50%, respectively ($P < 0.05$ and $P < 0.01$), compared to vehicle. Moreover, the anti-dyskinetic effect of granisetron was achieved without hindering L-DOPA anti-parkinsonian action.

Conclusions: Our results suggest that granisetron is a potential drug candidate to effectively alleviate L-DOPA-induced dyskinesia without impairing the therapeutic efficacy of L-DOPA. Because it is already available in the clinic, our results could quickly lead to clinical trials. Moreover, coupled with the companion Abstract on ondansetron, our results suggest that selective 5-HT3 receptor blockade is a potentially effective anti-dyskinetic approach likely to be well tolerated by PD patients.

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Inhibition of HDAC4 attenuates rotenone-induced abnormal increase of α-synuclein levels and affects autophagic flux in human dopaminergic SH-SY5Y cells

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Objective: To explore the effects of selective inhibition of HDAC4, on levels of α-syn and autophagy-related proteins in PD. To discuss the mechanism for the neuroprotective effects of HDAC inhibitors, to find a way to regulate autophagy and reduce neurotoxicity of α-syn moderately, and to give a new clue to the targeted therapy in PD.

Background: Parkinson's disease (PD) is one of the most common neurodegenerative diseases, and its predominant pathologies is the pathogenic aggregation of α-synuclein (α-syn) in vulnerable neurons. One of the degradation pathways of α-syn is the autophagy/lysosomal pathway, which is partially regulated by the acetylation of histones. Meanwhile, HDACs also affect the activation of autophagy. Multiple HDAC inhibitors have been proved to have neuroprotective effects in PD models. However, the HDAC inhibitors used in most present studies have broad spectrums and block multiple HDACs in a time. Thus it is difficult to figure out the specific mechanism implicated in their neuroprotective effects, as well as their complicated side effects and neurotoxicity.

Methods: Mc1568 was used to inhibit HDAC4 in rotenone-treated SH-SY5Y cells; then expression levels of α-syn were analyzed by Western blot, and cell viability was analyzed by Cell Counting Kit-8 (CCK-8). HDAC4 was knocked-down or overexpressed in SY5Y cells by shRNA or inserted HDAC4 sequence; after treatment with rotenone, expression levels of α-syn and autophagy-related proteins (such as LC3, Beclin-1 and p62) were analyzed by Western blot.

Results: Rotenone induced a significant increase in α-syn levels in SH-SY5Y cells. Mc1568 reduced α-syn in rotenone model in a dose-dependent manner. The overexpression of HDAC4 repressed the expression of autophagy-related proteins including LC3-Ⅱ and Beclin-1 in rotenone-treated SH-SY5Y cells, while the knockdown(KD) of HDAC4 upregulated the expression of autophagy-related proteins, and significantly abolished the abnormal increase in α-syn levels induced by rotenone.

Conclusions: The selective inhibition of HDAC4 had neuroprotective effects in cellular model of PD. HDAC4 repressed the autophagy pathway, and promoted the accumulation of α-syn in rotenone-induced cellular model of PD. However, Mc1568 reversed the abnormal increase in α-syn levels. Knockdown of HDAC4 expression also promoted the degradation of α-syn by activation of autophagy, and therefore abolished the rotenone-induced neurotoxicity.

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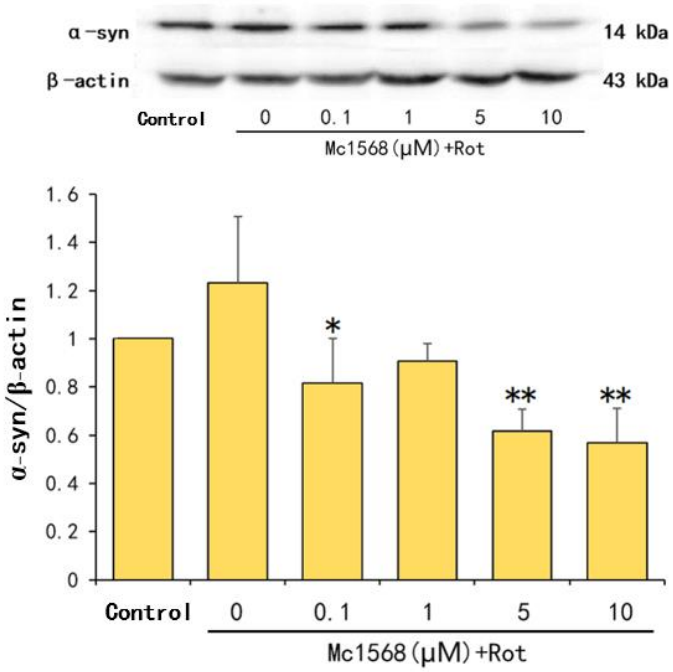

FIG. 1 (263) Mc 1568 reduced α-syn in rotenone model in a dosedependent manner.

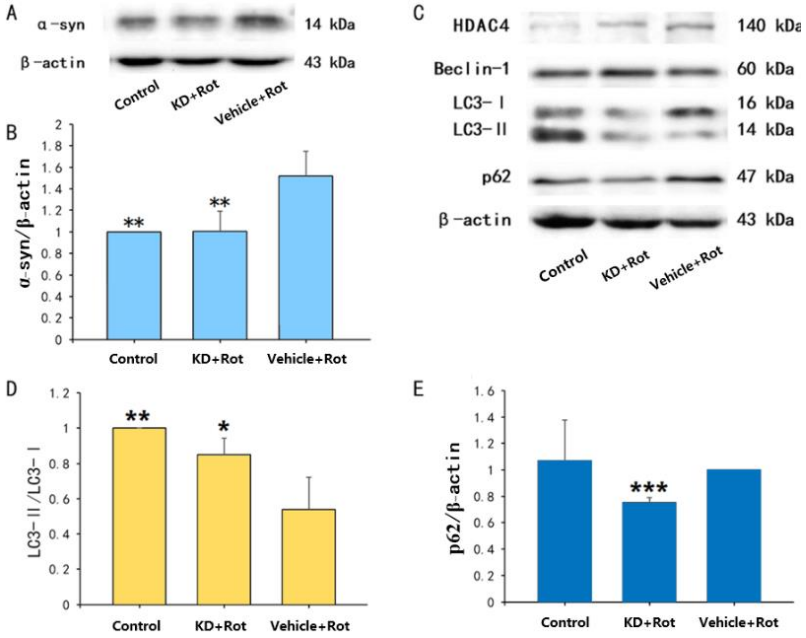

FIG. 2 (263) Rotenone induced a significant increase in α-syn levels in SH-SY5Y cells ("Vehicle+Rot" vs. "Control") The knockdown(KD) of HDAC4 upregulated the expression of autophagy-related proteins, and significantly abolished the abnormal increase in α-syn levels induced by rotenone.

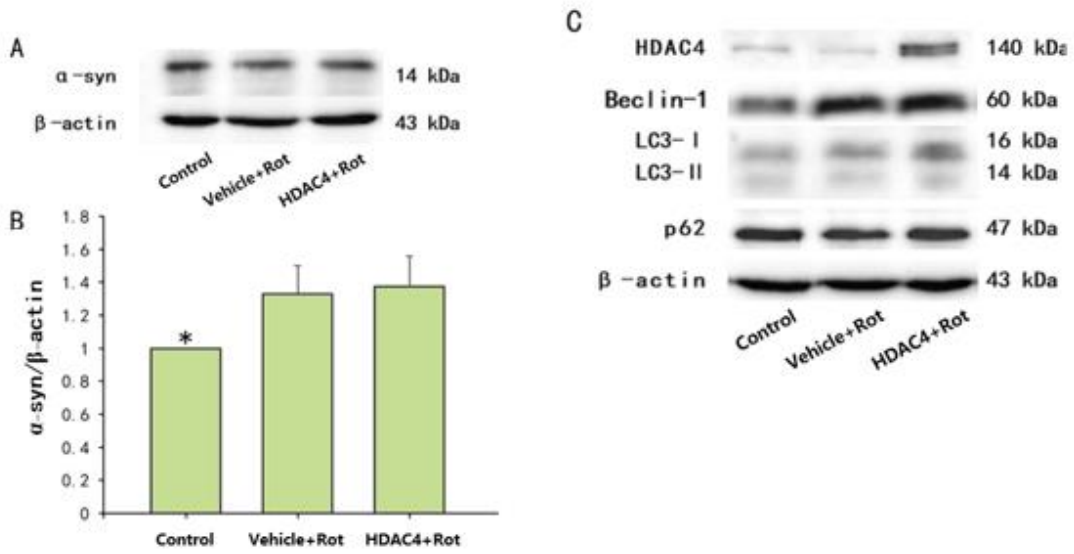

FIG. 3 (263) The overexpression of HDAC4 repressed the expression of autophagy-related proteins including LC3-II and Beclin-1 in rotenone-treated SH-SY5Y cells.

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Development of anti-amyloid drugs for Parkinson's disease by combination screening of high-throughput in vitro assay and phenotype-based C. elegans system

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Objective: To establish a new combination screening system consisted of high-throughput in vitro assay with phenotype-based in vivo assay and to develop anti-amyloid drugs truly effective for Parkinson's disease.

Background: Amyloid fibril made of alpha-synuclein (aSyn) is a pathological hall mark of Parkinson's disease (PD) and one of a good target for disease modifying therapy. Several compounds have been analyzed in vitro and reported to have an anti-amyloid effect to aSyn. However, few studies have performed high-throughput screening, because the growth of aSyn fibril is very slow and highly variable. In addition, few studies have performed second in vivo screening to confirm the effect of hit compounds, only focused on one or two compounds interested in. To overcome these limitations, we have developed a new combination screening system of high-throughput in vitro screening followed by in vivo phenotype-based second screening.

Methods: ASyn was purified as previously described (1). Thioflavin T was employed to monitor the kinetics of aSyn fibril formation.To obtain more uniform and faster kinetics, we used zirconia beads. We modified the multi-plate reader to control the shaking speed and interval. We employed 1263 FDA approved compounds library and 20 drugs approved for PD. After the first screening, we performed phenotype-based second screening using C. elegans model. Worms were incubated with drugs and the locomotion were analyzed by high-throughput Worm-Tracker system. Number of synuclein aggregations in the worms were also calculated.

Results: Our revised in vitro screening system showed an excellent value of Z' factor (> 0.7). Among 1280 drugs, we obtained 19 hit compounds inhibit synuclein aggregation in vitro. Interestingly, some of them were FDA approved drugs for PD or Alzheimer's disease. Second screening by C. elegans revealed that one of the PD drug inhibit synuclein aggregation in vivo and recovered the motor phenotype of the worm.

Conclusions: We have found that one drug approved for PD inhibited not only aSyn aggregation in vitro but also modified the C elegans model. It is currently planned to investigate the longitudinal changes in CSF before and after the treatment with the drug using our new device 'HANABI' (presented in International Congress of MDS 2017 (Ikenaka et al.) and 2018 (Kakuda et al.)).

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Level of serum Mortalin in Parkinson's disease correlating with □-Synuclein: Assessment of protein marker for early detection

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Objective: This study aims to quantify and correlate Mortalin and α-Syn protein in serum of Parkinson's disease
patients and develop it as protein marker for early detection of Parkinson's disease.

Background: Mortalin is associated with Parkinson's disease (PD) both physiological as well as clinical. Mortalin, a mitochondrial chaperone, plays significant role in reducing toxicity of lewy bodies. The earlier study reported lower expression of Mortalin in different models of PD patients. In PD, increased level of Mortalin suppresses the toxicity of aggregated α-Syn (1)

Methods: The expression of Mortalin and α-Syn in serum samples of 38 PD patients and 33 control group (CG) individuals were estimated by surface plasmon resonance (SPR). The receiver operating characteristic (ROC) curves were constructed to develop it as blood based protein marker. The level of Mortalin in serum was confirmed by western blot.

Results: The Mortalin level was found to be downregulated in PD patients (1.98±0.53 ng/μL) in comparison to CG individuals (3.13±0.48 ng/μL) whereas α-Syn level was found to be upregulated in PD patients (38.20±4.22ng/μL) than CG individuals (34.31±3.23 ng/μL) in serum. The negative correlation between Mortalin and α-Syn was revealed by statistical analysis

Conclusions: This preliminary study concluded that Mortalin plays a crucial role in PD with negative correlation with α-Syn. This study provides a new platform for the development of Mortalin as a potent serum protein diagnostic marker for PD.

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Challenging mitochondria in idiopathic Parkinson‘s disease fibroblasts

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Objective: Evaluate mitochondrial integrity in cultivated fibroblasts of patients with idiopathic Parkinson's disease (IPD) and healthy controls (HC).

Background: Mitochondrial dysfunction (MD) is considered an essential contributor to IPD pathophysiology and could become a future treatment target. Although MD is probably not limited to the basal ganglia, studies on other tissues have so far produced conflicting data. We had initiated a three cell type evaluation of MD covering platelets (doi:10.1002/acn3.151), submucosal enteric ganglia neurons (doi:10.1038/srep33117), and fibroblasts. Here, we report the results in IPD fibroblasts.

Methods: 41 IPD patients (mean disease duration: 6.5 years (st.dev. 5.5 years) and 21 healthy age-matched controls were recruited. Punch skin biopsy was performed and mitochondrial membrane potential (Ψm) was measured via TMRM assay and automated microscopy before and after challenging with the protonophore FCCP.

Results: [figure1]

Mitochondrial challenge test: (A) pooled single cell data histograms, (B) individually averaged data. At single cell level, basal Ψm is significantly higher in IPD than in HC ($p<0.001$). Under FCCP stress, the median cellular Ψm per person is also significantly increased in IPD patients.

Conclusions: The fibroblasts of IPD patients show increased mitochondrial membrane potential both at the basal state and after challenging with a potent protonophore. These findings are counterintuitive at first look but may reflect compensatory mechanisms upstream of mitochondrial quality control. Indeed, the maintenance of mitochondrial membrane potential could prevent the triggering of mitochondrial degradation via the lysosomal pathway.

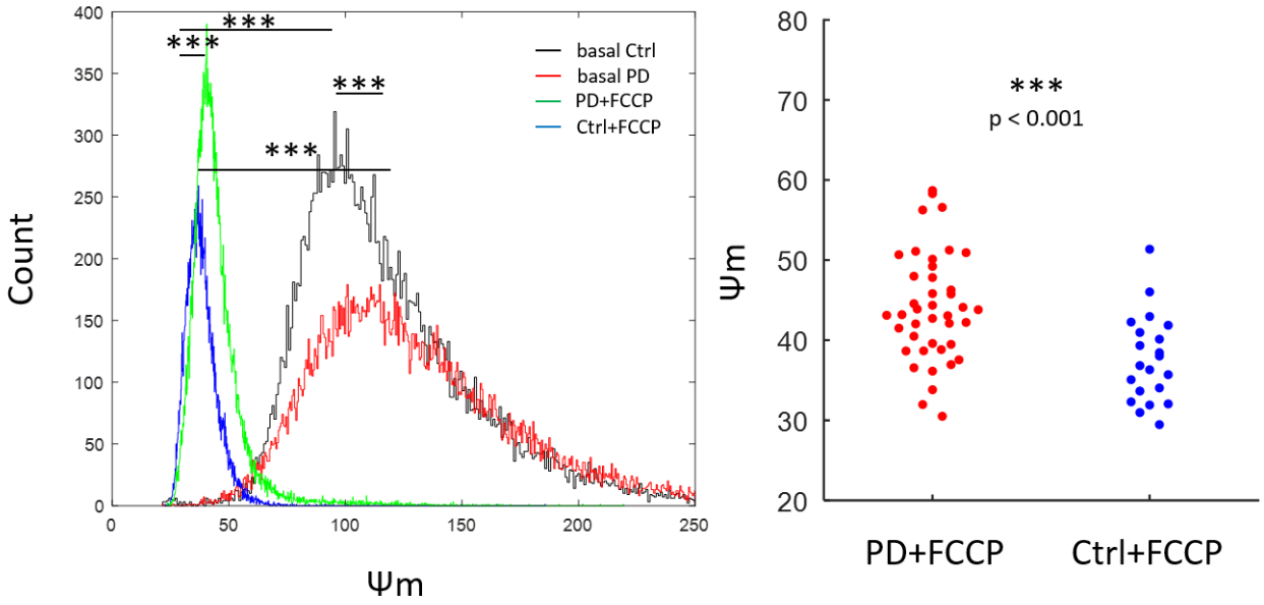

FIG. 1 (281)

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A novel mGlu4 PAM alleviates motor symptoms in primate models of PD and of LID

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Objective: Objective was to assess the therapeutic potential of our novel mGlu4 positive allosteric modulator (PAM), foliglurax, as an anti-parkinsonian treatment in gold-standard primate models.

Background: Levodopa remains the gold standard treatment for Parkinson's Disease (PD). However, as the disease progresses, dopaminergic treatments become less effective and produce debilitating side effects, including motor fluctuations and levodopa-induced dyskinesia (LID). Over the past decade, modulation of presynaptic metabotropic glutamate receptor 4 (mGlu4) has been proposed as a promising anti-parkinsonian approach but it has never been demonstrated in primates so far.

Methods: Foliglurax (PXT002331) was tested in three models of MPTP-induced parkinsonism in macaques: early stage, advanced parkinsonism and LID. Brain penetration of the compound has also been assessed using PET imaging in macaques.

Results: Foliglurax demonstrated consistent anti-parkinsonian efficacy in all models. Co-administration of foliglurax and levodopa resulted in a robust and dose-dependent reversal of parkinsonian motor symptoms in macaques. Moreover, foliglurax strongly decreased dyskinesia induced by levodopa, thus having therapeutic efficacy on both aspects: parkinsonian motor symptoms and LID.

Conclusions: This is the first demonstration that a mGlu4 PAM can alleviate the motor symptoms of PD and the motor complications induced by levodopa in primates. Supported by its unique preclinical profile, foliglurax has been the first mGlu4 PAM entering the clinics and is now being tested in a Phase IIa study.

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Combination Therapy of Ellagic Acid and Mucuna Pruriens Seeds Extract Improves Rotenone Induced Behavioural, Oxidative and Mitochondrial Deficits in Mice Model of Parkinson's Disease

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Objective: The present study was designed in order to explore the possible synergistic effect of two highly naturally occurring bio-active compounds viz. Ellagic acid (EA) and Methanolic extract of Mucuna Pruriens Seeds (MPM). The neuroprotective role of combinational therapy of EA and MPM was explored in rotenone induced behavioural, oxidative and mitochondrial dysfunction in mice model of Parkinson's disease.

Background: Ellagic acid (EA) and Mucuna Pruriens, are natural polyphenolic, powerful bioactive compounds used word wide. They exhibited numerous biological and pharmacological activities including potent antioxidant, cardiovascular disease, anticancer, anti-inflammatory effects and neurodegenerative disorders in cell cultures and animal models.

Methods: Chronic administration of rotenone (1 mg/kg i.p.) for a period of three weeks significantly impaired behavioural paradigm (Memory, learning and locomotor activity), oxidative defence (Decreased activity of superoxide dismutase, catalase and reduced glutathione level) and mitochondrial Complex-II-Succinate Dehydrogenase (SDH), Complex III- MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyl-H-tetrazolium bromide) enzymes activities as compared to normal control group in the brain of mice.

Results: Three weeks of EA and MPM combination (50, 100 and 200 mg/kg, p.o) treatment significantly improved behaviour parameters ($P < 0.001$) oxidative damage ($P < 0.001$) and mitochondrial enzyme complex activities (< 0.05, $P < 0.01$, $P < 0.001$) as compared to negative control (rotenone treated) group. We found that combination of Cur and MPM restored motor deficits and enhanced the activities of antioxidant enzymes suggesting its antioxidant and neuroprotective potential in vivo.

Conclusions: The findings of present study concludes neuroprotective role of combination of EA and MPM against rotenone induced Parkinson's in mice and offers strong justification for the therapeutic prospective of these compound in the management of PD.

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Contribution of ATP-sensitive potassium channels in the subthalamic nucleus neurons towards Parkinson's disease

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Objective: This study describe a quantitative investigation to explore the modulating effects of the ATP-dependent Potassium channel (KATP) ion channel conductance on spike patterns in subthalamic nucleus (STN) neurons towards Parkinson's disease (PD).

Background: It has been observed that deep brain stimulation (DBS) at the STN neuron site improves the PD as a new, effective and efficient treatment method. The firing properties of STN neurons in terms of single-spike or the burst modes modulate pathophysiological conditions in PD. Ion channel play dominant role in regulating firing properties of STN neurons.

Methods: Here the interpretation of KATP ion channel is based on conventional Hodgkin-Huxley formalism. Then the KATP ion channel model is incorporated into a published STN electrophysiological model. A brief square pulse of varied duration and magnitude is applied as an external stimulus current (Istim) to trigger action potential (AP) in the whole cell model. Then ATP internal concentration is varied to investigate the modulated response in AP and resting membrane potential.

Results: Voltage clamp protocol is used to generate KATP current for various test potentials in our model for validation. The electrical activities are generated in the whole cell model by applying a brief square pulse

of varying magnitude (0.1-0.6nA) and duration (1-5ms). Then, we investigated the modulating effects of KATP current in two ways. First, we increased the KATP channel maximum conductance by 50% of its control value to get the promising effects in action potential. Then, we reduced the ATPi (intracellular free ATP concentration) to 0.0001mM to get the same effects in action potential. The opening of KATP channel reduced the resting membrane potential from ─65mV to ─69mV before generation of the AP by the injected current in STN cell model. The rising phase of the AP is shifted towards right due to this hyperpolarization. Again, the negative peak of the action potential hyperpolarization is more negative due to KATP current.

Conclusions: The opening of KATP channel reduced the resting membrane potential from ─65mV to ─69mV before generation of the action potential by the injected current in STN cell model. As the KATP channel openers hyperpolarize the STN cell membrane by increasing K+ ion permeability, reduce the initiation of spontaneous action potentials to bursting patterns, the pharmacological targeting of these channels may shed light on treatment of the PD.

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Putative treatment of Parkinson's Disease using extract of Bacopa monnieri

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Objective: Bacopa monnieri is largely treasured as a revitalizing herb used by Ayurvedic medical practitioners for almost 3000 years. The herb has been mentioned in several Ayurvedic treatises including Charaka Samhita and Sushruta Samhita in the 3rd century AD. Bacopa monnieri may improve circulation to the brain and even protect brain cell hence could be used as a potent agent against development of PD.

Background: Parkinson's Disease (PD), is the contemporary name for a degenerative brain disease that has been recorded by humans. In ancient India, the practitioners of the medical system Ayurveda called it Kampavada. Bacopa monnieri is used in India since ancient times is also a day to day use herb.

Methods: A total of 50 adult male Wistar rats (NGBU, Laboratory Animal Center), weighting 200 g, were used. All model rats received 6-OHDA injection (6-OHDA injection: 6-OHDA, vitamin C powder, 0.3% 6-OHDA injection according to the ratio of 2.5:1 with saline. The injection was used when preparation and stored sealed, low temperature, and dark) stereotactic brain injection. After 6-OHDA administration 1d, 7d and 14d, 10 rats per group were observed every time. When the symptoms of PD progressed, we applied Bacopa monnieri seed powder extract in water to rodent models of Parkinson's.

Results: Bacopa monnieri extract exhibits interesting antioxidant properties, expressed by its capacity to scavenge superoxide anion and hydroxyl radical, and to reduce H2O2 induced cytotoxicity and DNA damage in human fibroblast cells. Results showed promise in conquering the disease and protecting the brain from damage by the antioxidant activity of the extract in the hippocampus, frontal cortex and striatum.

Conclusions: Bacopa monnieri helps in coping with combined hypoxic, hypothermic and immobilization stress that could lead to the onslaught of 'free radicals' during the progress of PD.

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Neurorestorative effects of glycine transporter 1-inhibitor and D-Serine in a mouse model of nigrostriatal dopaminergic degeneration

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Objective: We set out to evaluate potential neurorestorative effects of the GlyT1 inhibitor ACPPB vis-à-vis D-Serine in the intrastriatal 6-OHDA mouse model of Parkinson's disease. Efficacy endpoints included:

i) amelioration of animals' motor performance; ii) regrowth of dopaminergic fibers into the highly denervated motor region of the striatum.

Background: Synaptic NMDA glutamate receptor activity is required to stimulate axonal sprouting. Glutamate-induced NMDA receptor activation requires a glycine modulatory site to be occupied by either Glycine or D-Serine. A previous study has shown that pharmacological inhibition of the Glycine transporter (GlyT) stimulates sprouting of dopaminergic axons both in vitro and in vivo (Schmitz et al. J Neurosci 2013).

Methods: Mice sustained unilateral 6-OHDA injections in the lateral striatum. Treatment with either ACPBB or D-Serine was started 3 weeks post-lesion and was administered 3 times per week for 5 weeks (subcutaneous injections at doses previously tested in mice). Tests of forelimb use (cylinder test) and postural asymmetry (spontaneous rotations test) were carried out at the end of each treatment week; one test of sensorimotor neglect (corridor test) was performed at the end of the treatment. Brains were processed immunohistochemically to analyse striatal tyrosine hydroxylase (TH)-positive fibers and nigral dopamine cells.

Results: Both compounds significantly ameliorated forelimb use and sensorimotor neglect. Improvements occurred particularly during the last 2 weeks of treatment, suggesting a dependence on neurorestorative effects that are time-dependent. No improvement was seen in the spontaneous rotation test (a behavior highly dependent on nigral dopamine levels). Optical density analysis of striatal TH-positive fibers revealed a pronounced restorative effect by D-Serine. Mice treated with ACPPB showed trends towards an increased striatal TH optical density. Stereological counts of nigral dopamine cells compacta did not reveal group differences. Striatal dopamine fiber growth is currently being examined using sensitive methods.

Conclusions: Both of the compounds under investigation produced functional neurorestoration. Our results provide a rationale for considering this class of compounds for clinical evaluaton in patients with PD.

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Surface mechanised nano-formulation of naringenin as adenosine A2A receptor antagonist against in Neurological associated problem of Parkinson

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Objective: In the current experimental study, we intended to develop Surface mechanized nano-formulation of naringenin (NG) as potent A(2A)AR antagonists against parkinson's disease.

Background: One of the most common neurological problem which the world faces is Parkinson Disease (PD) associated progression, neurological degeneration and finally hampering the motor neuron with loss of drug action and expansion of disease. The need of hour is to go for novel chemicals entities in this disease. Adenosine receptors are the target molecules (A(2A)ARs) by researchers and opens different avenues in this field. Reactive oxygen or free radicals plays a significant role in pathogenesis of this disease .Naringenin (NG) is a free radical scavenger that can be used to antagonised the (A(2A)ARs) for the treatment of Parkinson Disease.

Methods: Emulsion solvent technique was employed for the development of NG loaded Nanoparticles of PLGA (NG-PLGA-NP). Drug release, particle size, poly-disparity and zeta potential were measured. Molinspiration software was used for elucidation of Lipiski's Rule of 5. NG was subsequently appraised for invitro anti-Parkinson's activity via free radical scavenging assay. Adenosine A2A receptor crystal structure was undertaken with the aim of selectively antagonize its effect.

Results: The prepared NG nano-formulation gave the outcome with spherical small surface with relative narrow distribution of the nanoparticles. The bioavailability of NG was in line with the Drug likeness recommendation for new entity of Chemical (NCE). It is observed that anti Parkinson activity of NG have 97% potential against DPPH free radical, respectively. NG selectively and catagorily interact with Ile80, Phe168, Ile66, Ala59, Ile274 and Tyr271 receptor at Ki ranging from 495.34nM to 5.04µM to mark adenosine A2A receptor interaction.

Conclusions: In short we can state that surface nano formulation of NG can provide some potential beneficial effect against PD by scavenging free radicals with best bioavailability.

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Novel COMT inhibitor opicapone shows sustained inhibition and improved L-DOPA availability in monkeys

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Objective: To evaluate the effect of repeated OPC treatments, COMT inhibition versus plasma opicapone level, dose response of COMT inhibition, and systematic and central bioavailability of L-dopa in cynomolgus monkeys.

Background: L-dopa continues to be the most effective agent for the symptomatic treatment of Parkinson's disease. Even fast metabolic elimination is inhibited by decarboxylase inhibitors, levodopa metabolism is predominantly shifted to COMT. Opicapone (OPC) is a high binding affinity and long-acting third generation nitrocatechol COMT inhibitor. The long-acting COMT inhibition was not based on pharmacokinetics improvement but considered to be depend tight-binding inhibition characteristics.

Methods: Adult cynomolgus monkeys (Macaca Fascicularis), 3-6 years old and weighing 2.4 - 4.5 kg were used. Subjects were dosed for 14 days with OPC (1-100 mg/kg/10ml/day, po) with vehicle, 0.2% hydroxypropylmethylcellulose. COMT activity was determined in erythrocytes. Plasma opicapone concentration was quantified with by LC/MS. Levodopa was quantified in deproteinized plasma by HPLC-ED.

Results: OPC was rapidly absorbed and eliminated, and not accumulated. In contrast, OPC continuously decreased COMT activity, >90% inhibition at maximum and ~60% at 24 h on day 1, and further inhibited COMT after repeated treatment. OPC increased L-dopa exposure by 2-fold in plasma and >1.4-fold in the brain, without affecting Cmax. OPC showed sustained COMT inhibition, and markedly increased systemic and central L-dopa bioavailability.

Conclusions: Opicapone (OPC) was rapidly absorbed and eliminated after oral administration, but showed sustained COMT inhibition, and markedly increased systemic and central L-dopa bioavailability. The increase of L-dopa bioavailability was accompanied with a shift in L-dopa tmax (time to Cmax) to a later time, but without significantly affecting L-dopa Cmax levels. The data suggests that OPC fulfils the unmet need for sustained COMT inhibition which will improve levodopa bioavailability in patients with Parkinson's disease.

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Combined administration of A2A receptor antagonist and 5-HT1A/1B receptor agonist reverses neuroinflammation in the 6-OHDA model of Parkinson's disease

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Objective: To investigate whether in a model of Parkinson's Disease (PD), an early treatment with the serotonin 5-HT1A/1B receptor agonist eltoprazine and the adenosine A2A receptor antagonist preladenant counteract neuroinflammation correlated to dyskinetic movements induced by L-dopa.

Background: Several evidence have implicated neuroinflammation in PD progression and L-dopa-induced dyskinesia 1. The serotonin 5-HT1A/1B receptor agonists suppressed L-dopa-induced dyskinetic movements in the unilateral 6-hydrossidopamine (6-OHDA)-lesioned rat model of PD, reducing, however, L-dopa efficacy 2. In contrast, the adenosine A2A receptor antagonists, which has neuroprotective effects, increased L-dopa efficacy without exacerbating dyskinetic-like behavior in animal models of PD 3. Our previous report demonstrated that combination of eltoprazine, with preladenat produced prevention and reduction of L-dopa-induced dyskinesia, without impairing the efficacy of L-dopa in relieving motor symptoms 4.

Methods: Unilateral 6-OHDA-lesioned rats, were sub-chronically treated with eltoprazine and/or preladenant, alone or in combination with L-dopa, and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated. Four days after the last drugs administration all rats were treated with L-dopa or vehicle. The immunoreactivity (IR) for the glial fibrillary acidic protein (GFAP), and the co-localization of the ionized calcium binding adaptor molecule 1 (IBA1), with interleukin (IL)-1β, tumor necrosis factor-α (TNF-α) and IL-10, were evaluated in the denervated caudate-putamen (CPu) and substantia nigra pars compacta (SNc). Finally, the IR for tyrosine hydroxylase (TH) and the dopamine (DA) transporter (DAT) was quantified.

Results: Combined pre-treatment with L-dopa plus eltoprazine plus preladenant induced a reduction of basal GFAP and IBA1 IRs in CPu and SNc. Moreover, a reduction of IL-1β in IBA1-positive cells in CPu and SNc and of TNF-α in IBA1-positive cells in SNc was observed. Besides, a significant increase in IL-10 in IBA1-positive cells was also observed in SNc. Finally, a significant reduction of DAT and TH IRs was found in all the experimental groups.

Conclusions: The present findings indicate that the combined administration of L-dopa plus eltoprazine plus preladenant reduced the neuroinflammatory responses in the nigrostriatal system of 6-OHDA-lesioned rats.

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Ondansetron, a highly-selective 5-HT3 receptor antagonist, alleviates L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease

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Objective: To determine the effectiveness of selective serotonin 3 (5-HT3) receptor blockade at reducing the severity of established, and preventing the development of, dyskinesia.

Background: L-3,4-dihydroxyphenylalanine (L-DOPA) therapy is the most effective treatment for Parkinson's disease, but with chronic L-DOPA administration, complications such as dyskinesia emerge. It has been shown 5-HT3 blockade reduces dopamine levels within the basal ganglia, suggesting that it could potentially lead to a reduction of dyskinesia. Here, we hypothesised that 5HT3 blockade would effectively alleviate L-DOPA-induced dyskinesia.

Methods: Rats were rendered hemi-parkinsonian by injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle. The degree of parkinsonism was assessed by the cylinder test. Two sets of experiments were then conducted. In the first set of experiments, rats were primed with L-DOPA to elicit stable and reproducible axial, limbs and oro-lingual (ALO) abnormal involuntary movements (AIMs), after which they were administered acute challenges of ondansetron (0.0001, 0.001, 0.01, 0.1 or 1 mg/kg) or vehicle in combination with L-DOPA and the severity of ALO AIMs was rated. In the second set of experiments, following 6-OHDA lesion, rats were administered ondansetron 0.0001 mg/kg or vehicle, started concurrently with L-DOPA, once daily for 22 days, during which the severity of ALO AIMs was assessed regularly. After a 2-day washout period, an acute L-DOPA challenge was administered and ALO AIMs severity was assessed. The effect of ondansetron on L-DOPA anti-parkinsonian action was also determined by the cylinder test.

Results: The addition of ondansetron 0.0001 mg/kg to L-DOPA resulted in a significant decrease of ALO AIMs duration and amplitude, by 53% and 51%, respectively (both $P < 0.01$), when compared to vehicle. Ondansetron, when started concurrently with L-DOPA, also attenuated ALO AIMs by 51% ($P < 0.05$), when

compared to vehicle. The anti-dyskinetic effect of ondansetron was achieved without impairing L-DOPA anti-parkinsonian action.

Conclusions: Our results suggest that the potent, highly-selective and clinically-available 5-HT3 antagonist ondansetron is a promising molecule to reduce the severity, and attenuate the development, of L-DOPA-induced dyskinesia.

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The highly selective 5-HT2A receptor antagonist EMD-281,014 alleviates L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease

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Objective: To investigate the effect of the highly-selective serotonin 2A (5-HT2A) receptor antagonist EMD-281,014 at alleviating L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia in the 6-hydroxydopamine (6-OHDA)-lesioned rat.

Background: Chronic administration of L-DOPA, the most effective symptomatic treatment for Parkinson's disease (PD), leads to motor complications such as dyskinesia in as many as 95% of patients. Previous studies have suggested that antagonising 5-HT2A receptors may alleviate dyskinesia in animal models of PD, but the drugs assessed interacted with targets other than 5-HT2A receptors and, as such, a 5-HT2A-selective mechanism may not be the only factor underlying their effectiveness. Here, we hypothesised that EMD-281,014, a clinically-ready compound that is currently the most selective 5-HT2A antagonist available, may effectively decrease dyskinesia in PD.

Methods: Rats were rendered hemi-parkinsonian by administration of 6-OHDA. They were then primed with L-DOPA to elicit stable and reproducible axial, limbs and oro-lingual (ALO) abnormal involuntary movements (AIMs). On experimental days, rats were administered L-DOPA in combination with EMD-281,014 (vehicle, 0.01, 0.03 and 0.1 mg/kg), after which ALO AIMs were assessed for 1 min, every 20 min, for 180 min. After a 72h washout period, animals were administered an acute challenge of EMD-281,014 in combination with L-DOPA and the degree of parkinsonism was assessed with the cylinder test.

Results: In combination with L-DOPA, EMD-281,014 mildly, but significantly, diminished the amplitude of dyskinesia, when compared to vehicle. Thus, EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) significantly reduced cumulative ALO AIMs amplitude, when compared to vehicle, by 20%, 14% and 13%, respectively (all $P < 0.01$). EMD-281,014 (0.01 and 0.1 mg/kg) also reduced cumulative axial AIMs amplitude, by 21% and 18% (both $P < 0.05$), respectively, when compared to vehicle. Lastly, EMD-281,014 (0.01 and 0.1 mg/kg) diminished cumulative limbs AIMs amplitude, by 40% and 20% (both $P < 0.05$), respectively, when compared to vehicle. Importantly, EMD-281,014 did not hinder the anti-parkinsonian action of L-DOPA.

Conclusions: These results suggest that the highly-selective 5-HT2A antagonist EMD-281,014 is a promising drug to reduce the severity of dyskinesia in PD.

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Quantifying the Reproducibility of an In Vivo Assay: Examination of Historical Amantadine Effects in the macaque Model of L-DOPA-Induced Dyskinesia

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Objective: This study was designed to assess the reproducibility of the amantadine anti-dyskinetic effect in the MPTP L-DOPA-Induced Dyskinesia (LID) macaque model for soundly defining the sample sizes required to test anti-dyskinetic compounds.

Background: Dyskinesia are side effects associated with chronic L-dopa treatment in Parkinson's patients that are partially alleviated by the weak NMDA antagonist amantadine. Although the current benchmark, response to amantadine is variable and effective only in a subset of patients. While recognizing that the MPTP macaque model is the gold-standard pre-clinical translational model, we provide critical information for designing and powering studies for testing of novel anti-dyskinetic strategies.

Methods: We conducted the meta-Analysis of 11 studies (n ranging from 7 to 24 individuals) involving NHPs treated with vehicle and amantadine in combination with levodopa. The effect was calculated by the difference in total dyskinesia score between amantadine and vehicle. The primary objective was to quantify the reproducibility of the study responses and to understand the variability present between studies and between animals within the studies. Secondly, we determined the expected effect size and variation to ensure appropriate statistical design and power for future studies with new compounds.

Results: The mean profiles over all animals in all studies show a reasonable efficacy window between vehicle and amantadine response profiles. However, the profiles in individual studies are less consistent. The meta-analysis of the effect suggested a study-to-study heterogeneity (effect from -2.6 ± 1.45 to -15.3 ±1.03). The meta-analysis of the within-subject standard deviation suggested that the heterogeneity between-study is negligible relative to the size of the variability within-study. From the two meta-analyses, we obtained an estimate of the 'average' amantadine effect of -8.8 and an estimate of the potential size of the within-subject SD of 3.6. The number of animals required to detect 100%, 67% and 50% of the amantadine effect is 4, 6 and 12 with the corresponding power of 85%, 84% and 83% respectively.

Conclusions: Result of this meta-analysis is of significance and confirms that the MPTP macaque model is a reliable translational model to assess the antidyskinetic ability of a novel mechanism. However, due to the intersubject variability using suitable sample size is essential to obtain consistent results.

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Effect of nicotinamide riboside-enriched diet on neurodegeneration in lactacystin PD mouse model

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Objective: The objective of this study is to investigate the effect of nicotinamide riboside (NR), a form of vitamin B3, NAD+ precursor, and FDA approved food supplement, on neurodegeneration in proteasome inhibitor lactacystin-induced Parkinson's disease (PD) mouse model.

Background: Failure in mitochondrial and/or proteostasis function is common in many neurodegenerative disorders, including in PD1. Several studies have reported that NR is neuroprotective and has mitochondrial function boosting properties2. However, whether NR is able to prevent PD progression and neurodegeneration is currently unknown. In this study, we evaluated the effect of NR-enriched diet preconditioning on neurodegeneration in lactacystin-induced PD mouse model.

Methods: One group of mice received diet enriched for NR (12g NR/5kg food) and the control group received normal food. Lactacystin (LC), was injected just above the substantia nigra. The extent of the lesion was evaluated before and after the LC injection using behavioral tests which assess motor function, including cylinder, elevated body swing, adhesive removal, and open field tests. Striatal mitochondrial oxygen consumption was measured using the Oroboros Oxygraph-2k.

Results: While the experiment is currently ongoing the results so far show that mice that received NR-enriched diet have significantly smaller behavioral deficits after LC injection, compared to the animals from the control group (unpublished), indicating that NR pre-treatment can ameliorate the motor symptoms of PD, especially after two weeks of LC injection. Specifically, we find that NR increases striatal oxygen consumption, suggesting that NR enhances mitochondrial function in the striatum. Whether NR treatment also influences the number or morphology of mitochondria, regulates the monoamine levels and the expression of genes involved in relevant pathways including mitochondrial stress, unfolded protein response, and mitophagy pathways, is currently under investigation.

Conclusions: Preliminary results from this study suggest that increasing levels of available NAD+ by NR supplementation protects mice from LC-induced PD like motor deficit and enhances striatal mitochondrial respiration. Further analysis is needed to understand how the observed effects manifest at the molecular and cellular level. I hope that our work will pave the way for the use of FDA accepted NAD+ precursors such as NR for the prevention of PD in humans.

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PLG restores the balance of autophagy and apoptosis by increasing BCL2 phosphorylation in rotenone-induced Parkinson disease models

J. Liu, H. Yang (Beijing, China)

Objective: Parkinson disease (PD) is the second most common neurodegenerative disorder after Alzheimer disease and there are few treatments currently available. The present study investigated the protective effects of PLG in rotenone-induced PD cell and mouse models.

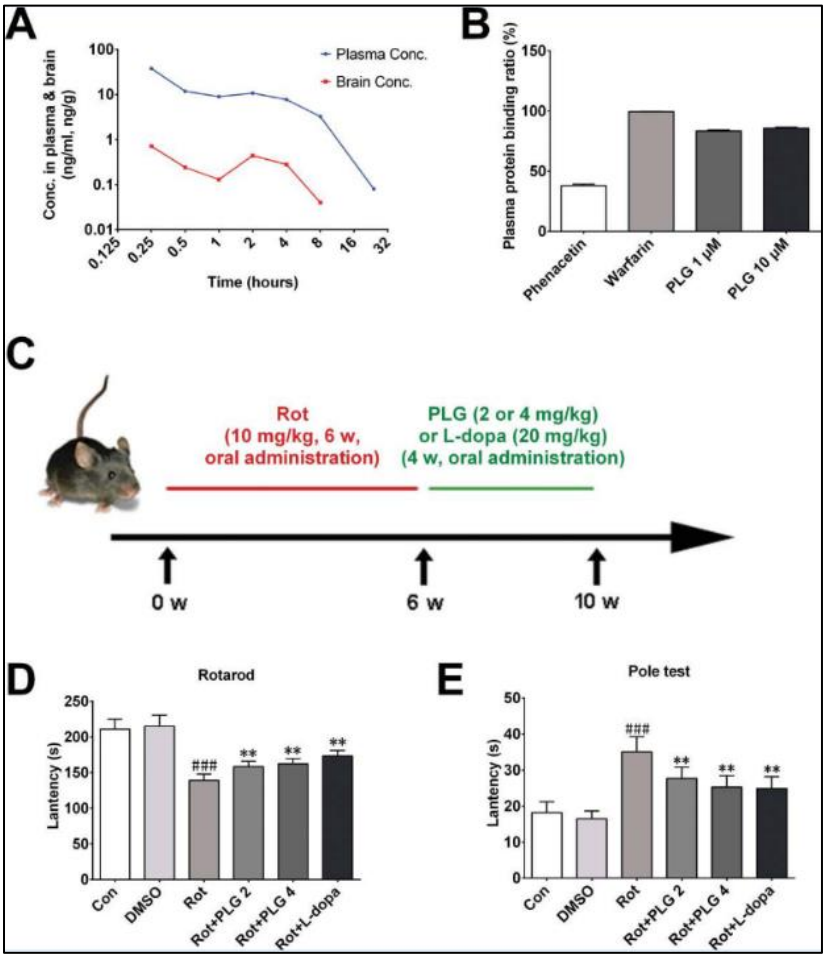
Background: Apoptosis and macroautophagy/autophagy play critical roles in PD pathogenesis; as such, modulating their balance is a potential treatment strategy. BCL2 (B cell leukemia/lymphoma 2) is a key molecule regulating this balance. PLG is an alkaloid extracted from Piper longum L. that has anti-inflammatory and anticancer effects. Our previous study concerned PLG has protective effects in PD models involve inhibiting mitochondrial dysfunction and apoptosis, although the underlying mechanism is unknown.

Methods: C57BL mice were orally administered rotenone and PLG, motor behavior was evaluated with the rotarod and pole tests. The number of dopaminergic neurons was measured by immunohistochemistry. In cell models, cell viability and cytotoxicity were measured by MTT and LDH assay, and mitochondrial function was evaluated with JC-1 and Calcein AM assay. The interaction of BCL2 and BAX or BECN1 was measured by co-immunoprecipitation to evaluate apoptosis or autophagy.

Results: We found that PLG administration (2 and 4 mg/kg) for 4 weeks attenuated motor deficits in mice and prevented the loss of dopaminergic neurons in the substantia nigra induced by oral administration of rotenone (10 mg/kg) for 6 weeks. PLG improved cell viability and enhanced mitochondrial function in primary neurons and SK-N-SH cells. These protective effects were exerted by inducing BCL2 phosphorylation at Ser70 via MAPK8 activation, which resulted in the dissociation of BCL2 and BECN1 and the stabilization of the BCL2 and BAX heterodimer, consequently enhancing autophagy and inhibiting apoptosis.

Conclusions: Our results demonstrate that PLG exerts therapeutic effects in a rotenone-induced PD models, and restoring the balance between apoptosis and autophagy by targeting BCL2 may be an effective treatment for PD.

References: Liu J, Liu W, Lu Y, Tian H, Duan C, Lu L, Gao G, Wu X, Wang X, Yang H. Piperlongumine restores the balance of autophagy and apoptosis by increasing BCL2 phosphorylation in rotenone-induced Parkinson disease models. Autophagy, 2018, DOI: 10.1080/15548627. 2017. 1390636.



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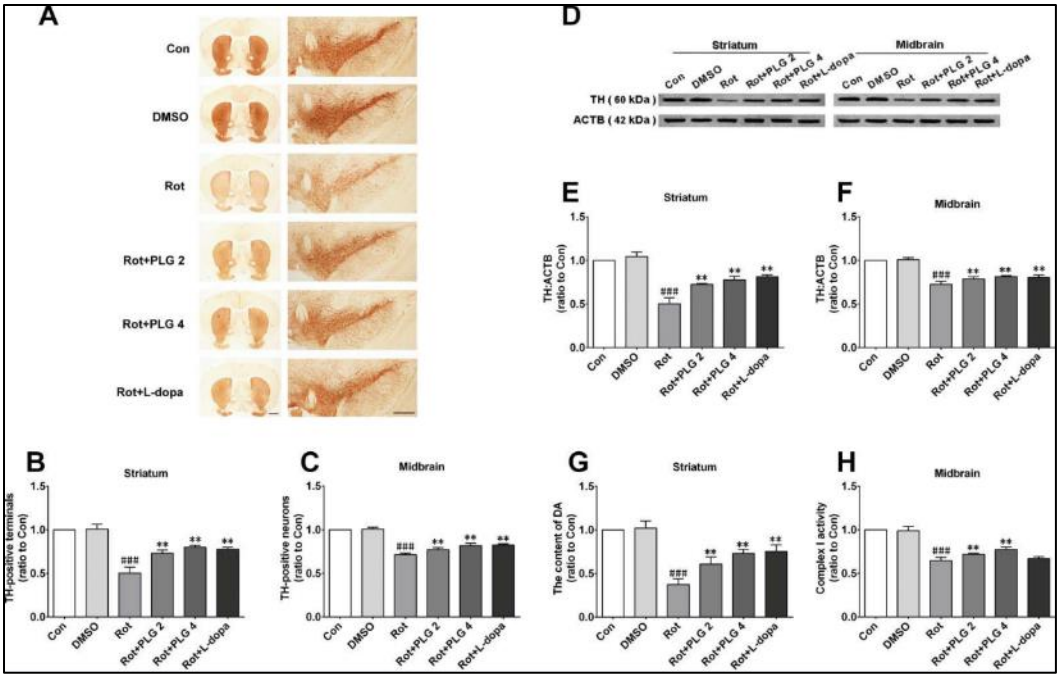

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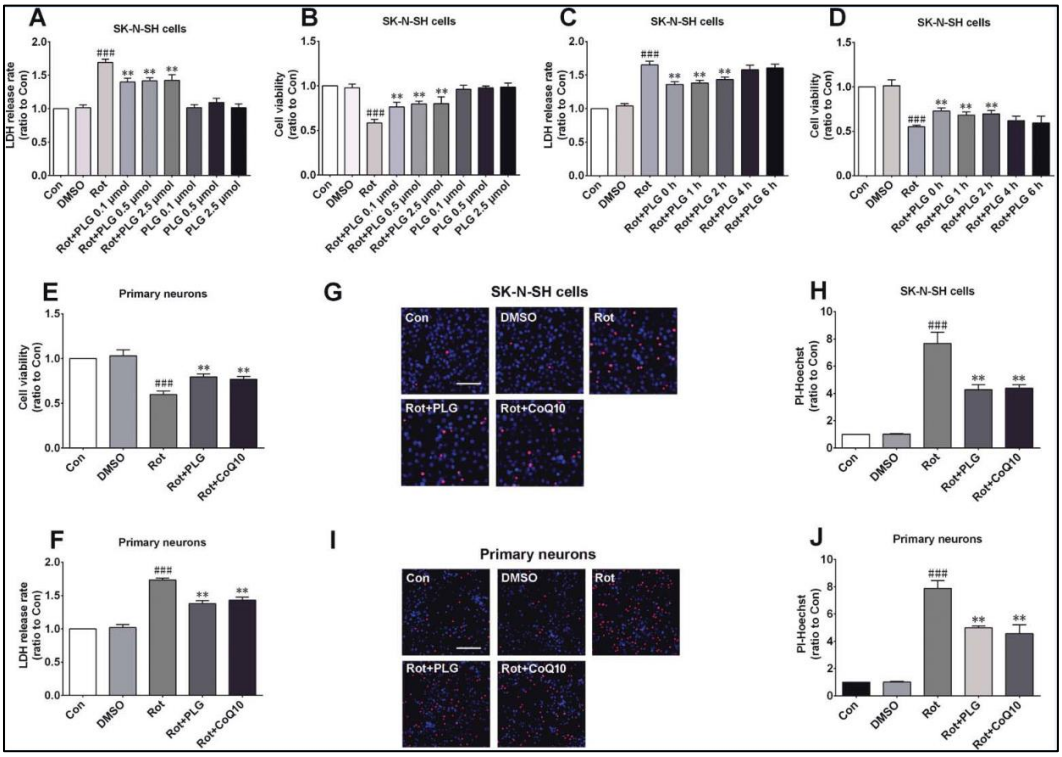

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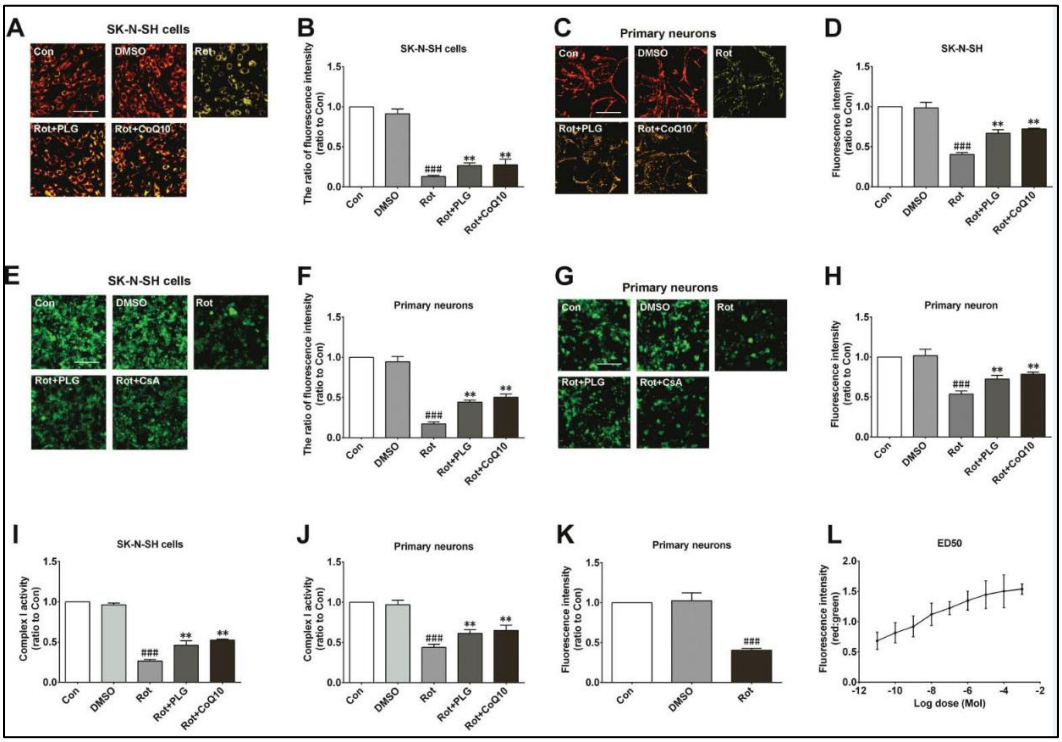

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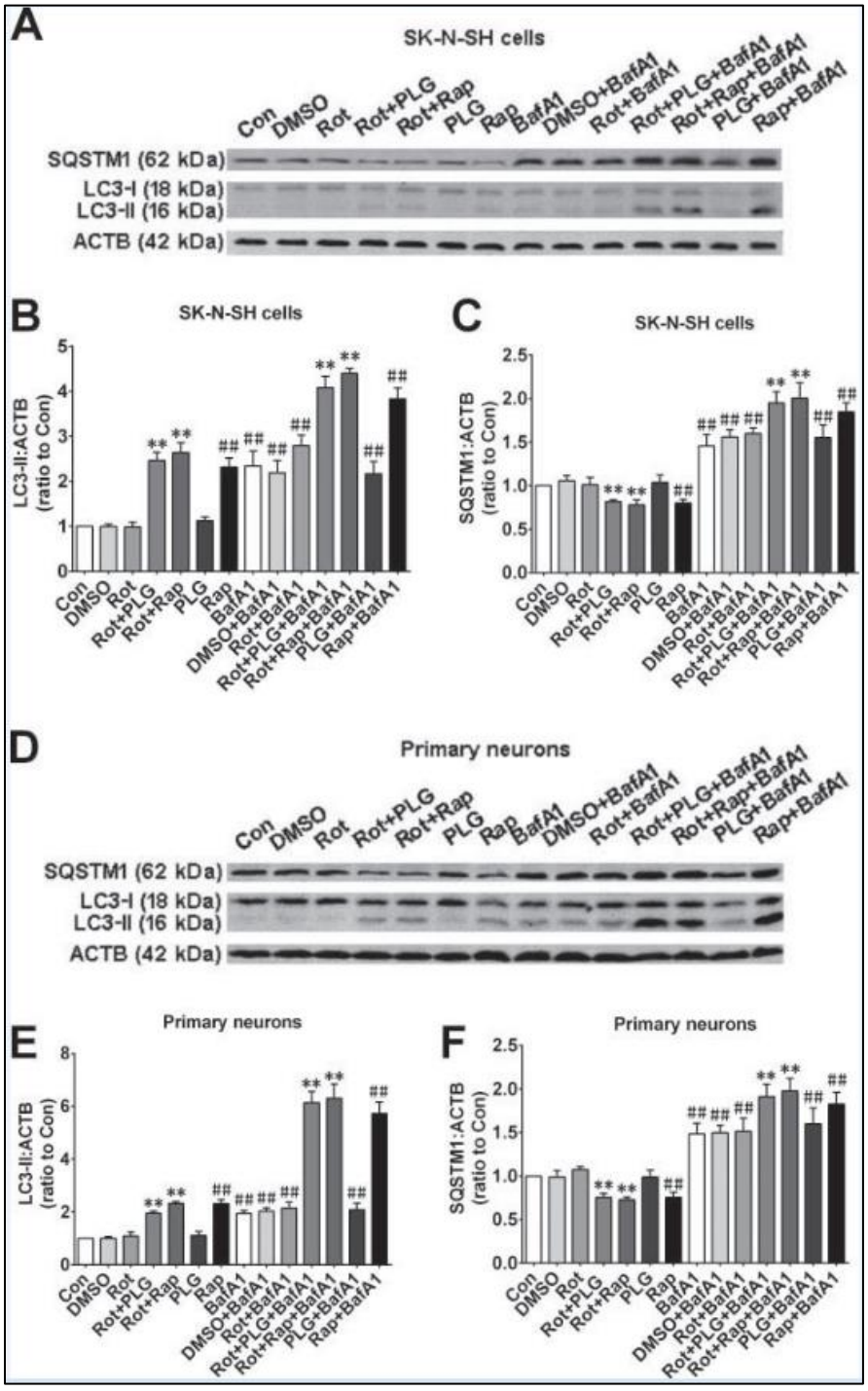
A
SK-N-SH cells
Con
DMSO
Rot
Rot+PLG
Rot+Rap
PLG
Rap
BafA1
DMSO+BafA1
Rot+BafA1
Rot+PLG+BafA1
Rot+Rap+BafA1
PLG+BafA1
Rap+BafA1
SQSTM1 (62 kDa)
LC3-I (18 kDa)
LC3-II (16 kDa)
ACTB (42 kDa)
B
SK-N-SH cells
LC3-II:ACTB (ratio to Con)
C
SK-N-SH cells
SQSTM1:ACTB (ratio to Con)
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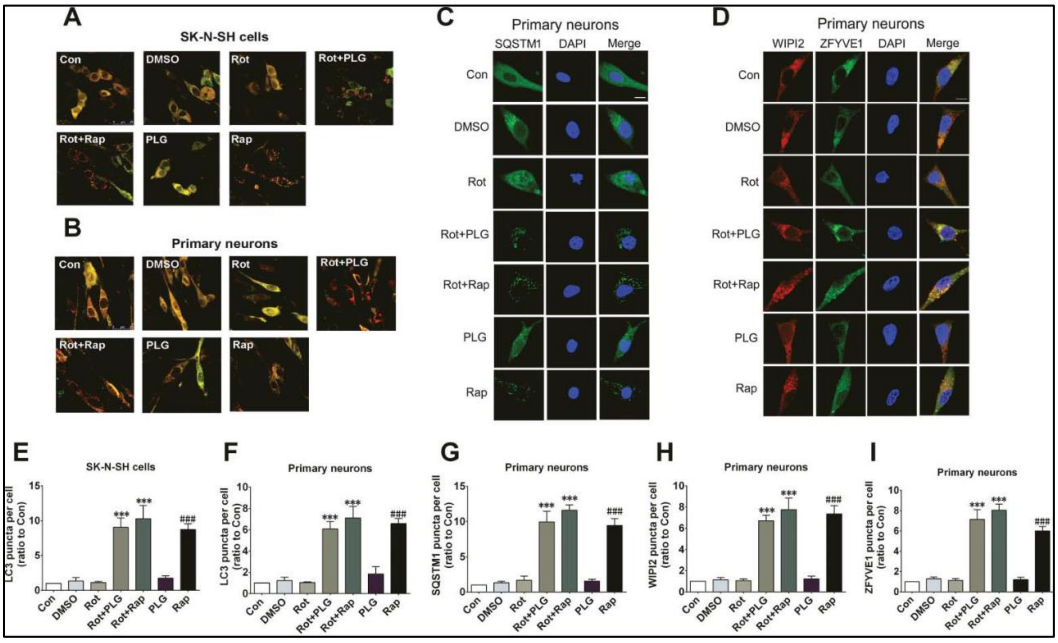
FIG. 5 (405)



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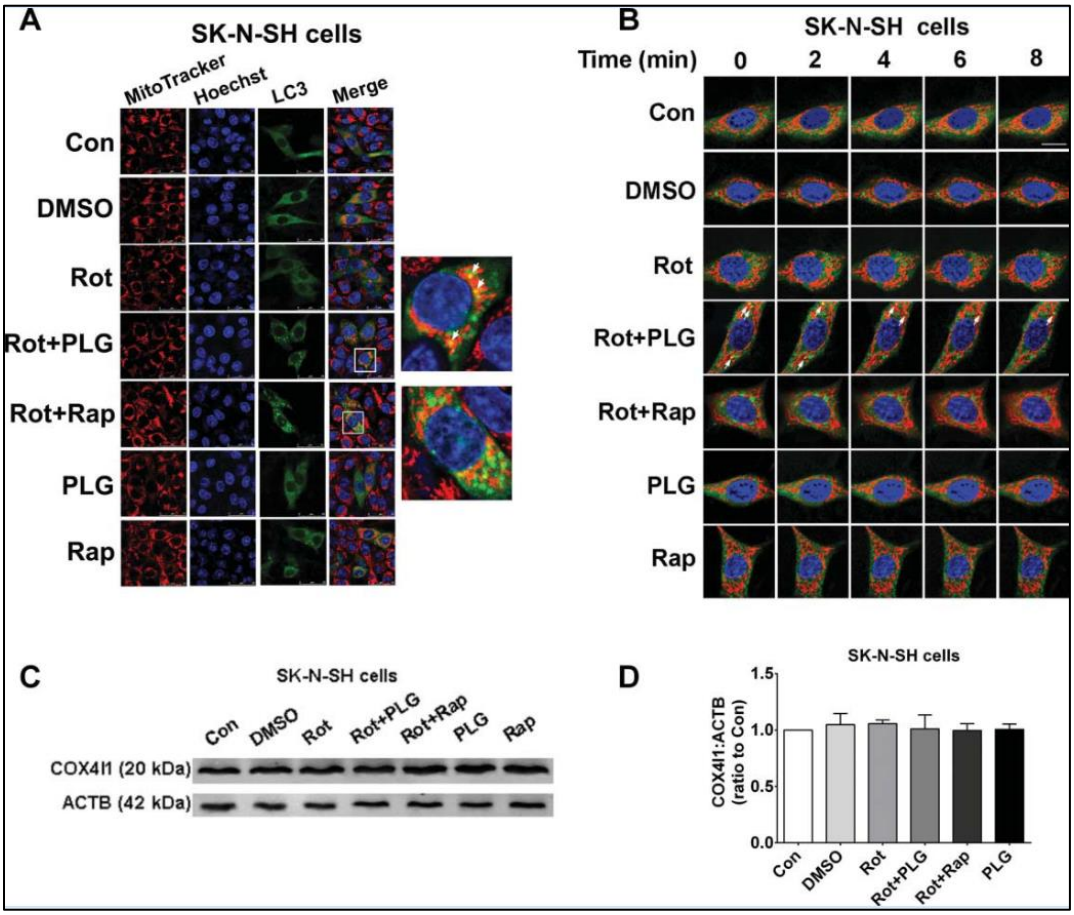

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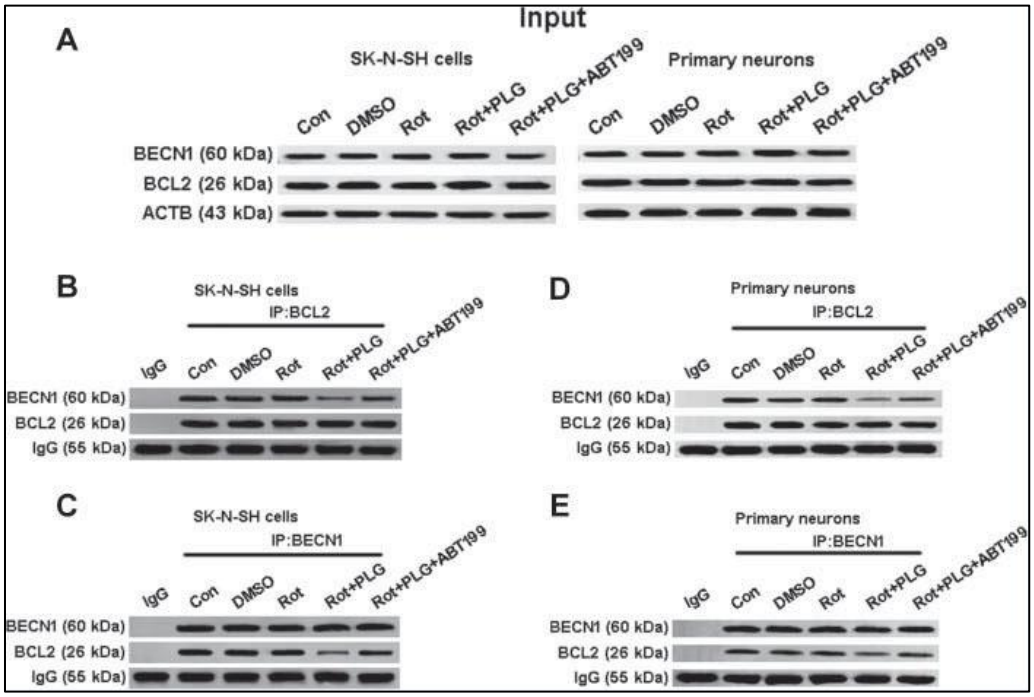

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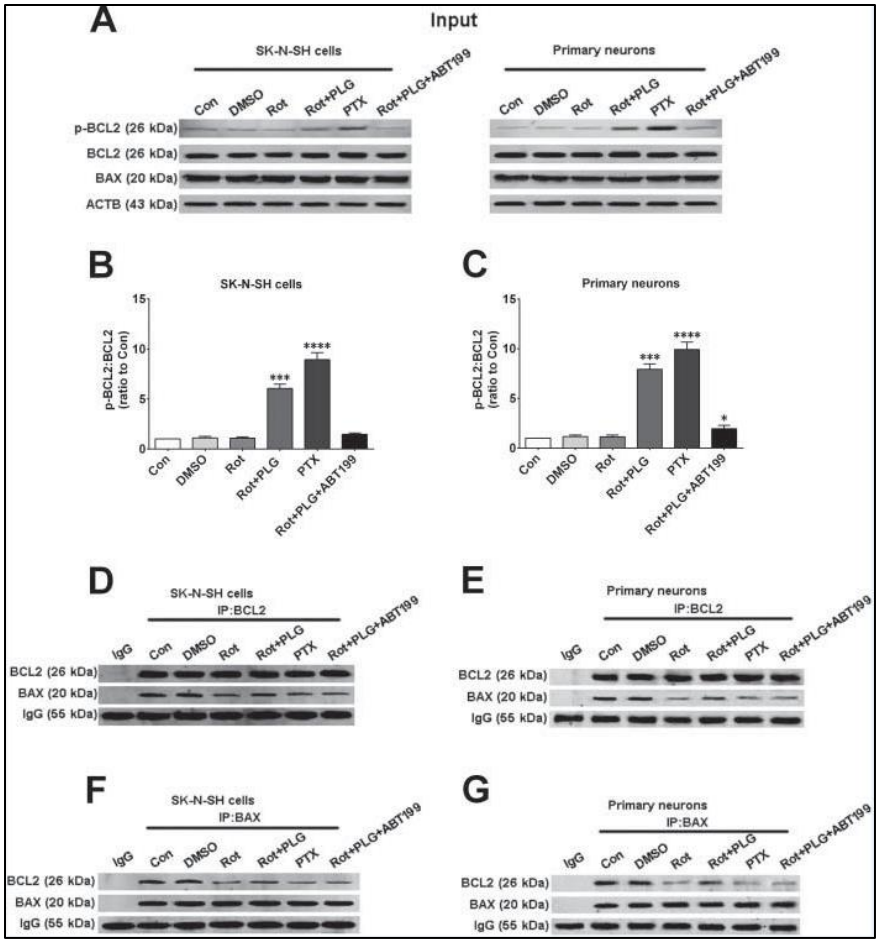

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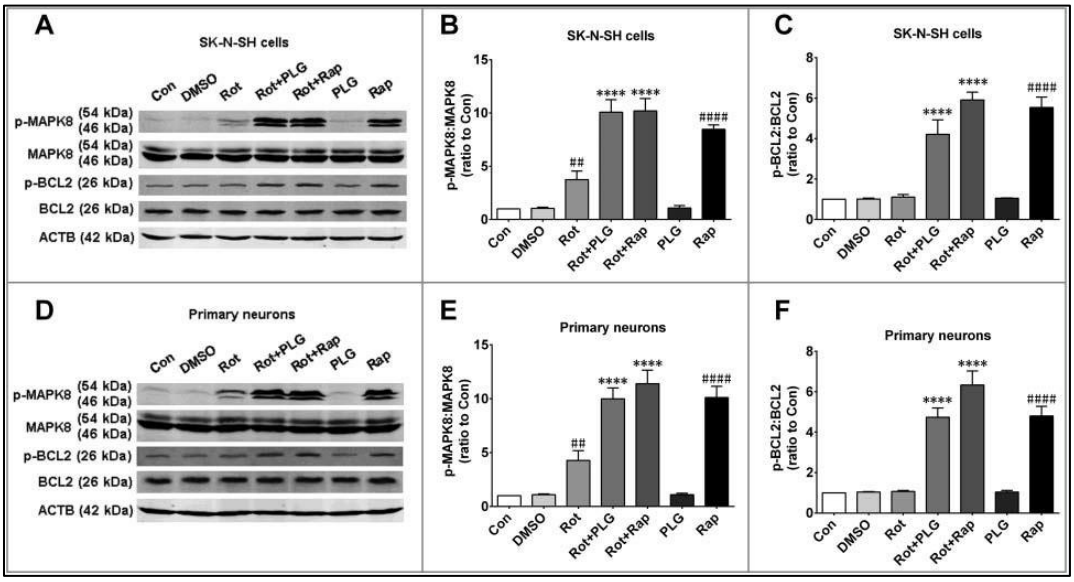

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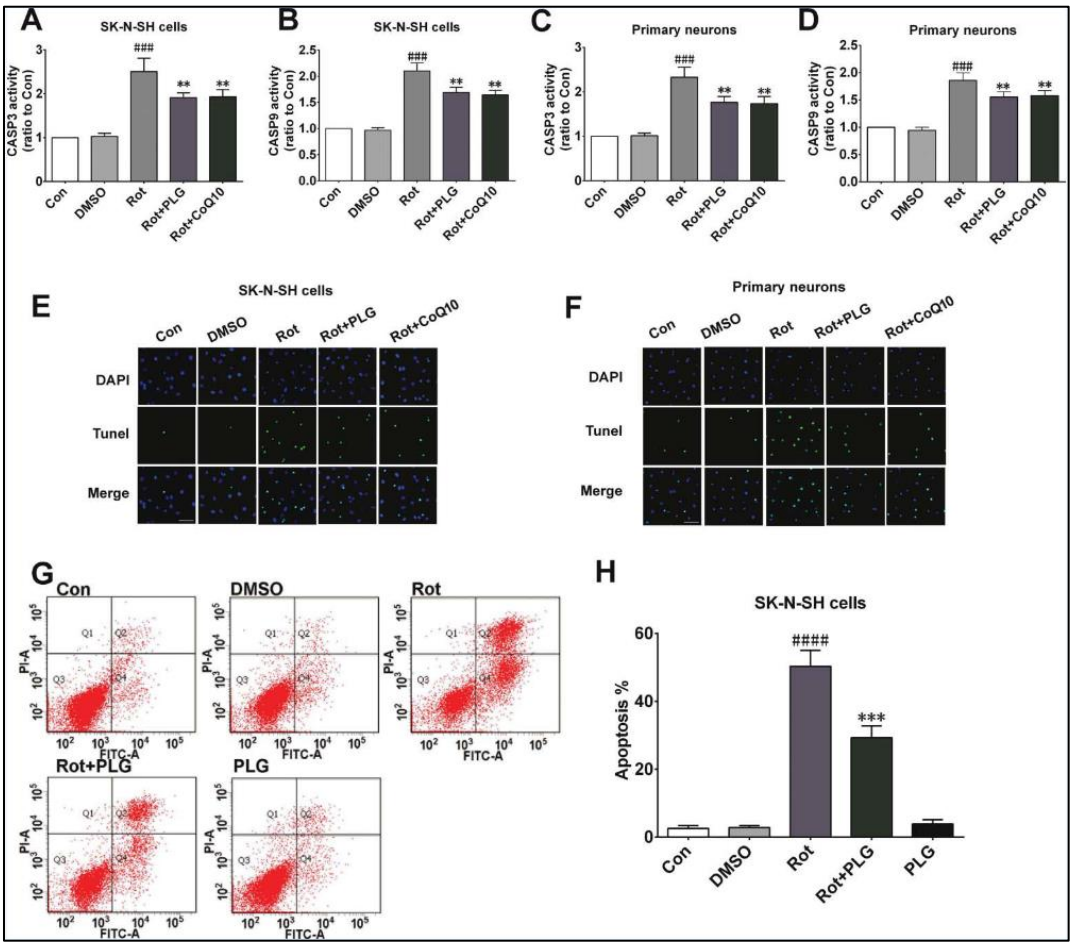

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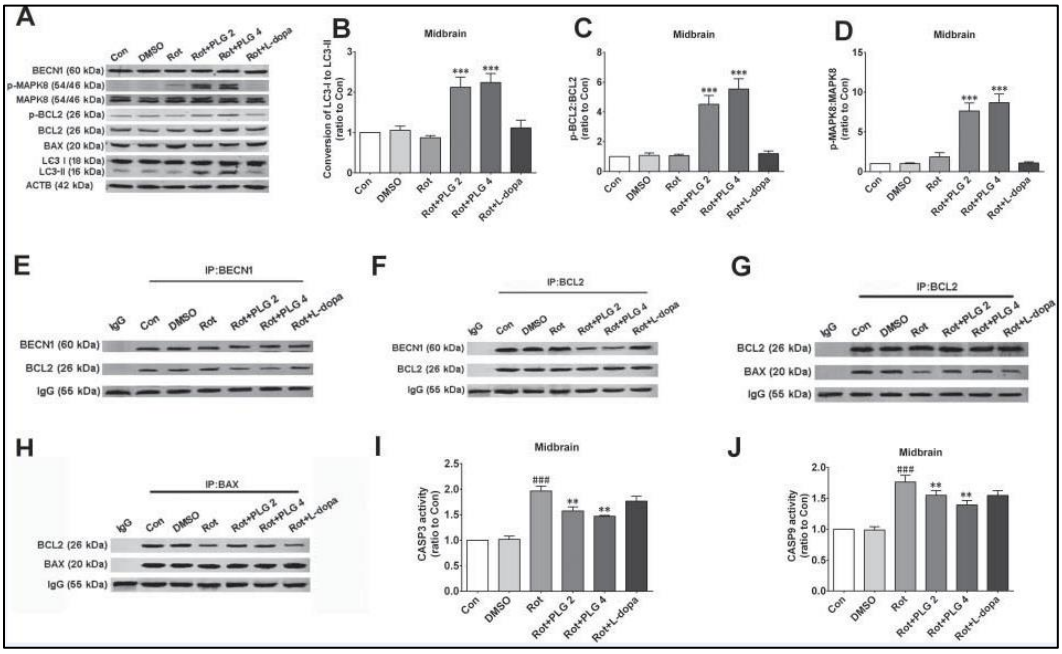

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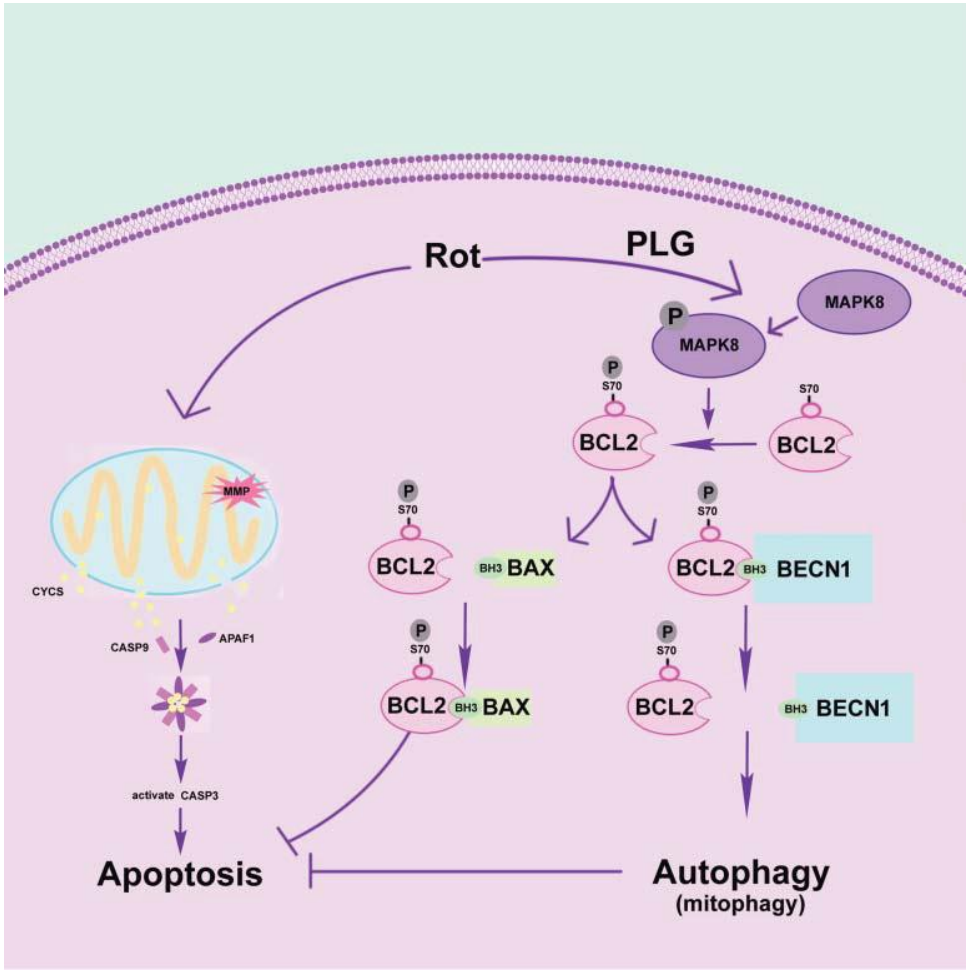

FIG. 13 (405)

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The Akt/mTOR/P70S6K/4EB-P1 signaling pathway is activated by metformin in a toxin-induced cellular model of Parkinson's disease

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Objective: To investigate the effects of metformin on the Akt/mTOR/P70S6K/4EB-P1 signaling pathway in a cellular model of Parkinson's disease (PD).

Background: Metformin is a drug used in treatment of type 2 diabetes. The neuroprotective effect of metformin has been investigated in MPP+-treated SH-SY5Y neuroblastoma cells, which showed that metformin could activate AMPK in these cells. Some studies suggested that the neuroprotective mechanism of metformin is not strictly dependent on induction of AMPK but via inhibition of mTOR signaling. mTOR plays a key role in regulating the balance between cell survival and autophagy in response to stress signals. The role of metformin, as an AMPK activator and mTOR inhibitor, in protection of neuronal death in models of PD remain controversial.

Methods: SH-SY5Y cells were differentiated with retinoic acid into tyrosine hydroxylase-expressed neuronal cells, and treated with MPP+. The effects of metformin on cell viability and protein expression of phospho-Akt, phospho-mTOR, phospho- p70S6K, and phospho-4E-BP1 in MPP+-treated differentiated SH-SY5Y cells were investigated using an MTT assay and Western blotting, respectively.

Results: Treatment with 500 or 2000 µM of metformin alone did not affect cell viability. Pre-treatment with metformin followed by exposure to 1000 µM MPP+ significantly increased cell viability of differentiated SH-SY5Y cells compared to MPP+ alone. Compared to MPP+ treatment only, pre-treatment with metformin prior to MPP+ exposure showed significant increases in the expression of p-Akt/Akt, p-mTOR/mTOR, p- p70S6K /p70S6K, and p-4E-BP1/4E-BP1.

Conclusions: The neuroprotective effect of metformin in MPP+-treated differentiated SH-SY5Y cells is mediated via the activation of the Akt/mTOR/P70S6K/4EB-P1 signaling pathway.

References: The neuroprotective effect of metformin in MPP+-treated differentiated SH-SY5Y cells is mediated via the activation of the Akt/mTOR/P70S6K/4EB-P1 signaling pathway.

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Highly-selective blockade of 5-HT2A receptors with EMD-281,014 reduces the severity of L-DOPA-induced psychosis and dyskinesia in the MPTP-lesioned marmoset model of Parkinson's disease

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Objective: To investigate the effect of the highly-selective serotonin 2A (5-HT2A) receptor EMD-281,014 on L-3,4-dihydroxyphenylalanine (L-DOPA)-induced psychosis-like behaviours (PLBs) and dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset model of Parkinson's disease (PD).

Background: Psychosis and dyskinesia undermine the quality of life of as many as 50-95% of patients with advanced PD. Available therapies are few, and they may elicit important side effects. Whereas there is mounting evidence indicating that antagonising 5-HT2A receptors is effective at alleviating both psychosis and dyskinesia, the drugs used so far, both pre-clinically and clinically, were not entirely selective for this target. Here, we have used the potent and highly-selective 5-HT2A antagonist EMD-281,014, which harbours □ 2,000-fold selectivity over its next target, and have assessed its effect on dyskinesia, PLBs and parkinsonism in the MPTP-lesioned primate.

Methods: Six marmosets were rendered parkinsonian by MPTP administration. Following repeated administration of L-DOPA to elicit stable PLBs and dyskinesia, they were administered acute challenges of EMD-281,014 (0.01, 0.03, 0.1 mg/kg) or vehicle, in combinaison with L-DOPA after which the severity of PLBs, dyskinesia and parkinsonian disability was rated.

Results: EMD-281,014 (0.03 and 0.1 mg/kg) significantly reduced the severity of peak dose PLBs, by ≈ 42.5% and ≈ 45.9% ($P < 0.05$ and $P < 0.001$), respectively when compared to L-DOPA/vehicle. Peak dose dyskinesia was also reduced, by ≈ 41.8% and ≈ 54.5% ($P < 0.05$ and $P < 0.001$), respectively, when EMD-

281,014 (0.03 and 0.1 mg/kg) was added to L-DOPA, compared to L-DOPA/vehicle. The anti-psychotic and anti-dyskinetic effects of EMD-281,014 were achieved without interfering with L-DOPA anti-parkinsonian action

Conclusions: Our results suggest that highly-selective blockade of 5-HT2A receptors is effective at alleviating psychosis and dyskinesia in PD, without interfering with L-DOPA anti-parkinsonian action. EMD-281,014 has already been tested in clinical settings and could therefore be advanced rapidly to Phase II trials in PD patients experiencing dyskinesia and psychotic features.

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Mitochondrial morphometrics in idiopathic Parkinson‘s disease fibroblasts

P. Antony, O. Boyd, K. Mommaerts, K. Sokolowska, M. Ostaszewski, A. Baumuratov, L. Longhino, F. Poulain, R. Krueger, R. Balling, N. Diederich (Belvaux, Luxembourg)

Objective: To identify mitochondrial morphometric phenotypes in fibroblasts derived from patients with idiopathic Parkinson’s disease (IPD).

Background: Mitochondrial dysfunction (MD) has been proposed as cellular phenotype in patients with idiopathic Parkinson’s disease (IPD). However, study results remain controversial for fibroblasts derived from IPD. Here we aim to identify mitochondrial morphometric changes that eventually can be used in cell based drug screening assays targeting IPD.

Methods: Axilla skin punch biopsy derived fibroblasts from 41 IPD patients with a mean disease duration of 6.5 years (st.dev. 5.5 years) and from 21 healthy age-matched controls (HC) were stained with Hoechst, CellMask, and TMRM. Mitochondrial morphometrics were analyzed via automated confocal microscopy and in house developed computational image analysis (figure).

Results: Significant morphometric changes were seen in IPD fibroblasts. The number of mitochondria was increased ($p=0.002$) whereas the mitochondrial morphometric complexity was substantially decreased as visualized by reduction of mitochondrial perimeter ($p=0.029$) and mitochondrial form factor ($p=0.013$). Further analysis with established algorithms from computer vision and graph theory confirmed mitochondrial fragmentation: The mitochondrial skeleton corresponding to the final chain of pixels remaining after step by step erosion of the mitochondrial mask was significantly reduced in IPD fibroblasts ($p=0.020$). The number of mitochondrial nodes in the sense of graph theory applied to the mitochondrial skeleton reflects reduced mitochondrial branching in IPD ($p=0.013$). Finally, the mitochondrial node degree representing the average branch length in the mitochondrial skeleton network was also reduced ($p<0.001$). [figure1] Figure: (white) mitochondrial shapes, (blue) skeleton, (green) branch points, (red) endpoints. Note the reduced branching in the IPD patient sample.

Conclusions: Morphometric changes of IPD mitochondria with increased number of mitochondria on one side and reduced branching on the other side reflect mitochondrial fragmentation. This may be due to impaired mitophagy and/or also – at least temporary efficient – compensatory mechanisms, as also indicated by increased mitochondrial membrane potential. The results are in agreement with similar changes observed in submucosal enteric ganglia neurons of IPD patients (doi:10.1038/srep33117).

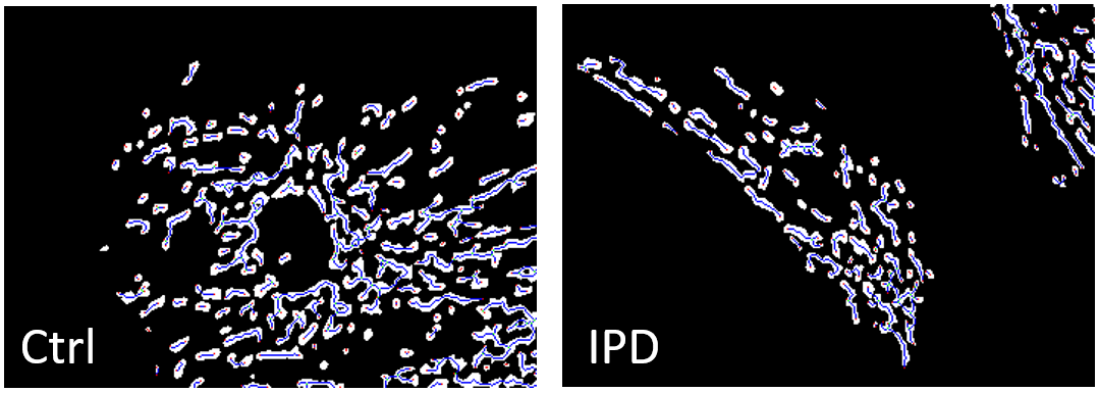

FIG 1. (420)

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Oral treatment with JNX3001 protects dopaminergic function in a non-human primate model of Parkinson's disease alpha-synucleinopathy

P. Howson, T. Johnston, J. Brotchie, M. Hill, J. Koprich (Toronto, ON, Canada)

Objective: We evaluated the efficacy of JNX3001 in an aSyn-based macaque model with plasma exposures associated with efficacy in rodents.

Background: Alpha-synuclein (aSyn) deposition is a pathological feature of PD. Reductions in aSyn, by enhancing autophagy, is an attractive disease modifying approach in PD. We, and others, have demonstrated that JNX3001 enhances autophagy and is efficacious in rodent models of PD. We recently demonstrated that JNX3001 was efficacious in an AAV aSyn rodent model of PD.

Methods: Pharmacokinetic Study. 3 female macaques were administered JNX3001 (2.67g/kg, p.o.) once daily for 7 days and plasma collected on days 1 and 7. In a subsequent step, macaques were administered JNX3001 for 2 days and brain and CSF samples collected 1 h post-administration. Trehalose levels were analysed by LC-MS/MS.

Efficacy Study. Female cynomolgus macaques (~9 y, ~3.5kg) received AAV1/2 A53T aSyn or empty vector into the SN. Once daily treatment with JNX3001 (2.67 g/kg, p.o.) or vehicle commenced the day after surgery and continued to day of necropsy (day 142). Brain samples were collected for postmortem measures; striatal DA by HPLC, striatal DA transporter by autoradiography and DA neuron numbers by stereology.

Results: Pharmacokinetic Study. Administration of JNX3001 (2.67 g/kg, p.o.) produced plasma and brain trehalose levels comparable to those associated with efficacy in the rat.

Efficacy Study. Five months of exposure to AAV1/2-produced A53T aSyn resulted in significant reductions in striatal dopamine (by 50%; HPLC), striatal DAT (by 45%; autoradiography) and dopamine neurons of the SN (by 38%; stereology). Once daily oral dosing with JNX3001 (2.67 g/kg, p.o.) commenced one day after AAV1/2 delivery and resulted in significant protection on striatal measures of DA function, including DA (deficit reduced to 31%) and DA transporter levels (deficit reduced to 17%), while SN DA neuron numbers were not protected.

Conclusions: AAV1/2 delivery of A53T aSyn results in significant deficits on measures of nigrostriatal dopaminergic function in which to evaluate efficacy of potential disease modifying treatments. Once daily treatment with JNX3001 is able to produce plasma and brain JNX3001 levels comparable to those associated with efficacy in a rodent model of PD. Once daily administration of JNX3001 for 5 months is well tolerated and results in significant protection on striatal measures of DA function. These data support the continued development of JNX3001 as a disease modifying therapy for PD.

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Intranasal Delivery of Bromocriptine loaded Lipid Nano Carriers for Enhanced Brain Delivery in Parkinson's disease

S. V G, P. Vavia (Mumbai, India)

Objective: The objective of this research work is to develop a lipid nanocarrier system of Bromocriptine and evaluate its increased efficacy in the management of Parkinson's Disease (PD). The developed system is administered via the non-invasive nasal route for efficient transfer to the brain.

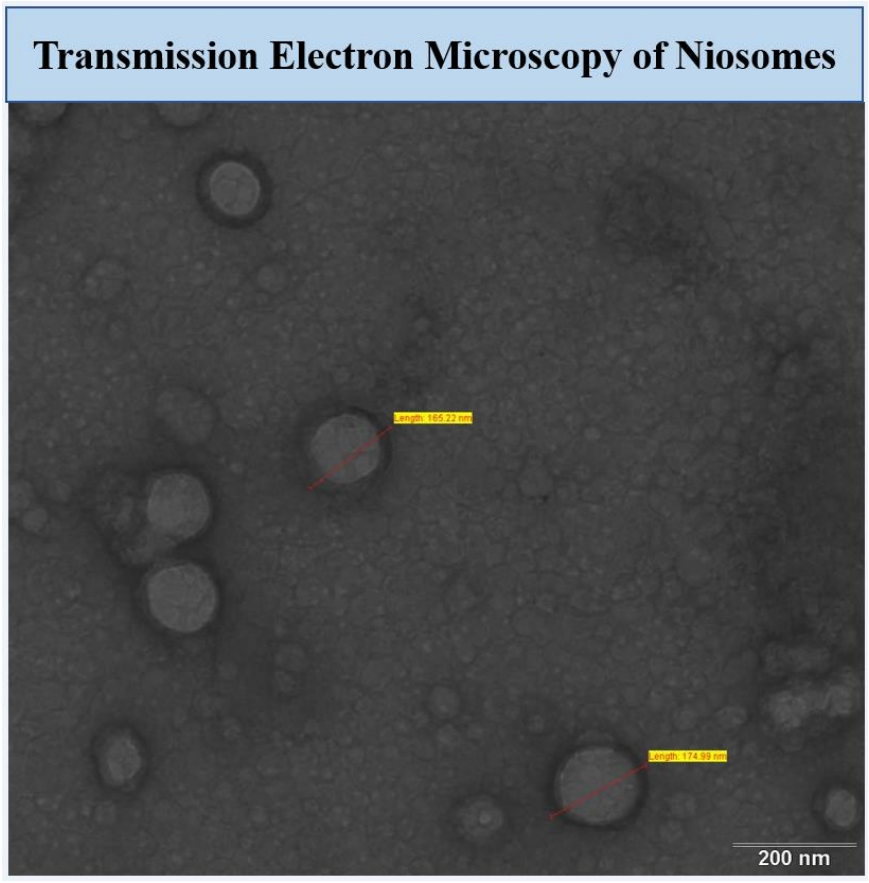
Background: Age-related Parkinson's disease (PD) is the most common neurodegenerative motor disorder in the world, causing tremor, rigidity, bradykinesia and gait impairment (1). One serious problem in administration of neuro therapeutics is its inability to cross the Blood Brain Barrier (BBB) and show its efficacy (2). Hence, novel nano formulations are administered via the nasal route for rapid and selective targeting to the brain by transport via the olfactory pathway bypassing the BBB and avoiding peripheral distribution and associated potential adverse effects.

Methods: Stable surfactant vesicles i.e niosomes were prepared by ethanol injection method. The optimized vesicular formulation was evaluated for its size, surface morphology by Transmission Electron Microscopy (TEM), % drug encapsulation and haemocompatability studies. Niosomes were evaluated for drug permeation across freshly excised goat nasal mucosa to understand the % enhancement in permeation as compared to plain drug solution. Cataleptic activity of the developed system was evaluated in a haloperidol induced rat model to establish enhanced efficacy of niosomes over plain drug suspension.

Results: Niosomes were found to be spherical in shape measuring 175.32 nm (figure 1). The encapsulation of drug was found to be 60 %. Niosomes were found to be hemo compatible. Ex – vivo permeation studies on goat nasal mucosa revealed nearly 3.2 times enhancement in permeation as compared to plain drug suspension (figure 2). This can be corroborated by fluorescent microscopic images (figure 3) of coumarin-6 loaded niosomes across nasal mucosa as compared to coumarin-6 aqueous suspension. Histopathological examinations of nasal mucosa showed no signs of necrosis and hemorrhage. Haloperidol induced rat models showed a marked improvement in reversal of catalepsy behavior treated with intranasal niosomes as compared to intranasal plain drug suspension.

Conclusions: Results from various studies suggest that niosomes are safe and effective delivery systems for targeted delivery of Bromocriptine following the direct nose to brain pathway. The composition of the vesicles facilitated enhanced transport of the drug across nasal mucosa. This nano-carrier system not only serves as a non-invasive therapeutic alternative in the management of PD but also is a platform technology that can be extrapolated to numerous other drugs to target the brain.

References: 1. Complexity of dopamine metabolism, Johannes Meiser, Daniel Weindl and Karsten Hiller, Meiser et al. Cell Communication and Signaling 2013, http://www.biosignaling.com/content/11/1/34. 2. Leonard AK, Sileno AP, Brandt GC, Foerder CA, Quay SC, Costantino HR. In vitro formulation optimization of intranasal galantamine leading to enhanced bioavailability and reduced emetic response in vivo. Int J Pharm. 2007;335(1-2):138-146.



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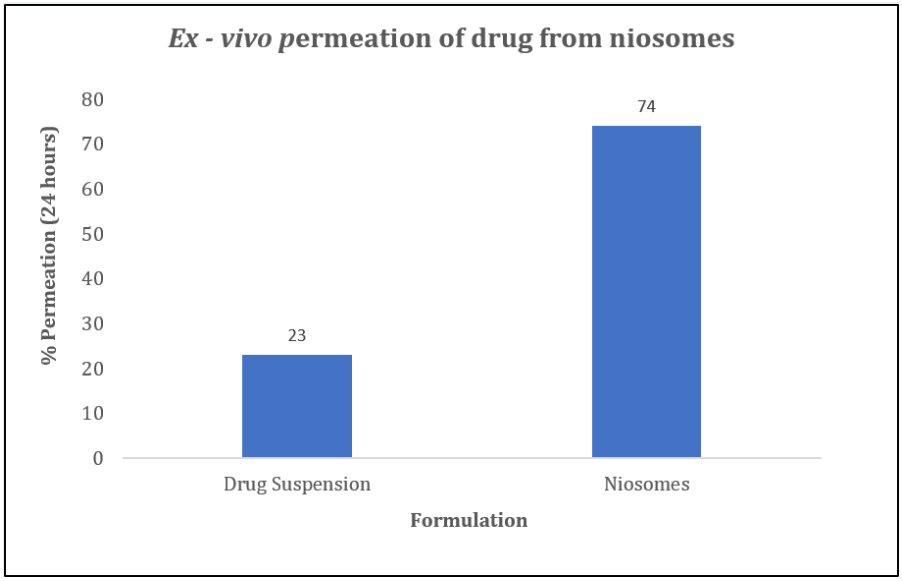

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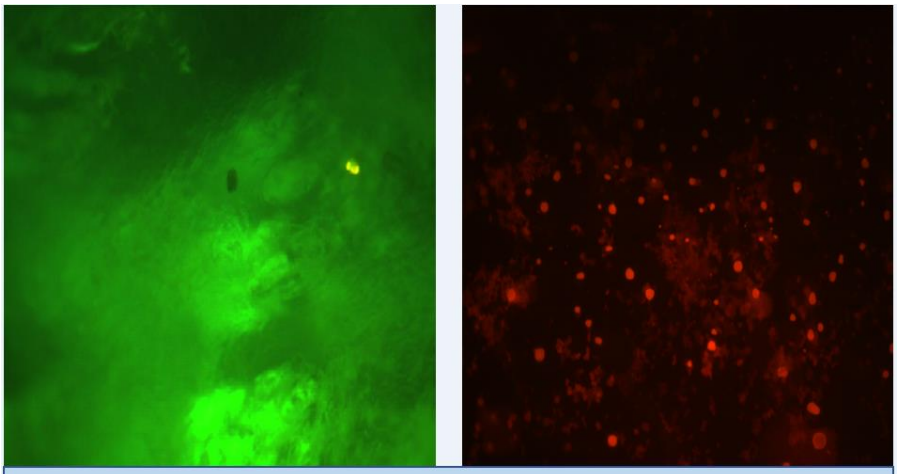

Fluoroscent Microscopic images of nasal mucosa: Coumarin 6 loaded noisome (left) and Aqueous Coumarin 6 suspension (right)

FIG 3. (431)

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Primary familial brain calcification – The impact of calcifications on the development of motor symptoms

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Objective: The aim of the present study was to investigate whether the nigro-striatal system in a genetic mouse model of primary familial brain calcification (PFBC) is more susceptible to dopaminergic toxins.

Background: Primary familial brain calcification (PFBC) is characterized by calcifications in the basal ganglia and other brain areas. It can be caused by mutations in at least four different dominantly inherited genes, namely SLC20A2, PDGFRB, PDGFB, or XPR1. Calcification of the brain is present in 100% of mutation carriers, whereas associated movement disorders, such as dystonia and parkinsonism, are present in only about 50%. The disease mechanism of calcification and even more importantly, the impact of these calcifications on the development of clinical symptoms, in particular movement disorders, remains unknown.

Methods: To this end, we used PDGFbret/ret mice that develop brain calcifications at the age of 4 months. We analyzed these mice in two different toxin-based PD models to test the integrity of the nigro-striatal system.

Results: First, we treated PDGFbret/ret mice and wild-type (WT) littermates with MPTP. We found increased levels of MPP+ in the brain of PDGFbret/ret mice, which supports the hypothesis that PDGF-B dysfunction leads to pericyte dysfunction and an altered blood brain barrier. Second, we intoxicated 8- and 12- month-old PDGFbret/ret mice and WT controls with a unilateral intrastriatal 6-OHDA injection and analyzed behavior and the integrity of the nigro-striatal system. We found that, although the nigro-striatal pathway is unaffected in aged PDGFbret/ret mice under basal conditions, the toxin-naïve animals showed behavioral alterations that increase between 8 and 12 months of age. After 6-OHDA injection, the nigro-striatal system of 12-month-old mice is more affected compared to controls, which is also reflected on the behavioral level. Again, these differences increased with age.

Conclusions: It is conceivable that the results of the MPTP treatment reflect the situation in humans, i.e. that the concentration of incidentally accumulated environmental toxins is higher in the brains of PDGFB mutation carriers compared to healthy controls. Regardings the results in aged 6-OHDA treated animals, it is tempting to speculate that, despite an alteration in striatal dopamine levels in aged PDGFbret/ret mice, the nigro-striatal system becomes more susceptible to brain damage with an increasing load of calcifications.

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Reduction of neuroinflammation by selective inhibition of the N-type calcium channel is beneficial in various animal models of neurodegeneration, here in the SOD1G93A transgenic mouse model of ALS

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Objective: Neuroinflammation is a pathological hallmark of several neurodegenerative diseases. We speculated that reduction of calcium ion influx specifically in neurons should reduce inflammatory cellular signaling and ultimately lead to reduced neurodegeneration and to deceleration of disease phenotype.

Background: We have identified orally available compounds that specifically inhibit the N-type calcium channel CaV2.2 (NCC) in nanomolar concentrations without affecting the L-type calcium channel. Target engagement has been shown in vivo in a spinal nerve ligation (SNL) rat model and a sciatic inflammatory neuritis (SIN) rat model. The observed acute and therapeutic reversion of tactile allodynia shows that the orally applied compound hits the NCC also in vivo. The same compound and a second NCC inhibiting compound were tested also in different neurodegenerative animal models. There, we found very significant deceleration of the neurodegenerative phenotype as shown for example in the SHIRPA assay. Here, we present efficacy of one of our compounds in the SODG93A transgenic mouse model of ALS.

Methods: SOD1G93A mice were treated with the compound either intraperitoneally or orally and compared with placebo. In pre-tests and during treatment, the mice were tested longitudinally in different behavioral and motor coordination tests (SHIRPA test, open field test, rotarod test, pole test). Brain, cervical spinal cord and the M. gastrocnemius were analyzed histologically for ALS pathology. Several pro- and anti-inflammatory cytokines were analyzed in plasma samples.

Results: Treatment led to slower developing neurodegenerative phenotype based on the performance in the SHIRPA, rotarod and pole tests. Last not least, we observed retardation of the average disease onset in the treated animals. In addition, we will present treatment influence on pathology and cytokine levels in plasma.

Conclusions: We were able to show that the investigated NCC-inhibiting compound shows beneficial efficacy in the SOD1G93A mouse model, also upon oral application. These findings qualify the compound as a compound to be considered for further development and testing towards a disease modifying ALS treatment.

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Rose Anthocyanins protect against Parkinson's pathophysiology: Behavioral and biochemical changes in young Drosophila

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Objective: To test the hypothesis that Rose Anthocyanins, owing to their biological properties in vitro, protect against Parkinson's pathophysiology in young Drosophila melanogaster.

Background: Anthocyanins are polyphenolic pigments found among colored fruits, vegetables and flowers. Vast classes of Rose Anthocyanins have been reported and possess antioxidant properties. Owing to their potential biological in vitro, and no data about the neuroprotective effects, it was envisaged to test their properties against a Parkinson's phenotype in Drosophila.

Methods: Rose Anthocyanins (RA) were prepared using acidified water method from the red rose petals according to standard protocol, dried under pressure. Drosophila melanogaster larvae (2d old, 100 larvae/ group) maintained on a diet enriched with Rose anthocyanins (0.01-0.05% w/v in medium) were co-challenged with Parkinson toxins Rotenone and Paraquat (Rot-0.5mM). Larvae were monitored for behavioural manifestations and terminally for biochemical index.

Results: Larvae treated with RA demonstrated modulated redox status evident from increased thiol levels and reduced hydroperoxides levels in the homogenates. Further, there was induction of hsp70 levels among Bg9 larvae. Rot induced locomotor deficits in terms of number of line crossings on the grid in a concentration dependent manner. Interestingly, RA larvae performed better in an open field grid in terms of improved locomotor function as opposed to Rot groups. Biochemically dopamine levels were partially

restored with Rose anthocyanins among Rot larvae. Further, RA reduced the Rot-induced oxidative stress among larvae in a concentration dependent manner.

Conclusions: Our data from drosophila are suggestive of potent neuro-attenuatory propensity of Rose anthocyanins against chemically-induced Parkinsonian phenotype among drosophila however studies are warranted for long term implications.

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Exosomal alpha synuclein secretion is beneficial for cellular models of Kufor-Rakeb syndrome

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Objective: The purpose of this study is to investigate the effect of induction of exosomal a-syn secretion on PARK9-mediated neurotoxicity.

Background: Kufor-Rakeb syndrome (KRS) is a rare hereditary neurodegenerative disorder in which patients show parkinsonism with other neurological manifestations. KSR is caused by loss of function mutations in ATP13A2 (PARK9) which codes lysosomal type 5 P-type ATPase. Loss of PARK9 leads to lysosomal dysfunction, subsequent α-synuclein (α-syn) accumulation and abnormal mitochondrial respiration. We and others have shown that PARK9 deficiency also impaired secretion of exosomes and a-syn.

Methods: We used H4 cells and midbrain DA neurons differentiated from patient-derived iPS cells. First, we silenced the expression of ESCRTIII-associated protein ALIX, which is known to play an important role in multivesicular body (MVB) formation. We also tried neutral sphingomyelinase (n-SMase), which is known to induce exosome secretion in the ESCRT machinery independent manner. We then conducted a series of experiments including exosome measurement, a-syn immunoblotting and mitochondria respiration.

Results: Both ALIX silencing and n-SMase treatment were able to increase the number of exosomes. While ALIX silencing lead to decreased a-syn levels through exosomal a-syn secretion, n-SMase treatment failed to attenuate a-syn accumulation in KRS DA neurons. While both did not affect lysosomal proteolysis, ALIX silencing improved mitochondria respiration.

Conclusions: These results demonstrate that α-syn is secreted by exosomes that are produced through ESCRT-dependent MVB biogenesis. Enhancing this pathway can reduce intracellular α-syn levels. These results highlight the importance of exosomal secretion to reduce intracellular protein levels and normal cellular functions. Therefore, targeting exosomes offers potential therapeutic opportunities not only for KRS, but also diseases characterized by the accumulation of pathological proteins.

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Impact of Subthalamic Nucleus Stimulation on Striatal Dopamine Transporter in Parkinsonian Rat Models

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Objective: To investigate the impact of subthalamic nucleus deep brain stimulation (STN-DBS) on striatal dopamine transporter (DAT) of Parkinsonian rat models.

Background: STN-DBS has become an effective treatment option in advanced Parkinson's disease (PD). Whether STN-DBS would affect the striatal dopamine levels remains unclear.

Methods: Fourty healthy male Sprague-Dawley rats were randomly divided into four groups: PD rat model group, normal rat group, PD rat model treated with DBS group and normal rat treated with DBS group. PD rat model received unilaterally administered 6-OHDA in the right MFB with two points. Deep brain stimulation electrode was stereotactic implanted in STN at right side. The rats treate with DBS received stimulation for two weeks (frequency 130Hz, pulse width 60us, voltage 1.0V, 30 minutes/day). Apomorphine rotation test and Cylinder test were used to assess the behavioral changes in rats. [11C]CFT PET was performed to assess the changes of dopamine transporter in rats. Tyrosine hydroxylase (TH) immunohistochernical staining was used to detect the change of dopaminergic neurons in all groups of rats.

Results: After two weeks of STN stimulation, there was a significant reduction of apomorphine-induced rotation in PD rat models. Cylinder trials suggest that the number of forelimb activity significantly increased from 0.4 ± 0.3 beats / min at pre-stimulation state to 0.8 ± 0.5 beats/min after stimulation, and the ratio of limb-use asymmetry significantly decreased from 91.3 ± 9.5% at pre-stimulation state to 75.1 ± 8.6% after stimulation. [11C]CFT PET imaging showed significantly decreased DAT binding in the lesion side (right side) of striatum of PD rat models while DAT binding was symmetry in bilateral striatum of normal rats. After two weeks of STN stimulation, there was a significant increase of DAT binding in the right side striatum in both normal rats and PD rat models. Average asymmetry index (AI) changed from -5.58 ± 2.66 at pre-stimulation state to -9.42 ± 3.91 after stimulation in normal rats. Average AI decreased from 28.54 ± 8.66 at pre-stimulation state to 19.10 ± 6.60 after stimulation in PD rat models. There was no significant change of TH immunohistochemistry after STN stimulation in both normal rats and PD rat models.

Conclusions: STN-DBS could improve the behavior function of PD rat models. STN-DBS could increase striatal DAT in both normal rats and PD rat models, suggesting it could influence the release or metabolism of striatal dopamine.

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Controlled neural organoids grafting promotes functional recovery in experimental parkinsonism

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Objective: We propose that the striatal transplantation of 3D-neural organoids of controlled cell size and cell content, named "Controlled Neural Organoids" (CNOs), could circumvent the limitations of current cell therapy and allow functional recovery in Parkinson's disease (PD).

Background: A major impetus for research in PD is centered on cell therapy strategies that aim at replacing the dysfunctional or dying neuronal cell populations. In the past decade, pluripotent stem cells have provided unprecedented access to various human cell types, especially to central nervous system neurons. The availability of patient-specific cell lines associated with the development of highly efficient protocols to in vitro generate specific neuronal cells is an important step in overcoming the ethical and logistical challenges associated with the use of embryonic stem cells. However, mature neuronal transplantation leads to poor survival due to their detachment sensitivity and the fragility of neuritic extensions. Similarly, the transplantation of neuronal precursors does not allow in situ tight control of the neuronal identity and carries a tumor risk.

Methods: CNOs were generated through cell capsules technology developed in the lab associated with differentiation protocol of dopaminergic neurons (DN) from human pluripotent stem cells. Following the neuronal transplantation in immunocompromised hemiparkinsonian rats (6-OHDA), motor functions were evaluated by stepping test, cylinder test and amphetamine-induced rotations. CNOs characterization was carried out by immunostaining. In this study, we compared the therapeutic efficacy of 3D-neural organoids versus individual neurons transplantation.

Results: From eight weeks onwards after the transplantation, CNOs allowed functional recovery associated with tyrosine hydroxylase positive neurons into the graft whereas the transplantation of dopaminergic individual neurons did not induce any effect.

Conclusions: Our CNOs constitute more efficient and safer cell therapy products than individual neurons. Pending further validation, this innovative cell therapy approach for the treatment of PD could become a real alternative to drug-based symptomatologic treatments.

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A wireless brain-spine interface alleviating gait deficits of non-human primates model of Parkinson's disease

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Objective: While levodopa and deep brain stimulation alleviate most of the symptoms associated with Parkinson's Disease (PD), axial gait disorders are less responsive to these treatments. Impairments include short and slow steps, balance deficits and freezing of gait. Using MPTP-treated non-human primates, we studied the impact of a brain-spine interface on alleviating axial gait deficits observed in PD.

Background: We have established a mechanistic and technological framework that guided the design of electrical spinal cord stimulation protocols engaging extensor and flexor muscle groups. We created an interface that linked gait events decoded from leg motor cortex activity to spatially selective stimulation protocols that reinforced the movements associated with these events. As early as 6 days after spinal cord injury, this brain-spine interface restored weight-bearing locomotor movements of a paralyzed leg in a non-human primate model of spinal cord injury [1].

Methods: Three MPTP-treated Rhesus macaque monkeys, the gold standard model for PD symptomatology, were implanted with the wireless brain-spine interface. Recordings of multi-unit activity from the left and right leg motor cortex were used to detect neural states related to flexion and extension movements of both legs while the animal walked freely overground or over a horizontal ladder. The detection of these gait events triggered the delivery of spatially selective electrical stimulation protocols that reinforced the extension and flexion movements of the legs. Stimulation protocols were delivered using an implantable pulse generator with real-time triggering capability that was connected to a custom-made electrode array. The electrode layout was based on a computational model that estimated optimal locations to target the dorsal roots of each lumbar spinal cord segment.

Results: MPTP-treated monkeys exhibited moderate to severe axial gait deficits, including short and slow steps, balance deficits, freezing of gait, and poor precision of paw placement when traversing the horizontal ladder. The brain-spine interface instantly alleviated these deficits, allowing the monkeys to increase their walking speed, improved their balance and regained coordinated gait patterns. Moreover, the brain-spine interface enabled the monkeys to regain the ability to position the paws precisely on the rungs of the horizontal ladder.

Conclusions: These preliminary results illustrate the ability of the brain-spine interface to alleviate axial gait deficits and restore visuomotor control of leg movements in MPTP-treated non-human primates, standard model for PD symptomatology. These findings open promising avenues for targeting gait deficits in people with PD, which are still resistant to current treatments.

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Transplantation therapy of human iPS cell-derived dopamine neural progenitor cells for Parkinson’s disease

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Objective: To examine the efficacy of midbrain dopaminergic progenitors derived from human iPS cells, as the cell source for Parkinson's disease model mouse, by analyzing distribution of differentiated cells and evaluating improvement of motor function in host animals.

Background: It has been reported that the fetal cell transplantation could improve motor symptoms of Parkinson's disease patients. However, it is not used as a general treatment due to dyskinesia or ethical problems. Recently, midbrain dopaminergic progenitors derived from human iPS cells are expected as a potential cell source for future regenerative medicine because they are effective for rodent and primate PD models. In this study, we differentiated human iPS cells into highly-enriched dopaminergic neural progenitor

cells without cell sorting used in previous studies and transplanted these cells into PD model mice, to evaluate safety and effectiveness of these cells as a source of regenerative medicine.

Methods: To establish unilateral Parkinson model mice, we injected 6-OHDA into the striatum of immunodeficiency model mice. Severity of model mice were evaluated by rotation behavior of them after subcutaneous injection of apomorphine. Human iPS cells from healthy subjects are differentiated into dopamine neural progenitor cells by using a neural differentiation protocol we recently established. After the transplantation, we continuously evaluated the apomorphine-induced rotation behavior of recipient. Four months after transplantation, we sacrificed the animals and analyzed their brain section to evaluate differentiation properties of transplanted cells in that.

Results: We established 19 6-OHDA injected PD model mice that met the criteria of PD symptoms. Then dopaminergic progenitors were transplanted into 12 of them and saline was injected into 7 of them as a sham group. No tumor formation was observed in the brain section at four months after transplantation. At three months after transplantation, the transplant group began to recover from apomorphine-induced abnormal rotation behavior while the sham group did not.

Conclusions: Highly-enriched dopaminergic neural progenitor cells differentiated from human iPS cells by our neural induction protocol could be transplanted safely even without purification by cell sorting. Further transplantation and investigation are ongoing to confirm statistical significance of improved motor function in host animals.

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An investigation of the potential neuroprotective role of curcumin in Parkinson's disease patient-derived fibroblasts

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Objective: To investigate if the protective effects of curcumin previously observed in SH-SY5Y cells are also found in patient-derived fibroblasts from Parkinson's disease patients.

Background: Parkinson's disease (PD) occurs in 1-2% of individuals older than 60 years and is recognized by the loss of balance, resting tremors and bradykinesia. Potential underlying mechanisms of PD include mitochondrial dysfunction, oxidative stress and abnormal protein processing. To date, no cure exists for PD while various drug treatments only treat the symptoms and not the underlying cause of the neurodegeneration. Therefore, there is a need for a therapy directed at the underlying mechanisms of PD. Curcumin is a polyphenol, attributed with antioxidant, anti-inflammatory and antimicrobial properties. A recent study by our group showed that curcumin protected against mitochondrial dysfunction and cell death in a siRNA-mediated knockdown of PINK1 in SH-SY5Y cells [1]. The aim of this study was, therefore, to follow-up on the PINK1 cell model of PD and investigate the protective effect of curcumin in PD patient-derived fibroblasts.

Methods: Dermal fibroblasts were obtained from PD patients with LRRK2 genetic mutations. A MTT assay was used to determine the effect of paraquat and curcumin treatment on cell activity. Concentration and treatment duration curves for paraquat treatment were performed to obtain 50% cell viability loss in fibroblasts.

Results: We previously showed that paraquat (25 μM) decreased cell viability, increased apoptosis and disrupted mitochondrial function in the PINK1 SH-SY5Y cell model [1]. Notably, curcumin rescued cell viability, decreased apoptosis and increased mitochondrial respiration in these cells [1]. In the current study, we found that a higher concentration of paraquat (35 mM) was needed to decrease cell viability in the

primary fibroblasts. Studies are in progress to determine whether we see the same protective effect of curcumin in the fibroblasts.

Conclusions: The preliminary results indicate that primary fibroblasts are less sensitive to paraquat toxicity, than SH-SY5Y cells. We previously showed that curcumin was protective in SH-SY5Y cells and it would be interesting to observe the effect in patient fibroblasts. Successful results for curcumin in fibroblasts can further elucidate the role of curcumin as a potential neuroprotective agent against cell loss in PD.

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Preclinical evaluation of a novel autologous substrate for cell-based therapy

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Objective: Evaluate the survival and potential of brain-derived progenitor cells as a cell-based therapy(CBT) for Parkinson's disease in a mouse xenograft and syngeneic rat model.

Background: Brain biopsies from living PD patients, taken during deep brain stimulation surgery, can yield large numbers of progenitor cells with key merits of being both host- and brain-derived progeny (Xu et al., 2013). Interestingly, these brain-derived progenitor cells express a broad array of neurotrophic factors (NTFs) that include the most promising and potent cytoprotective agents against PD neurodegeneration. The colocalization of multiple NTFs (e.g., GDNF, BDNF, CDNF) with progenitor and neural proteins raises the intriguing prospect that BDPCs may effectively integrate into the brain to confer broad and enduring therapeutic function in PD, but a preclinical transplant models are a necessary step to evaluate this potential.

Methods: Human BDPCs were xenografted into immunocompromised NSG mice and Fischer rat BDPCs transplanted into syngeneic rodents. Before transplantation both cell populations were transduced to express a fluorescent luciferase construct and labelled with the iron oxide contrast agent, Molday ION Rhodamine-B™ (MIRB). The MIRB allowed in vivo BDPC tracking using a 3T MRI unit, in addition to bioluminescence imaging(BLI) via the luciferase reaction. Imaging data was correlated with histological findings to ascertain the accuracy and sensitivity of the two tracking modalities, as well as the long-term survival of the BDPCs in vivo.

Results: Xenografted human BDPCs in a pilot cohort of NSG mice show a persistent stable BLI signal and MRI signal void for 6 weeks. Fischer rat syngeneic grafts also showed a robust BLI signal for 2 weeks and were easily localized on MRI. Histological examination of both groups revealed expression of the transduced fluorescent tag highly colocalized with the MIRB tracking agent.

Conclusions: Human BDPCs may provide a novel substrate for personalized CBT in neurological disease. This work provides novel insight into the viability of BDPCs from living PD patients in a xenograft model and from rodents in a syngeneic model. It also offers the key advantage of using brain tissue from living PD patients and has potential to provide unparalleled contributions that expand the field of cellular imaging of the brain and the development of personalized therapeutics for PD.

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Vitamin B3 and Neurodevelopment in Parkinson's Disease

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Objective: This study aimed to investigate the potential of vitamin B3 to promote the conversion of stem cells to mature central nervous system (CNS) neurons.

Background: Vitamin B3 has been shown to play an important role during embryogenesis. Specifically, there is growing evidence that nicotinamide, the biologically active form of vitamin B3, plays a critical role as a morphogen in the differentiation of stem cells to mature cell phenotypes, including those of the CNS. Nicotinamide levels must be tightly regulated, with excess leading to neural damage, and deficiency leading to the condition, Pellagra, which manifests in a number of neural symptoms similar to Parkinson's. Detailed knowledge of the action of small molecules during neuronal differentiation is not only critical for uncovering mechanisms underlying lineage-specification, but also to establish more effective differentiation protocols to obtain clinically relevant cells for regenerative therapies for neurodegenerative conditions such as Parkinson's disease.

Methods: Nicotinamide was applied to developing mouse embryonic stem cells (mESCs) during their conversion towards a neural fate. Cells were assessed for survival, proliferation, differentiation and maturation.

Results: In the presence of an optimal dose of nicotinamide (10 mM), mESCs showed accelerated neural and neuronal differentiation, to yield higher numbers of dopamine neurons. Nicotinamide was shown to act in a dose-dependent manner in a defined time-window - with high doses (20mM) however causing toxicity. The mechanism of nicotinamide's action was through reduction in cell proliferation (i.e. cell cycle exit), rather than increased survival of neural progenitors. Nicotinamide alone was sufficient to generate dopaminergic neurons in similar numbers to current cell culture additives that have been used in previously published methods.

Conclusions: Our results show that, within an optimal dose range, nicotinamide is able to positively influence the conversion of embryonic stem cells to dopamine neurons, and therefore may be a critical factor for normal brain development. Thus, nicotinamide may offer a simple effective alternative supplement to enhance the use of stem cells as therapeutics for Parkinson's. An optimal maternal to foetal dietary dosage of nicotinamide - neither too low nor too high - could also be linked to protection from Parkinson's disease later in life.

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Biofabricated tissue constructs to recapitulate the anatomy and functionality of the nigrostriatal pathway in Parkinson's disease

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Objective: 1) Biofabricate constructs that mimic the cytoarchitecture and function of the nigrostriatal pathway, and 2) Microinject constructs to assess restoration of dopaminergic input to the striatum in a rat model of Parkinson's disease.

Background: Currently available therapeutic strategies to ameliorate the loss of nigrostriatal input to the striatum either replace dopamine systemically or place dopaminergic neurons directly into the striatum, both of which have significant limitations.

Methods: Dopaminergic neurons were dervived from either rat embryonic ventral mesencephalon or human stem cells. Green florescent protein expression on the tyrosine hydroxylase promoter enabled fluorescent activated cell sorting (FACS). Neuronal micro-spheres were generated via "forced cell aggregation" and then seeded within hydrogel micro-columns to facilitate axonal tract extension in vitro as described previously (Struzyna, et al. 2017). Constructs were stereotaxically microinjected into rats with the aggregate end placed in the substantia nigra and the fully-grown axonal terminals ending in the striatum. Constructs were assessed via phase contrast microscopy, immunochemistry, and confocal microscopy, and dopaminergic release was assessed using fast-scanning cyclic voltammetry (FSCV).

Results: Dopaminergic micro-sphere seeding resulted in axonal extension at rates exceeding 350μm/day. Micro-tubular constructs were generated with unidirectional dopaminergic axonal tracts spanning >1cm by 1 month in vitro. FACS-based enrichment resulted in >50% dopaminergic neurons. Electrical stimulation resulted in evoked dopamine release as measured by FSCV. Similar constructs were also fabricated using human stem cell derived neurons, with >50% dopaminergic neurons. Following stereotaxic microinjection in

rats, implanted neurons survived in the substantia nigra and integrated via local neurite extension, while the axonal projections to the striatum were maintained to >1 month post-transplant.

Conclusions: Implantable tissue engineered nigrostriatal pathways are capable of physically replacing lost dopaminergic neurons in the substantia nigra as well as their long-projecting axonal inputs to the striatum. Ongoing studies are assessing the ability of this strategy to restore striatal dopaminergic tone from the substantia nigra, thereby anatomically and functionally recapitulating the native pathway.

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MicroRNAs Unveil Metabolic Imbalance in Spinocerebellar Ataxia Type-2

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Objective: To correlate differentially expressed non-coding microRNA of peripheral blood mononuclear cells (PBMCs) with SCA2 pathogenesis

Background: Spinocerebellar ataxia aype-2 (SCA2), the most common SCA of India, is caused by expansion of uninterrupted CAG triplet repeats in first exon of ATXN2 gene. The ATXN2 is involved in the regulation of cellular processes such as RNA metabolism/stabilization, translation regulation, calcium homeostasis, and cytoskeleton reorganization etc. ATXN2 encompasses 25 exons occupying ~147 Kb of genomic space and harbouring long 3'-UTR stretch which altogether can be template for binding of several microRNAs. The non-coding miRNAs are established modifiers of gene expression in several neurological diseases but yet unexplored in SCA2.

Methods: Total RNA from PBMCs of four confirmed SCA2 patients and four matched healthy controls was analysed for differential miRNA expressions using SurePrint-Human-miRNA-Microarrays (Agilent Technologies, USA). The bioconductor "limma" package was used to calculate miRNA expression and unpaired t-test was applied to find significant ($p<0.05$) differential expression. Selected miRNAs were validated by qRT-PCR. Fisher's Exact Test was used for hypergeometric distribution. Online Dianna tool was used to identify correlation of miRNAs with cellular pathways.

Results: Total 421 miRNA were found to be differentially expressed; 6 were upregulated and 16 were down regulated (Figure-1). Some of deregulated miRNAs were found affecting cellular pathways like, lipid metabolism, Phosphatidylinositol signalling, biotin metabolism, calcium homeostasis etc. (Table-1) Target scanning also identified genes associated with altered Ca2+ channels, IP3 pathway etc.

Conclusions: Identified miRNAs were able to demonstrate altered metabolic processes and crucial pathways associated with SCA2 pathogenesis in concordance with our earlier proteomic work and other studies. Few miRNAs can be potential biomarkers or targets to develop new therapeutic approaches.

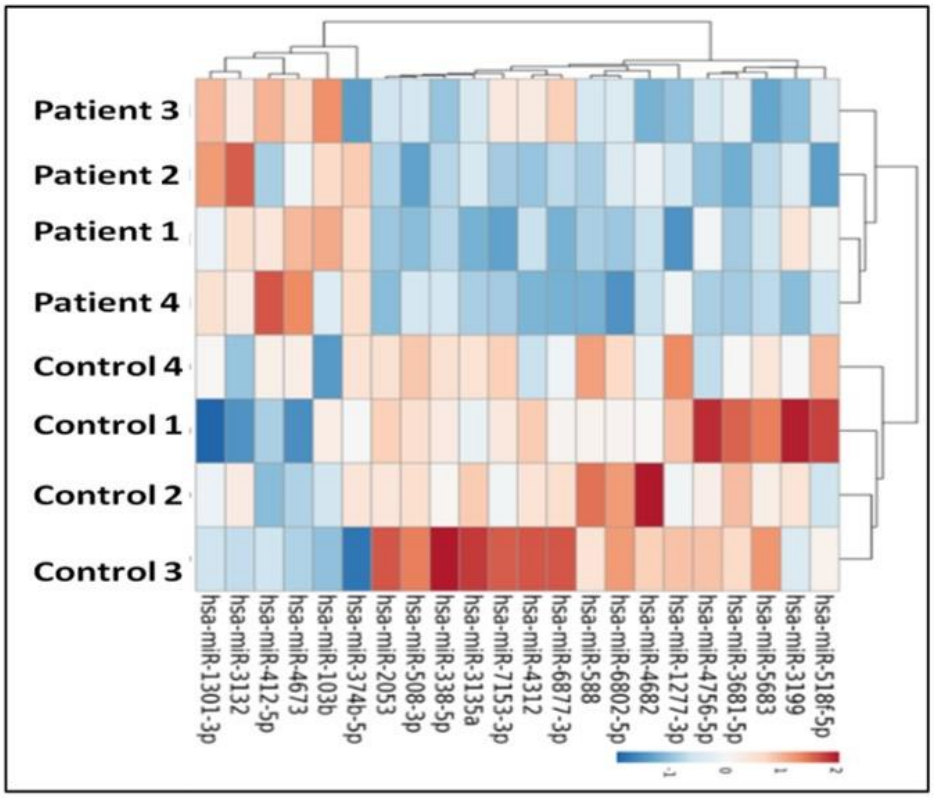

FIG. 1. (631) Heat map of differentially expressed significant miRNAs in SCA2 patients

TABLE 1. (631) Affected metabolic pathways related to altered miRNA expression

KEGG Pathway	miRNA	p-Value	Genes (Interaction Score)	p-Value
Biotin metabolism (hsa00780)	hsa-miR-6877-3p	1.9E-11	HLCS (0.85)	2.9E-05
Phosphatidylinositol signalling system (hsa04070)	hsa-miR-3132	3.8E-02	IMPAD1 (0.92), ITPR2 (0.96), DGKI (0.84)	5.6E-04
	hsa-miR-3135a	6.1E-06	PLCD4 (0.85), INPP4A (0.81), MTM1 (0.89)	
Lysine degradation (hsa00310)	hsa-miR-508-3p	2.6E-07	CAMKMT (0.92), SETD7 (0.99), PIPOX (0.86), WHSC1 (0.84)	1.3E-03
Fatty acid degradation (hsa00071)	hsa-miR-508-3p	6.9E-04	ACOX1 (0.894)	1.8E-02
	hsa-miR-3135a	5.7E-04	HADHB (0.801)	
Fatty acid elongation (hsa00062)	hsa-miR-3135a	1.7E-06	HADHB (0.801)	1.8E-02
Thyroid hormone signalling pathway (hsa04919)	hsa-miR-508-3p	5.2E-05	MED12L (0.854), THRB (0.802), NCOA1 (0.941)	3.2E-02
	hsa-miR-3135a	4.6E-02	PLCD4 (0.852), SLCO1C1 (0.897), PFKFB2 (0.941)	

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Transcriptional profiling of peripheral blood monocytes from child Friedreich's ataxia patient: New molecules and patterns of gene expression

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Objective: To explore peripheral biomarkers related to Friedreich's ataxia and identification of pathophysiological insights of complex phenotype

Background: Friedreich's ataxia(FRDA) causes nervous system damage and movement problems which starts in children leading to early death. Although reduction in frataxin level is responsible for FRDA, other deregulated bio-markers may also contribute in disease phenotype mediated by cellular pathways.Therefore, identification of such biomarkers can be helpful in order to understand disease etiology and development of future medications. In the present study genome-wide expression analysis was performed in FRDA child patients as compared to normal children.

Methods: Transcriptome expression profiling of FRDA and controls peripheral blood cells was performed on a cohort of 28 patients and 10 controls that was extracted from GSE11204. To calculate mRNA expression, the bioconductor R package "limma" was used. Significantly deregulated genes were filtered using unpaired Student-t test and bonferrani test. The gene list associated with FRDA was extracted from DisGeNET database (http://www.disgenet.org). The ontology analysis of significantly altered genes was done using String Database (string-db.org).

Results: A total of 144 genes were found to be significantly altered ($p<1.0E-06$) in children manifest FRDA phenotype (Figure 1). Out of 144 genes, 76 genes were found to be downregulated and 68 genes were upregulated. It is also interesting to note that the identified genes were not reported earlier in FRDA provided at DisGeNET database. Genes associated with metabolic processes such as cellular metabolism of the transition metal, lipid metabolism etc (Table 1) were found to deregulated leading to altered metabolic processes damage and affect tissues leading to neuro-and cardio-degeneration.

Conclusions: The results suggest gene expression pattern consistent with metabolic process damage leading to neuro- and cardio-degeneration complexities in FRDA. Although the development of effective therapeutics enhanced analysis is required, identified biomarkers could also provide insight into pathway based etiology and may have predictive value in future clinical trials.

TABLE 1. (655) Gene ontology analysis of differentially expressed genes (n=144) in peripheral blood cells in Friedreich s ataxia

BIOLOGICAL PROCESS (GO)			
Pathway ID	***Pathway description***	***Count in gene set***	***FDR***
GO:0043933	Macromolecular complex subunit organization	32	0.004
GO:0044267	Cellular protein metabolic process	43	0.004
GO:0019538	Protein metabolic process	44	0.039
GO:0043412	Macromolecule modification	35	0.039
GO:0008064	Regulation of actin polymerization or depolymerization	07	0.045
MOLECULAR FUNCTION (GO)			
GO:0044822	Poly(A) RNA binding	24	0.001
GO:0003723	RNA binding	27	0.002
GO:0004713	Protein tyrosine kinase activity	07	0.036
CELLULAR COMPONENT (GO)			
GO:0070062	Extracellular exosome	34	0.029
GO:0044421	Extracellular region part	40	0.048
GO:0030529	Ribonucleoprotein complex	13	0.049
GO:0044444	Cytoplasmic part	64	0.049

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Disease Modeling of DYT1 Using Patient Induced Pluripotent Stem Cells

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Objective: To create a resource of induced pluripotent stem cells (iPSCs) as models for exploring mechanisms of pathogenesis in DYT1 dystonia.

Background: DYT1 an autosomal dominant early-onset movement disorder characterized by involuntary muscle contractions which force body parts into abnormal postures and or movements. DYT1 is caused by a 3-base-pair deletion (GAG mutation) in the Torsin1A gene. The function of the Torsin1A protein is only partially understood, but many studies point to a problem with dopaminergic neuron function.

Methods: Fibroblasts from 3 healthy controls and 3 DYT1 patients(with GAG mutations in Torsin1A) were reprogrammed to iPSCs. Two independent lines were created for each case. Immunocytochemistry was performed on iPSCs to confirm expression of pluripotency markers. We nest confirmed the karyotype of the iPSCs. Pluripotency was verified by testing ability to differentiate to the three germ layers: ectoderm, mesoderm, and endoderm. Gene expression and protein composition profiles were then determined for iPSCs from cases and controls.

Results: All 12 lines had immunostaining profiles consistent with pluripotency, and possessed the ability to differentiate into all 3 germ layers. All lines had normal karyotypes. All lines expressed high levels of genes typical of pluripotent cells. RNA-Seq identified a total of 81 differentially expressed genes between DYT1 and healthy controls with nominal $p<0.001$. After correcting for multiple comparisons, none of the genes remained significantly differentially expressed at a FDR<0.1. Studies addressing protein expression profiles and metabolic abnormalities are underway, as well as studies addressing their ability to differentiate into neurons.

Conclusions: We have validated multiple iPSC lines from patients with LND and matched controls. The lines from patients show consistent abnormalities in gene expression profiles and are being used now to evaluate dopamine neuron function.

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Screening study of COL6A3 in sporadic isolated dystonia

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Objective: To verify the role of COL6A3 in isolated dystonia.

Background: COL6A3 has recently been suggested to cause a form of autosomal recessive isolated dystonia. Until now, there is limited information about Col6A3 gene test in Asian population. Herein, we performed comprehensive COL6A3 mutation screenings in 179 sporadic isolated dystonia patients from Chinese population, including 57 early-onset cases.

Methods: We collected blood samples and used Polymerase chain reactions (PCR) to amplify 43 coding exons and intron 41 of COL6A3 (NM_004369.3). Mutational screening was performed by direct sequencing. All patients were negative for the genetic testing of TOR1A, THAP1, CIZ1, GNAL and ANO3.

Results: None of the reported disease-causing mutations were found. As a result, we identified one patient with compound heterozygotes of maternal and paternal origin (c.5000A>G[p.D1667G] and c.7570C>T[p.P2524S](Fig 1). Bioinformatics analysis demonstrated that both variants exhibit highly conserved residues across species. Structural analysis revealed hydrogen bonding interaction disruption in D1667G and free energy increase in P2524S. Besides, 8 other variants were found in 8 dystonia patients separately.

Conclusions: Our study revealed that mutation in COL6A3 is not a major cause of sporadic isolated dystonia, at least in Chinese population. Functional analysis D1667G and P2524S and validation in a larger study from a more diverse ethnic population are needed to ascertain the role of COL6A3.

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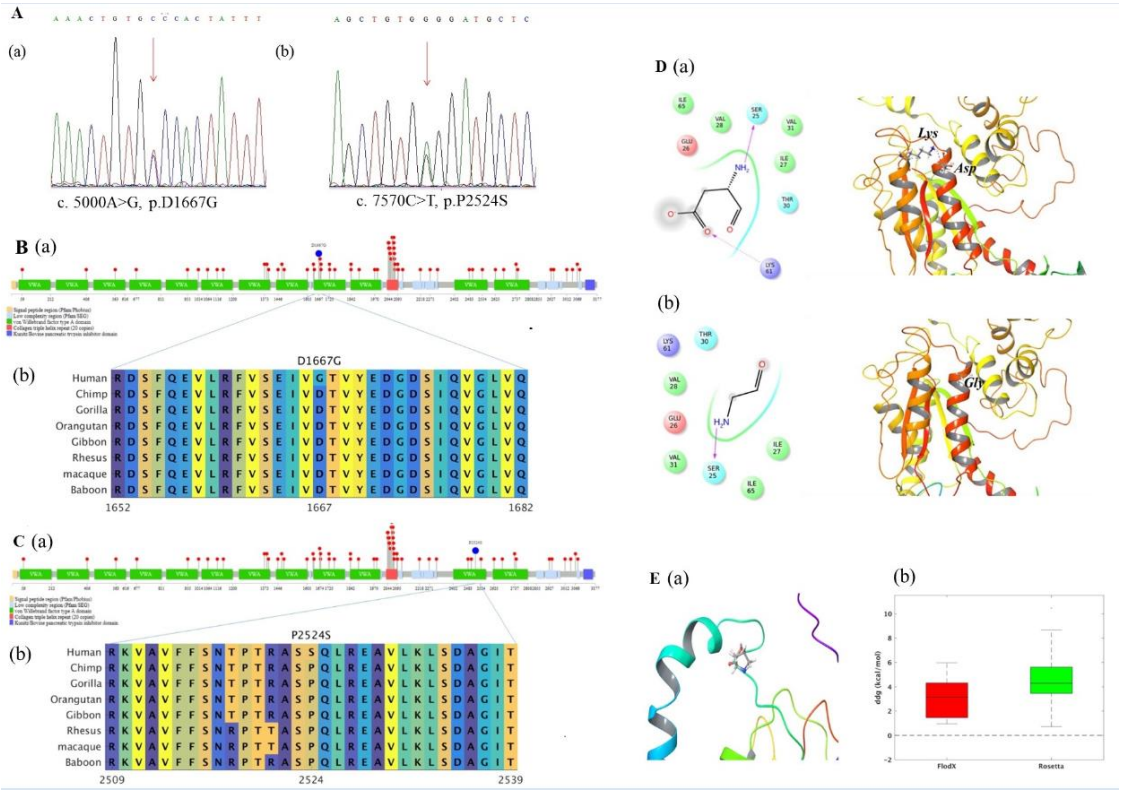

FIG. 1 (710)

730

Generation and in-depth characterization of induced pluripotent stem cell (iPSC) lines from 10 affected and unaffected carriers of THAP1 mutations

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Objective: To establish induced pluripotent stem cells (iPSCs) of affected and unaffected Mutation carriers to investigate disease mechanisms of THAP1 in dystonia.

Background: Mutations in the THAP1 (THAP domain containing, apoptosis associated protein 1) gene may cause a form of early-onset, generalized dystonia (DYT-THAP1, also known as DYT6). Of note, some mutation carriers remain unaffected throughout their life (reduced penetrance). THAP1 encodes a transcription factor and it has been suggested that THAP1 regulates its own expression and TOR1A expression. Of note, TOR1A is also mutated in some patients with dystonia (DYT-TOR1A, DYT1). However, little is known about the gene expression in human iPSCs. Therefore, this study aims to investigate the mRNA expression levels of THAP1 and TOR1A in iPSCs of 4 affected and 6 unaffected members from three different families carrying pathogenic THAP1 variants (p.Arg13His, p.Ser21Cys, p.Leu159fs180X).

Methods: Cultured skin fibroblasts were reprogrammed into iPSCs using Sendai virus. Two clones per patient were comprehensively characterized by testing for the mutation using Sanger sequencing, by expression analysis of four pluripotency markers using quantitative PCR and immunocytochemistry, and by their ability to differentiate into all three germ layers. Genomic rearrangements were excluded by single nucleotide polymorphism (SNP) array analysis. Expression of THAP1 and TOR1A was tested by quantitative PCR compared to 10 iPSC controls while ß-Actin served as a reference gene.

Results: We generated 2 iPSC lines each of 10 affected and unaffected members of three families carrying pathogenic THAP1 variants (p.Arg13His [4 lines], p.Ser21Cys [10 lines], p.Leu159fs180X [6 lines]). THAP1 expression was reduced for Arg13His and TOR1A expression in Arg13His and Leu159fs180X mutant cell lines compared to controls.

Conclusions: We report the generation and characterization of 20 lines from 10 human THAP1 iPSC lines as well as alterations in THAP1 and TOR1A expression in these cells. These stem cells can further serve as an ideal model to investigate the mechanism of reduced penetrance by transcriptomic analysis in affected and unaffected THAP1 mutation carriers on the stem cell and differentiated neuron level.

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Peripheral trauma elicits a dystonic phenotype in a DYT1 transgenic mouse model

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Objective: To induce a dystonic phenotype in DYT1 (hΔGAG3) mice through peripheral trauma in order to analyze metabolic and structural alterations in the striatum.

Background: DYT1 is the most common form of hereditary dystonia caused by mutations in the Tor1a gene with a penetrance of only 30%. hΔGAG3 mice express the human torsinA mutation and do not show a dystonic phenotype in their naïve state. We aimed to examine whether environmental factors have a lasting effect on the development of dystonic symptoms supporting a two hit hypothesis. Therefore, we induced a peripheral nerve trauma in genetically predisposed hΔGAG3 mice.

Methods: We analyzed wildtype (wt) and hΔGAG3 mice (tg) subdivided into naïve, sham and groups with nerve trauma. Dystonia-like movements were defined as clenching and retraction of hindlimbs. Before and after unilateral sciatic nerve crush injury dystonia-like movements were scored during tail suspension test (TST) according to frequency and duration with a 0-5 points scoring system. Rotarod Performance Test (RPT) was applied before nerve injury and afterwards over 12 weeks. After brain dissection at the end of week 12 the striatum was analyzed via HPLC to determine the quantity of catecholamines.

Results: While both tg and wt mice developed dystonia-like movements 2 weeks after nerve injury during TST, tg mice displayed a significantly higher dystonia score starting at week 3 compared to wt mice (week 12: 2,2±0,6 vs 0,4±0,2 points, $P<0,05$). Dystonic movements in wt mice declined after week 11 almost to control levels. RPT showed no significant differences in the latency to fall in tg and wt mice after nerve trauma. Dopamine (DA) level of the contralateral striatum showed a 23.3% reduction in sham-operated tg mice compared to wt mice. Crush injury led to a non-significant increase of DA levels by 42.4% in tg mice and mild decrease in wt mice.

Conclusions: Our study indicates that peripheral nerve trauma is able to trigger dystonic movements in genetically predisposed, asymptomatic hΔGAG3 mice. Preliminarily, monoamine analysis hints at a possible striatal dopamine dysregulation in hΔGAG3 mice. In summary, this study supports a potential two hit hypothesis in DYT1 dystonia.

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Disease Modeling of Lesch-Nyhan Disease Using Induced Pluripotent Stem Cells

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Objective: To create a resource of induced pluripotent stem cells (iPSCs) as models for exploring mechanisms of pathogenesis in Lesch-Nyhan disease (LND).

Background: LND is an X-linked metabolic disorder with characteristic abnormalities that include dystonia, intellectual disability, and self-injurious behaviors. LND is caused by mutations in the HPRT1 gene, which encodes the purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGprt). The mechanisms responsible for neurological and behavioral abnormalities are unclear. Because there are no signs of degeneration in the LND brain, the neurobehavioral syndrome is thought to be due to neural dysfunction.

Methods: Fibroblasts from 3 healthy controls and 3 LND patients (with nonsense mutations c.151C>T or c.508C>T, or frame-shifting insertion 371insTT) were reprogrammed to iPSCs. Two independent lines were created for each case. Immunocytochemistry was performed on iPSCs to confirm expression of pluripotency markers. Karyotyping was performed. Pluripotency was verified by testing ability to differentiate to the three germ layers: ectoderm, mesoderm, and endoderm. Cells were then evaluated for gene expression and protein expression profiles.

Results: All 12 lines had immunostaining profiles consistent with pluripotency and possessed the ability to differentiate into all 3 germ layers. All lines had normal karyotypes. All lines expressed high levels of genes typical of pluripotent cells. RNA-Seq identified a total of 144 differentially expressed genes between LND and healthy controls at nominal $p<0.001$. After correcting for multiple comparisons, 16 genes remained significantly differentially expressed at FDR<0.1, including the HPRT1 gene. Gene Set Enrichment Analysis (GSEA) analysis revealed a number of biological pathways significantly altered. Studies addressing protein expression profiles and metabolic abnormalities are underway, as well as studies addressing ability to differentiate into neurons.

Conclusions: We have validated multiple iPSC lines from patients with LND and matched controls. The lines from patients show consistent abnormalities in gene expression profiles, and are being used now to evaluate dopamine neuron function.

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Striatum of the ouabain-induced pharmacological DYT12 mouse model is affected by structural and metabolic abnormalities

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Objective: To study changes in neuronal morphology and neurotransmitter metabolism in the striatum of a pharmacological DYT12 mouse model after induction of a dystonic phenotype by motor stress.

Background: Patients with Rapid-Onset Dystonia-Parkinsonism (DYT12) carry a mutation of the α3 isoform of the Na+/K+-ATPase, which can be pharmacologically simulated through perfusion of ouabain into the striatum and cerebellum of wt mice. As seen in DYT12 patients, the development of a dystonic phenotype in ouabain-treated mice was induced by physical stress.

Methods: Striatum and cerebellum of wt mice were perfused over 72 h with NaCl (control) or ouabain via osmotic pumps, at a 24 h interval one group of ouabain-treated mice were subjected to motor stress in form of a Rotarod performance test and Pole test. A Dystonia Rating Scale (DRS) assessed the frequency and distribution of dystonia-like movements from 0-4 points. Mice were scored in a tail suspension test (TST) from 0-8 points for dystonia-like movements in forelimbs, hind limbs and trunc. A Golgi-Cox staining visualized medium spiny neurons (MSN) in the striatum and was evaluated via Sholl-analysis. Monoamine activity in the striatum was analyzed via HPLC.

Results: The DRS score and TST score in stressed mice were significantly higher than in unstressed mice (DRS: 3.0 ± 0.20 vs 2.1 ± 0.19, $p < 0.01$; TST: 5.50 ± 0.32 vs 3.77 ± 0.53, $p < 0.005$). Golgi-Cox staining showed a reduction in spine density, number of spines and dendrite lengths of MSN in both ouabain-treated groups compared to NaCl-perfused control mice. Stress application did not lead to any additional structural alterations. HPLC analysis revealed that ouabain treatment only led to significant reduction in striatal dopamine (DA) levels in comparison to the control group (relative level: 0.55 ± 0.09 vs 1.0 ± 0.034, $p < 0.05$), however motor stress in ouabain mice restored DA activity almost to control levels (0.90 ± 0.08). A significant increase in striatal DOPAC and HVA levels as well as DA turnover ratios in both ouabain-treated groups was recorded compared to NaCl-perfused mice.

Conclusions: A dystonic phenotype was successfully induced by application of motor stress in this DYT12 mouse model. Alterations in the major striatal output neurons are hinting at significant functional abnormalities. Furthermore, DA dysregulation may play a central role in DYT12 disease development.

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Neuropathology of dopa-responsive dystonia due to Tyrosine hydroxylase deficiency

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Objective: To describe detailed neuropathology findings from an autopsy case of dopa-responsive dystonia (DRD) with genetically confirmed tyrosine hydroxylase deficiency (THD).

Background: DRD comprises a group of rare monogenic conditions, characterized by a relatively static dystonic phenotype and disease course with marked and sustained response to low doses of dopamine. Previously, only three autopsy cases of confirmed GTP Cyclohydrolase-1 (GCH-1) related DRD had been reported. THD is a rare form of DRD and it`s histological changes are unknown.

Methods: Paraffin embedded brain tissue sections from a patient (male, 49 years) with genetically confirmed compound heterozygous mutation in the TH gene (c.1127C>T, p.A376V and c.1493A>G, p.D498G), previously published (Schiller et al., 2004), were examined histologically. In addition to haematoxylin & eosin staining, immunohistochemistry with following antibodies and dilutions were applied to respective brain tissue: glial fibrillary acidic protein (GFAP; 1:1,000, Dako), tau (1:600, AT8, Autogen Bioclear, Calne, UK), Aβ (1:200, Dako), α-synuclein (1:50, Novacastra, Newcastle, UK), ubiquitin (1:200, Dako, Ely, UK) and p62 (1:100, BD Bio-science, Oxford, UK).

Results: The salient histological feature was a striking pallor of neuromelanin containing neurons in the midbrain substantia nigra without obvious decrease in cell density, whereas neuromelanin content in the neurons of the motor nucleus of the vagus nerve appeared preserved. Immunohistochemical studies provided no evidence of pathological accumulation of proteins such as a-synuclein and amyloid-β, showed no ubiquitin or p62 positive intranuclear inclusions to suggest trinucleotide repeat expansion disorder and also showed no evidence of cerebral amyloid angiopathy. Hyperphosphorylated tau pathology was restricted to rare neuropil threads in the transentorhinal cortex with no apparent neurofibrillary tangle or glial tau pathology.
Quantification of neuromelanin-containing midbrain neurons as well as TH-positive striatal terminals comparing this case to patients with confirmed GCH-1, DYT1, sporadic dystonia, Parkinson´s disease and healthy controls is currently ongoing.

Conclusions: This work provides the first description of histological changes in THD, indicating a predominant loss of neuromelanin-pigment from midbrain neurons. It remains to be seen in how far this might constitute a DRD-specific change in comparison to other dystonic conditions.

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806

Elevated serum α-synuclein levels in Huntington's disease patients

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Objective: To investigate serum α-synuclein levels in Huntington's disease (HD) patients.

Background: HD is a heredodegenerative disease caused by mutations in HTT coding for huntingtin (Htt). A cross-talk between Htt aggregation and α-synuclein has been reported, even though the mechanism underlying such interaction is still obscure. Elevated serum α-synuclein has been demonstrated in other neurodegenerative diseases such as Parkinson's disease, in contrast to the declining levels of serum α-synuclein observed with normal ageing.

Methods: In total, 32 consecutive symptomatic patients with molecularly confirmed HD, 4 presymptomatic subjects positive for the HD expansion and 14 control subjects were recruited from the Neurogenetics outpatient clinic. Peripheral blood was collected during clinic visits, allowed to coagulate and centrifuged for serum collection. Serum α-synuclein was measured using our in house ELISA. For statistical analysis symptomatic and presymptomatic HD patients were grouped together. Chi-square tests, ANOVA

and ANCOVA were used as appropriate to investigate differences between groups. Correlations between groups were investigated using Pearson's method. All statistical analysis was performed on SPSS v.20.

Results: HD and control groups were well matched for sex, but not for age (HD patients on average 10 years older than controls) or medication (69% of HD group on symptomatic treatment vs. 0% of controls). Serum α-synuclein levels were significantly higher in HD patients vs. controls (p=0.004). To control for the age difference between HD and controls, we used ANCOVA with age as covariate, which did not affect the significance of the result (p=0.010). Serum α-synuclein levels did not differ significantly between male and female HD patients (2.45 ± 0.94 vs 2.43 ± 1.46 ng/ml; p=0.972, ANOVA). Serum α-synuclein levels did not differ significantly between HD patients receiving medication and HD patients not receiving medication (2.33 ± 1.19 vs 2.56 ± 1.53 ng/ml; p=0.639, ANOVA). Within the HD group, serum α-synuclein levels did not correlate significantly with CAG2, UHDRS motor score, age or disease duration.

Conclusions: Our results provide evidence for elevated serum α-synuclein levels in HD patients. Insights on α-synuclein levels may shed further light on the mechanism of pathological Htt aggregation and contribute to the identification of robust biomarkers of disease progression in HD. Further investigation in larger populations is needed to support our findings.

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Cerebrospinal fluid proteomics reveals alterations of the renin-angiotensin system in Huntington's disease

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Objective: To identify new biomarkers of disease stage in HD.

Background: Proteomic approaches for CSF studies in Huntington's disease (HD) have so far yielded limited information, but with more advanced techniques we aimed to discover new proteins involved in the pathophysiology of HD.

Methods: The study was conducted in accordance with the declaration of Helsinki and was approved by the local research ethics committee in Uppsala, Sweden. All participants signed an informed consent. A standardized protocol for lumbar puncture was applied. A clinically well characterized HD cohort was enrolled. Relative protein levels were measured with liquid chromotography-mass spectrometry. The study enrolled manifest HD-patients (n=13) and premanifest HD gene expansion carriers (n=12). Repeated samples after 1-4 years were obtained (n=10), and age- and sex matched gene negative controls (n=45).

Results: Protein level differences clearly separated the three groups. Upregulated proteins were mainly pro-inflammatory with roles in both innate- and adaptive immunity. Other upregulated proteins were involved in tissue remodeling, and plasticity. Downregulated proteins were involved in synaptic regulation, cell adhesion, neurite growth, and axonal transport. Alterations in proteins regulating calcium homeostasis, and the renin-angiotensin system were also discovered.

Conclusions: The results highlight the continued value of untargeted CSF proteomics approaches to discover disease related mechanisms. We aim to validate markers from selected pathways of interest with targeted assays.

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Pharmacological investigation of biochemical and behavioral effects resembling Huntington's disease utilizing selective calcium and calmodulin dependent phosphodiesterase inhibitor

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Objective: To investigate the effects of selective phosphodiestrase (PDE) inhibitors in Nimodipine induced neuro degeneration (animal models of Huntington's disease) in rats.

Background: Nimodipine is a dihydropyridine that antagonizes/blocks specifically L-type Ca2+ channel, and was first described as a PDE 1 inhibitor. This effect is not related to its calcium antagonist property since it inhibits, in micromolar range, basal and calmodulin stimulated purified PDE1. Since nimodipine at lower

concentrations blocks the L-type calcium channel, it can only be used to estimate PDE1 participation in tissue and cell homogenates. Nimodipine acid is a mycotoxin reported to inhibit succinate dehydrogenase resulting in mitochondrial dysfunction and cellular energy deficit. Associated memory deficit could be related to its selective striatal and hippocampal neuronal damage, but the reason for such selectivity is not known. The levels of both cAMP and cGMP are also reported to be decreased in neuropathological conditions which can be modulated by utilizing specific PDE inhibitors.

Methods: Nimodipine and PDE-5 inhibitor were co-administered i.p. for 21 days in separate groups, and the effects of such drug administration's were assessed on Morris Water Maze test, Spontaneous Locomotor Activity, Limb Withdrawal Test, and String test for grip strength. Biochemical parameters measured were: Succinate Dehydrogenase, Malondialdehyde, Reduced glutathione, Nitrites and Lactate Dehydrogenase. Morris water maze test data were analyzed by repeated measure two way ANOVA & rest of the parameters by one way ANOVA followed by Tukey's post-hoc test by using statistical software.

Results: Chronic administration of Nimodipine significantly reduced body weight, caused cognitive, motor deficits and increased oxidative–nitrosative stress, relating to/indicates neurodegeneration. Pharmacological inhibition of PDE-4, and to a lesser extent, PDE-5 significantly improved cognitive, motor functions and decreases the oxidative-nitrosative stress and increased the reduced glutathione, Nitrites and Lactate Dehydrogenase in Nimodipine administered rats.

Conclusions: The experimental results suggest that PDE-I inhibition is more beneficial than PDE-5 inhibition in offering neuroprotection against Nimodipine induced cognitive and motor deficits.

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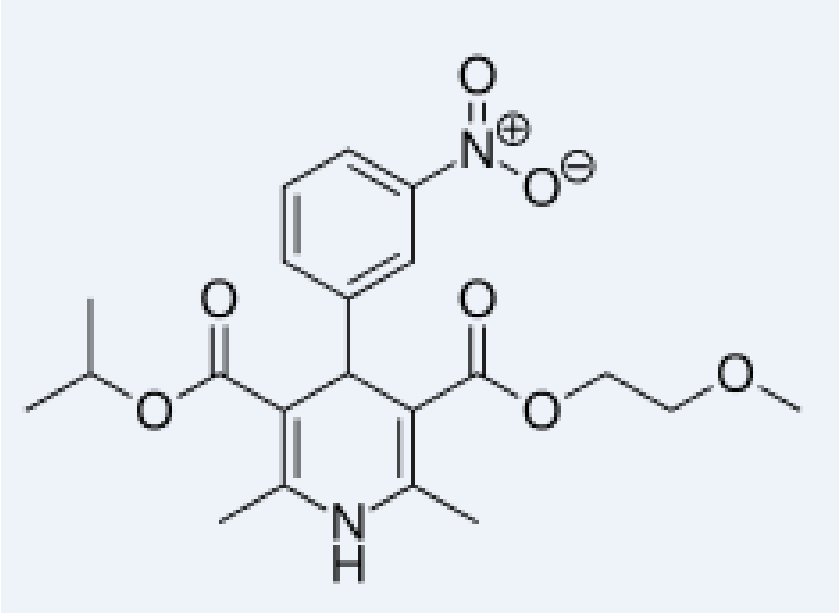

FIG. 1 (811)

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Neurofilament light protein: An emerging clinical and translational biofluid biomarker for Huntington's disease

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Objective: To investigate whether neurofilament light protein (NfL) a potential prognostic marker of neurodegeneration with Huntington's disease.

Background: NfL, a component of the axonal cytoskeleton, has been shown to be increased in cerebrospinal fluid (CSF) and blood and to respond to successful treatment in several neurological diseases.

Methods: We studied NfL in plasma from 298 participants, in plasma and CSF in 37 participants, and in the R6/2 HD mouse model.

Results: NfL concentration was increased in plasma at every stage of HD including premanifest mutation carriers, rose with progression and had a striking relationship with HTT CAG repeat length. In premanifest HD, baseline plasma NfL predicted subsequent motor onset even after adjustment for age and CAG repeat length. NfL predicted clinical, cognitive and neuroimaging progression, and CSF and plasma levels were strongly associated (Byrne et al, Lancet Neurology 2017). VBM analysis revealed that NfL level predicted atrophy throughout the white matter and in the occipital grey matter (Johnson et al, Neurology 2018). In the R6/2 mouse model, NfL was increased in plasma and CSF and associated with brain volume and clinical measures (Soylu Kucharz et al, Scientific Reports 2017).

Conclusions: NfL is a promising clinical and translational biomarker for HD.

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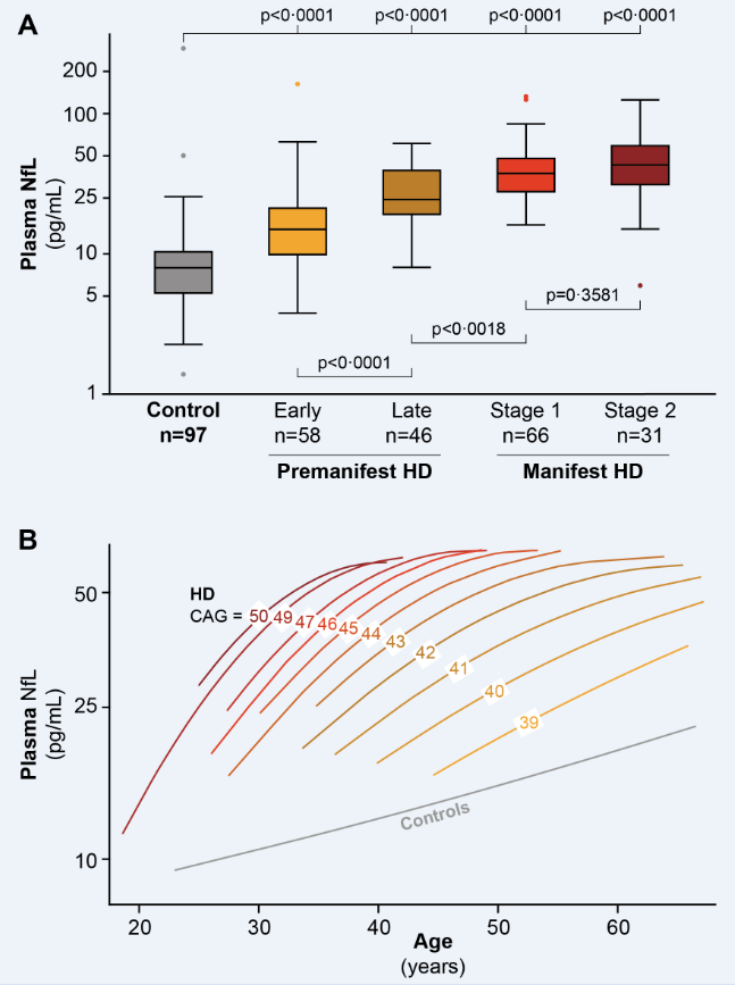

FIG. 1 (826)

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Neuroprotective role of cinnamaldehyde against 3-nitropropionic acid-induced oxidative stress in a rat model of Huntington's disease

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Objective: The present study examined the potential therapeutic effects of CA against 3-nitropropionic acid (3-NP)-induced oxidative stress in a rat model of HD and explored the mechanisms of action.

Background: Huntington's disease (HD) is a neurodegenerative disorder characterized by symptoms like chorea and dementia, results from the destruction of neurons in the basal ganglia, and oxidative stress has been implicated in its pathogenesis. Cinnamaldehyde (CA) is a diterpene with a wide range of anti-inflammatory, cognitive enhancer, neuroprotective, anti-diabetic effects thus may be advantageous in the treatment of neurological disease.

Methods: 50 Male SD rats were pretreated with CA (20 and 40 mg/kg b.w.) orally prior to the intraperitoneally (i.p.) administration of 3-NP (12 mg/kg b.w.) for 15 days. Nimodipine (12 mg/kg, po) was used as positive control drugs. The body weight, grip strength and behavior were monitored within 5th, 10th and 15th day after 3-NP treatment. Then the animals were sacrificed, neuronal damage in striatum was estimated using Nissl staining. Hsp70 expression was detected with immunohistochemistry. Reactive oxygen species (ROS) generation was measured using dihydroethidium (DHE) staining. Memory (Morris water maze) , antioxidants enzymes and lipid peroxides were analyzed in rat model of HD.

Results: Present results shown that administration of 3-NP resulted in a marked reduction in the body weight, memory, grip strength locomotion activity and significantly increased lipid peroxidation and depleted antioxidant enzyme accompanied by progressive striatal dysfunction. CA (20 and 40 mg/kg) treated animals exhibited a significant ($p < 0.01$) improvement in behavioural, biochemical, histological alterations and oxidative stress parameters in comparison to only 3-NP treated animals. Present results shown dose-dependently improved 3-NP-induced behavioral, biochemical, and enzymatic changes ($P < .001$). Similar effects were obtained with the positive control drugs nimodipine.

Conclusions: CA exerts a protective action against 3-NP-induced oxidative stress in the rat model of HD, which is associated with its anti-oxidant activity, and consequently improves behavioral deficits.

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Pronounced synucleinopathy and nigrostriatal degeneration result in forelimb use deficits in the rat preformed alpha-synuclein fibril model

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Objective: Generation of a synucleinopathy model in rats using injections of α-synuclein preformed fibrils (α-syn PFFs) resulting in robust and widespread α-syn pathology and at least 50% nigrostriatal degeneration.

Background: Genetic, viral vector, and neurotoxicant models of Parkinson's disease (PD) fail to recapitulate all the key features of PD. We have previously demonstrated that intrastriatal injections of sonicated α-syn PFFs result in widespread α-syn pathology and progressive bilateral nigrostriatal degeneration (≈30-40%) in rats. This degree of degeneration was not sufficient to produce motor deficits. The present study utilizes optimized stereotaxic coordinates in the dorsal striatum and an increased concentration of α-syn PFFs in attempt to increase the magnitude of nigral α-syn pathology, neurodegeneration, and elicit motor deficits.

Methods: Male Fischer 344 rats (n=88) were injected with 8 or 16 total µg of α-syn PFFs, 16 µg α-syn monomer or an equal volume of vehicle at two sites of the dorsal striatum. Post-mortem pathology and motor performance was evaluated at 2, 4, and 6 months after surgery.

Results: At 2-months post-injection (p.i.), the peak of phosphorylated α-syn inclusions was observed in the SNpc with approximately 35% of nigral dopamine neurons accumulating phosphorylated α-syn. Increased amounts of α-syn PFF concentration resulted in significant bilateral nigrostriatal degeneration with

substantia nigra pars compacta (SNpc) loss as high as ~59% ipsilateral and ~55% contralateral to injection at the 6-months p.i. Ipsilateral degeneration preceded contralateral degeneration, with significant degeneration of 35% of the ipsilateral SNpc neurons observed at 4 months compared to only 4% loss contralaterally at this same time point. Rats receiving the highest concentration of α-syn PFFs exhibited significant contralateral forelimb akinesia (~20% reduction, $p = 0.0254$) as assessed via the adjusting steps task.

Conclusions: Increased amounts of α-syn PFFs and optimized striatal injection coordinates can exacerbate the magnitude of synucleinopathy and nigrostriatal degeneration in rats, resulting in significant motor impairments. The α-syn PFF rat synucleinopathy model can serve as a valuable platform for allowing new therapies to be tested in a preclinical model of PD.

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Models of Parkinson's disease in vitro

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Objective: The problem for experimental studies of the pathogenesis of Parkinson's disease is the impossibility of taking material from patients with this pathology, therefore, it is almost completely impossible to observe the processes occurring in living human cells in BP. Models of Parkinson's disease are known in vivo (on living organisms) and in vitro ("in vitro"). The most approximate to the processes of human cells are models of pathology in vivo in animals, but when using them there are difficulties with the reproducibility of results, the difficulty of keeping pure lines of animals and justifying the use of models in vivo before the ethical committee. At the same time, recently in vitro models have been increasingly used. Thus, cell cultures are a universal method for studying "physiological" and pathological phenomena, clarifying the mechanisms of signal transmission, regulation of gene expression, cell proliferation, as well as the mechanisms of their death. These models do not completely exclude the model in vivo, but are a good addition to them, allowing to study physiological phenomena and pathogenesis mechanisms of diseases.

Background: To find out the mechanisms of signal transmission, regulation of gene expression, cell proliferation and death.

Methods: Among the models of Parkinson's diseases in vivo are models of genetic (knockout and transgenic models), neurotoxic (systemic administration of neurotoxins) and stereotaxic (stereotaxic administration of rotenone, paraquat, 6-OHDA, MPP +, MPTP, methamphetamine, degeline and other neurotoxins). Models with exogenous applications (rotenone, paraquat and MPTP) and endogenous (6-hydroxydopamine, MPP +, L-DOPA) neurotoxins are mainly used in in vitro models. For the modeling of Parkinson's disease in vitro, cultures of neurons, astrocytes and microglial cells are used that make up a functional network in the cytoarchitectonics of the brain that contact each other through neuronal-glial interactions and support the brain homeostasis.

Results: In the near future, possible models of Parkinson's disease in vitro may be models with the reproduction of previously studied pathophysiological mechanisms of BP development in vivo (AFK effect, creation of conditions for reactive astrogliosis and microgliosis, creation of mitochondrial dysfunction) and in vitro observations production of ROS, lipid peroxidation, activation of microglial NADPH oxidase, enhancement of expression of pro-inflammatory cytokines-IL-1β, TNF-α, IL-6 and / or nitric oxide-NO).

Conclusions: To develop new models of Parkinson's disease in vitro, interesting observations can also be taken into account in other models: according to Imam S.Z. and others, glucose protects dopaminergic neurons in vitro and in vivo from neurotoxin-mediated cytotoxicity. Based on these data, in new models of Parkinson's disease, glucose deprivation can be used as an additional damaging factor, which is used to create models of cerebral ischemia in vitro.

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Metabolomic Profiling of Activated Microglial Cells

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Objective: To investigate metabolomic changes in SIM-A9 microglia in vitro following exposure to neuroinflammatory insult using high resolution mass spectrometry.

Background: Microglial cells play an essential role in the CNS, acting as the primary immunological response to stress within the cellular environment. As the macrophages of the CNS, microglial cells are activated during the progression of several neurodegenerative diseases. Microglial are either cytotoxic M1 or the neuroprotective M2 phenotype. In PD, change in polarization to the M1 phenotype is not well understood at the molecular level. We focus on deciphering the metabolic signature of microglial cells after activation by a neurotoxin, lipopolysaccharide (LPS) using LC-HRMS.

Methods: SIM-A9 microglial cells were passaged into T-80 flasks, at 10 million cells per flask in a 5% CO2 incubator at 37 °C for 24 hours. Cells were stimulated with LPS at 2.5 ng/mL and metabolite extraction was performed on cell pellet after an ammonium formate cell pellet wash using 80% ice-cold methanol in water, followed by homogenization on a bead beater. Cells were dried down with liquid nitrogen and reconstituted for subsequent mass spectrometric analysis. Samples were run via reversed-phase LC-HRMS using a Dionex UHPLC interfaced with a Thermo Scientific Q-Exactive mass spectrometer.

Results: Data analysis was performed using MZmine, with accurate mass and retention time matched to an internal laboratory library. After filtering, 955 features in positive ion mode and 698 in negative ion mode were used for analysis. Statistical analysis using MetaboAnalyst revealed noteworthy differences between activated and quiescent microglial cells. One elevated feature in LPS-induced cells was phosphocholine, an intermediate between choline and cytidine-diphosphocholine in the phosphatidylcholine biosynthetic pathway. Cytidine, a precursor for the biosynthesis of cytidine triphosphate (CTP), was also increased in cells induced with LPS. Further studies will focus on identifying other significant features in this dataset and determining the biological role they play during the LPS-induced activation of microglial cells, as well as analyzing the lipidomic profile of LPS activated cells to shed light on changes in lipid biosynthesis.

Conclusions: As the sentinels of the CNS, it is likely that microglia respond to changes in brain metabolism and disease state. To our knowledge, this is the first mass spectrometry based metabolomic profiling of activated microglial cells. This study will allow us to uncover links between bioenergetics factors and activation states of microglia.

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CRISPR/Cas9-based fluorescent tyrosine hydroxylase-reporter lines

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Objective: To generate and characterize TH-GFP and -mCherry reporter iPSC lines.

Background: Human induced pluripotent stem cells (iPSCs)-based dopaminergic neuronal (iDA) cultures emerged as a valuable resource for disease modeling and development of therapies for Parkinson's disease (PD). In the last decade, several protocols for generating iDA cultures have been published. However, although efficient, these protocols result in mixed neuronal cultures containing only up to 30% of tyrosine hydroxylase (TH)-positive neurons.

Methods: Commercially available iPSCs from a healthy control were transfected with a vector coexpressing gRNA targeting a sequence of the TH gene right before the stop codon and Cas9 mRNA. In addition, a plasmid containing a Puromycin resistance cassette and GFP or mCherry DNA sequence flanked by 1kB-long DNA sequences of the TH gene upstream and downstream of the stop codon was used as a donor vector. Upon transfection, iPSCs were plated on the matrigel-coated plates and treated with Puromycin for seven days. Single colonies were picked and correct knocking in was confirmed by sequencing.
The "original" and TH-mCherry/-GFP reporter iPSCs were differentiated into dopaminergic neurons according to a previously published protocol by Kriks et al., 2011.

Results: 95% of Puromycin-resistant colonies showed correct, in-frame integration of the reporters. Among them, 20% had integrations in both TH alleles. Expression analysis showed comparable levels of the floorplate marker FOXA2, of the dopaminergic midbrain progenitor marker LMX1A, as well as of markers for mature dopaminergic neurons, i.e. PITX3 and NR4A2 between the "original" and the reporter iPSCs. Biallelic knock-in of the reporters did not alter TH mRNA expression during differentiation. In differentiated iDA cultures, only GFP- or mCherry-positive cells were positively immunostained using an antibody against TH. Finally, we were able to select out mCherry-positive cells at day 17 using fluorescence-activated cell sorting and to plate them onto Poly-L-ornithine/laminin/fibronectin-coated plates for further differentiation.

Conclusions: We developed an efficient, high-throughput method for generating TH reporter iPSCs lines. Differentiated reporter lines can be sorted to obtain pure dopaminergic neuronal cultures and thereby diminish the commonly observed heterogeneity-induced variability of iPSC-derived dopaminergic cultures.

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Surface Modified Solid Lipid Nanoparticles for the targeted delivery to brain: Management of HIV-1 Associated Dementia

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Objective: In this study, nifedipine loaded solid lipid nanoparticles (SLN) were developed for targeting drug into the central nervous system, the site of action of drug to block the apoptosis by HIV-1 virus. This would decrease the process of neurodegeneration and increase the survival time of neuronal cells. Also, this targeted delivery to brain will minimize the systemic effect of nifedipine, avoiding its delivery peripherally.

Background: HIV-Associated Dementia (HAD) is a significant neurological complication which occurs years after the acute viral sero-conversion reaction responsible for progressive Immuno-suppression and high viral loads. Many patients infected with HIV-1 suffer cognitive impairment ranging from mild to severe HAD which may result from neuronal death in the basal ganglia, cerebral cortex, and hippocampus. With Present available treatment system, there is no satisfactory treatment for HAD available, despite of advancement in the therapeutics.

Methods: The uncoated SLN were prepared by Solvent Injection Method & then coated with tween 80 and Lyophylized. Shape & surface morphological studies were done by Scanning Electron Microscopy (SEM) & Transmission Electron Microscopy (TEM). The in-vitro release profile of entrapped drug was studied using dialysis membrane. The Ex-vivo studies consisted of DNA fragmentation followed by in-vivo studies on albino rats.

Results: The SEM & TEM images show the smooth & spherical surface of SLN. The in-vitro release profile of drug shows more than 90% of drug release in 48hrs. DNA fragmentation was determined in presence and in absence of gp120 mimicking agent which shows no DNA fragmentation thus the developed carrier system works properly in releasing the drug and blocking apoptosis in the cortical cells. The fluorescence microscopy shows the qualitative uptake and localization pattern of the coated SLNs in brain.

Conclusions: In-vitro & in-vivo studies results shows more specific delivery of the Nifedipine to the Brain. The DNA Fragmentation & Cell Viability studies shows dementia blocking activity on brain cells. Brain specific delivery of Nifedipine could reduce the dose and potential systemic side effects, thus providing site specific delivery to brain. Thus, CNS delivery of these Nifedipine loaded SLNs via Intra Venous delivery will also open new opportunities for other Anti-Retroviral drug delivery to brain.

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Dopaminergic neurons in Substantia Nigra pars compacta code the vigor of movement sequences

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Objective: Assess the correlates of movement vigor in Substantia Nigra dopaminergic neurons

Background: Current basal ganglia models have focused on the role of dopamine in movement initiation. This contrasts with what is observed in Parkinson's Disease (PD) where chronic dopamine depletion leads not only to loss of movements (akinesia) but also to reduction in their speed and amplitude (bradykinesia).

Methods: Inspired by the repetitive finger tapping manoeuvre used to assess PD patients we developed a new self-paced operant task, in which mice learn to perform a sequence of fast actions using only one forepaw at a time. We collected data on speed, acceleration and spatial position of the lever and mouse paw. A miniature epifluorescence microscope (~1.9g) was used to image GCaMP6f fluorescence (a calcium indicator) in dopaminergic Substantia Nigra pars compacta (SNpc) cells while TH-cre mice performed the task. After animals learned the task, partial dopamine depletion is induced by unilateral intrastriatal 6-Hydroxydopamine (6-OHDA) injection.

Results: Healthy mice learned the task, with a progressive improvement in performance and a reduction in variability. During task performance, we identified distinct populations of SNpc neurons specifically modulated by reward consumption or movement sequence initiation. Phasic activity of a subpopulation of movement-modulated SNpc neurons preceded the start of a learned lever-press sequence and was related to the upcoming sequence vigor (higher activity related with faster movements and/or longer sequences). Unilateral SNpc depletion with 6-OHDA lead to a side specific loss of movement vigor (with slower movement and shorter sequences).

Conclusions: We developed a clinically-relevant task for skilled movements assessment in mice, and identified SNpc correlates of movement vigor. Dopamine depletion caused slower movement speeds and shorter movement sequences. Ongoing analysis will allow us to clarify the role of SNpc dopaminergic neurons in healthy and chronic dopamine depleted conditions. This will increase our understanding of basal ganglia dysfunction in PD.

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A53T mutant human α-synuclein BAC transgenic mice as a model for Parkinson's disease

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Objective: The aim of this study is to create mouse models reproducing the course of PD pathology and symptoms.

Background: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by a variety of motor and non-motor symptoms. The important pathologies of PD are the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the consequent loss of dopamine in the striatum, in association with Lewy bodies and Lewy neurites that are mainly composed of abnormally aggregated α-synuclein (α-syn). An appropriate animal model is essential not only to explore the pathogenesis but for the therapeutic intervention in PD. So far, genetic PD animal models have not completely recapitulated the whole process of aggregated α-syn progression in association with dopaminergic neuron loss.

Methods: We generated human α-syn bacterial artificial chromosome transgenic mice harboring the entire human α-syn gene and its gene expression regulatory regions with the A53T mutation which is a causative gene mutation for familial PD, a functional repeat polymorphism (REP1) in the α-Syn promoter region and single nucleotide polymorphisms (rs3857059 and rs11931074) which increase the risk of sporadic PD identified in GWAS-PD.

Results: A53T BAC tg mice expressed α-syn in a similar pattern with that of human α-syn. Abnormal phosphorylated α-syn was accumulated in the olfactory bulb, the cerebral cortex, the striatum, the substantia nigra pars compacta (SNc) and the dorsal motor nucleus of the vagus nerve. The number of tyrosine hydroxylase positive cells in SNc was decreased in an age-dependent manner. In the behavioral analyses, A53T BAC tg mice showed hyposmia, which is considered as prodromal symptom of PD.

Conclusions: A53T BAC tg mice can recapitulate the PD pathogenesis, especially in the early stage of PD. This novel tg mouse model is expected to be a valuable tool to tackle with the PD pathogenesis.

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Rotenone model of Parkinson's disease: The dosing options in mice revisited

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Objective: In the present study we have used various dosing protocols to induce the PD-like symptoms with the administration of rotenone in mice.

Background: Parkinson's disorder (PD) is among one of the most studied neurodegenerative disorders. PD affects the quality of life and renders patients with severe mobility issues in later stages of the disease. Among pharmacological anti-Parkinson drug targets, researcher are focusing on various receptors such as dopaminergic, cholinergic, adenosine etc. In order to develop better and refined new compounds targeting these receptors, appropriate animal model plays an important role.

Methods: Male NMRI mice were procured from the animal house of Dow University of Health Sciences, Karachi, Pakistan. Animals were divided into various groups, control, rotenone 1.5 mg/kg, rotenone 2.5 mg/kg, rotenone + levodopa (100 mg/kg). The drugs were injected using different protocols as follows: GROUP 1: animals were given daily 3 mg/kg dose of rotenone subcutaneously. GROUP 2: animals were given S/C 3 mg/kg dose of rotenone at alternate days. GROUP 3: animals were given daily 3 mg/kg dose of rotenone I/P. Following parameters were observed:

- number of box crossed
- time for corner sitting
- rearing: number of wall hugging

Results: The results showed that wall hugging was markedly reduced in group 1 from 32 (day 1) to 9 (day 5). However, in group 2 it was decreased by half from 34 (day 1) to 16 (day 5). Group 3 showed slight reduction in wall hugging i.e. from 35 (day 1) to 27 (day 9). Similarly, in box crossed group 1 showed significant effect at day 1 (177) and at day 5 (22). Group 1 and 2 showed significant reduction the no. of box crossed compared to control i.e. half (group 2) to four time (group 1) decrease. The data was analyzed using SPSS v20. (p-values were 0.005, 0.001 unless otherwise mentioned).

Conclusions: The results of our study indicate that daily protocol of rotenone injection via SC route is more effective in causing PD like symptoms as compare to I/P route. While, SC route with alternative dosing protocol is superior to I/P route but lesser in action than daily regimen. Conclusively, we may propose to use SC route in daily dosing for induction of PD symptoms using rotenone.

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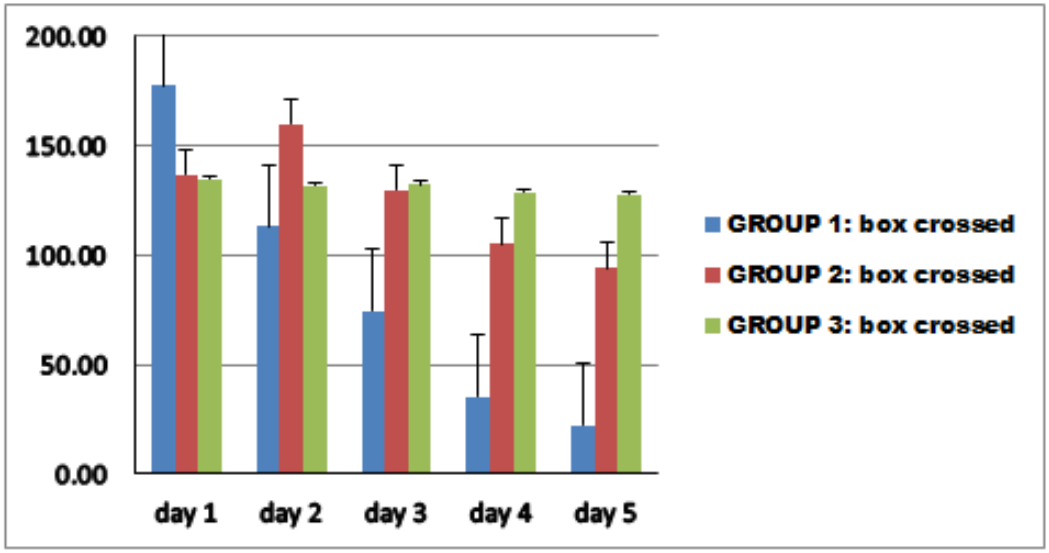

FIG. 1 (899)

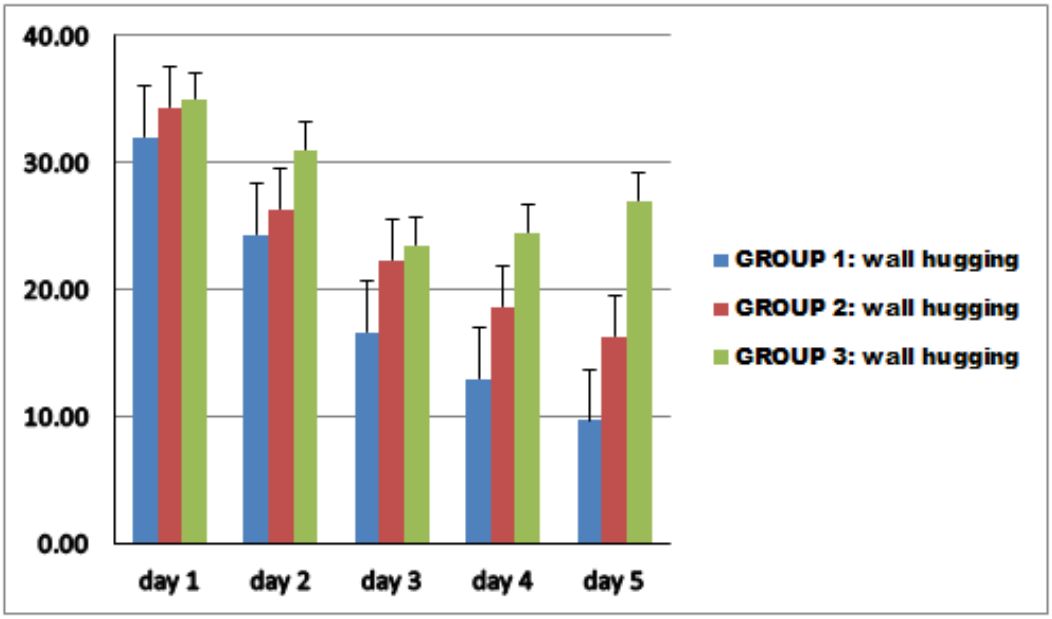

FIG. 2 (899)

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Apolipoprotein E and multiple system atrophy

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Objective: This study evaluated genetic associations of Apolipoprotein E alleles with risk of multiple system atrophy (MSA) and α-synuclein pathology, and also examined whether apolipoprotein E isoforms differentially affect α-synuclein uptake in a oligodendrocyte cell.

Background: Dysregulation of the specialized lipid metabolism involved in myelin synthesis and maintenance by oligodendrocytes has been associated with the unique neuropathology of MSA. We hypothesized that apolipoprotein E, which is associated with neurodegeneration, may also play a role in the pathogenesis of MSA.

Methods: One hundred sixty-eight pathologically confirmed MSA patients, 89 clinically diagnosed MSA patients, and 1,277 control subjects were genotyped for Apolipoprotein E. Human oligodendrocyte cell lines were incubated with α-synuclein and recombinant human apolipoprotein E, with internalized α-synuclein imaged by confocal microscopy and cells analyzed by flow cytometry.

Results: No significant association with risk of MSA or was observed for either Apolipoprotein E ε2 or ε4. α-Synuclein burden was also not associated with Apolipoprotein E alleles in the pathologically confirmed patients. Interestingly, in our cell assays, apolipoprotein E ε4 significantly reduced α-synuclein uptake in the oligodendrocytic cell line.

Conclusions: Despite differential effects of apolipoprotein E isoforms on α-synuclein uptake in a human oligodendrocytic cell, we did not observe a significant association at the Apolipoprotein E locus with risk of MSA or α-synuclein pathology.

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Motor and cognitive deficits in a mouse model of tauopathy with tau isoforms imbalance: Potential therapeutic strategies

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Objective: This work investigates motor phenotypes and neurochemical changes in a mouse model of tauopathy linked to tau mis-splicing. We sought to validate a potential therapeutic intervention to preclude disease progression.

Background: The microtubule-associated protein TAU controls neuronal functions such as microtubule dynamics and axonal transport. The alternative splicing of tau primary transcript produces isoforms with 3 or 4 microtubule binding repeats (3R and 4R), in equal amounts in the normal adult human [1]. Tauopathies are neurodegenerative diseases, with presence of insoluble tau aggregates and loss of tau function. In tauopathies with movement disorders, such as Progressive Supranuclear Palsy (PSP) the endogenous 3R:4R tau balance is altered. Here we investigated motor phenotypes and neurochemical changes in the basal ganglia of a mouse model linked to tau mis-splicing and assessed potential strategies to restore tau isoforms balance.

Methods: Transgenic mice bearing 3R:4R imbalance (Htau mice), and their wild type and Tau knock-out controls were tested in the open field and rotarod to assess motor coordination. Cognitive performance was tested in the novel object recognition (NOR) task. Western blot and immunohistochemistry were performed to assess tau pathology in the basal ganglia. Finally, a trans-splicing RNA reprogramming strategy [2] was used to modulate the 3R:4R tau ratio in young htau mice and phenotypic rescue was assessed after treatment.

Results: Both TauKO and htau mice showed poor performance in the rotarod compared to WT mice. Cognitive tasks were also severely impaired in aged htau model. Analysis of tau isoforms contents indicated that 3R >4R tau in the PFC and striatum of htau, yet, tau deposits were detected in the cortex but not in the striatum. Trans-splicing rescue of tau isoforms imbalance in the PFC and striata of young mice precluded cognitive impairment and motor deficits. In addition, local modulation of isoforms balance reduced insoluble and hyperphosphorylated tau contents.

Conclusions: Either lack of tau function or tau isoforms imbalance are detrimental for motor activity, however our results evidence that the (dys) functional consequences of tau 3R:4R imbalance would have a stronger impact in the development of motor and cognitive impairments than mutations that induce the lack of function. In addition the results obtained using tau isoforms modulation in the htau model rise the potential use of RNA reprogramming to correct tau mis-splicing in human tauopathies.

References: 1. Spillantini MG, Goedert M (2013) Tau pathology and neurodegeneration. Lancet Neurol 12:609–622. 2. Avale ME, Rodríguez-Martín T, Gallo JM (2013) Trans-splicing correction of tau isoform imbalance in a mouse model of tau mis-splicing. Hum Mol Genet 22:2603–2611.

964

Nilotinib for treating MSA: A preclinical proof of concept study

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Objective: To assess the effects of nilotinib on motor behavior, α-synuclein burden and surrogate markers of neurodegeneration in a transgenic mouse model of multiple system atrophy (MSA).

Background: The pathological hallmark of MSA is the presence of α-synuclein bearing glial cytoplasmic inclusions. Treatment is available for some symptoms, in particular autonomic dysfunction, while disease modification remains an urgent unmet need. Activation of the tyrosine kinase c-Abl protein is increased in Parkinson's disease (PD) and alpha-synuclein has been identified as one of its substrates. Through C-Abl inhibition, nilotinib (a commercially available treatment for a type of leukemia) is thought to potentially counteract α-synuclein accumulation and to protect neurons from degeneration. In this regard, positive effects of nilotinib on the neurodegenerative process have been reported in preclinical models of PD. The aim of this study was to evaluate the effects of nilotinib in PLP-SYN mice, a transgenic mouse model of MSA.

Methods: Wild-type (WT) mice received daily intraperitoneal injections of vehicle and transgenic PLP-SYN received daily intraperitoneal injections of either vehicle, nilotinib 1mg/kg or 10mg/kg, for 12 weeks since age 6 weeks. Motor behavior was assessed at baseline and every 4 weeks until termination. The histopathological analysis included cell survival in the substantia nigra pars compacta (SNpc) as assessed by the number of tyrosine hydroxylase and Nissl positive neurons. Immunoblotting was performed to measure α-synuclein load.

Results: Nilotinib was safe and well tolerated at both doses by PLP-SYN mice. There was no difference in motor performance between the different treatment groups of PLP-SYN mice. As expected, placebo-treated PLP-SYN mice showed dopaminergic cell loss in the SNpc compared to WT mice, while nilotinib failed to protect neurons from degeneration and to attenuate α-synuclein burden in PLP-SYN mice.

Conclusions: Nilotinib failed to demonstrate positive effects in a transgenic mouse model of MSA.

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Effects of mannitol treatment in a mice model of multiple system atrophy (MSA)

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Objective: The aim of this study was to examine the effect of mannitol treatment in a mice model of MSA.

Background: Alpha-synuclein aggregation represents the pathological hallmark of α-synucleinopathies like Parkinson`s disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). MSA is a fatal neurodegenerative disease characterized by pathological accumulation of alpha-synuclein aggregates in oligodendrocytes. No effective treatments are currently available for MSA patients. Recently, the ability of mannitol to interfere with the aggregation process of α-synuclein was shown in vitro and in mice and drosophila models of Parkinson's disease.

Methods: We hypothesized that mannitol treatment will lead to decreased α-synuclein pathology in the brain of transgenic mice overexpressing α-synuclein in oligodendrocytes under the proteolipid protein promoter (PLP-α-synuclein mouse model of MSA). Six months old wild type and PLP-α-synuclein mice were allocated to four groups and received 5 weekly intraperitoneal injections of mannitol or vehicle. In some of the experiments PLP-α-synuclein mice were also treated with the mitochondrial toxin 3-nitropropionic acid (3-NP) to model full-blown MSA. During a 12-week treatment period, motor behavior was assessed. Brains samples were collected for neuropathological analysis.

Results: Chronic systemic mannitol treatment of PLP-α-synuclein mice led to partial motor improvement. Pathological analysis demontrated rescue of nigral dopaminergic and striatal neurons and decreased astroglial and inflammatory responses.

Conclusions: Our findings demonstrate beneficial effects of chronic mannitol treatment in transgenic PLP-α-synuclein mice. However, the translatability of these results into human patients is yet to be determined.

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Rapamycin for treating MSA: A preclinical proof of concept study

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Objective: To assess the effects of rapamycin on motor behavior, α-synuclein burden and surrogate markers of neurodegeneration in a transgenic mouse model of multiple system atrophy (MSA).

Background: Growing evidence suggests impairment of the autophagy-lysosomal pathway in MSA. Since this pathway has a major role in the degradation of α-synuclein, its impaired function may contribute to the accumulation of α-synuclein in glial cytoplasmic inclusions in MSA. The mammalian target of rapamycin complex 1 (mTORC1) is a key protein complex regulating autophagy. Rapamycin is an mTORC1 inhibitor that enhances autophagy, by increasing autophagosomes and boosting lysosomal biogenesis. We

here assessed if rapamycin exerts neuroprotective effects by enhancing autophagic α-synuclein clearance in a transgenic mouse model of MSA.

Methods: Wild-type (WT) and PLP-SYN transgenic mice were fed either with normal food or food enriched with 14mg/kg of rapamycin for 16 weeks since age 6 weeks. Motor behavior was assessed at baseline and every 4 weeks until termination. Histopathological analysis included cell survival in the substantia nigra pars compacta (SNpc) as assessed by the number of tyrosine hydroxylase and Nissl positive neurons, and the amount of α-synuclein aggregates in oligodendrocytes in the SNpc and the striatum after incubation of sections with or without proteinase K. Additional immunoblotting was performed to measure α-synuclein load.

Results: Rapamycin was safe and well-tolerated. There was no difference in motor performance between groups. As expected, placebo-treated PLP-SYN mice showed dopaminergic cell loss in the SNpc compared to WT mice. Rapamycin provided partial neuroprotection since the number of Nissl positive neurons in the SNpc was similar compared to WT mice, while the number of tyrosine hydroxylase positive neurons was significantly lower and not different from placebo-treated PLP-SYN mice. Rapamycin also significantly reduced the amount of α-synuclein aggregates in the SNpc, while only a trend was observed for the striatum.

Conclusions: Rapamycin partially rescued neurons in the SNpc, although they remained dysfunctional as suggested by the down-regulated tyrosine hydroxylase expression. The partial rescue was paralleled by a positive effect on the amount of α-synuclein aggregates.

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PBT434 prevents the accumulation of glial cell inclusions and insoluble alpha-synuclein in a mouse model of Multiple System Atrophy

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Objective: To evaluate PBT434 in a transgenic mouse model of multiple system atrophy (MSA)

Background: PBT434 is a novel quinazolinone inhibitor of iron-mediated protein accumulation and aggregation. In multiple animal models of Parkinson disease, PBT434 reduces alpha-synuclein aggregation and oxidative stress, preserves neurons and improves motor function (DOI 10.1186/s40478-017-0456-2). A transgenic model of MSA (PLP-alpha-Syn) overexpresses alpha-synuclein, has parkinsonian deficits and manifests oligodendroglial pathology (DOI:10.1016/j.expneurol.2010.05.008). Orally administered PBT434 readily penetrates the blood brain barrier and is well-tolerated in rat and dog toxicology studies. The affinity of PBT434 for iron is greater than that of alpha-synuclein but lower than that of endogenous iron trafficking proteins, e.g., ferritin.

Methods: PBT434 or vehicle was administered orally for 4 months at 30 mg/kg/day starting at age 7 or 12 months. Mice were culled at 11 or 16 months of age. Nigral neuron counts were assessed at 11 and 16 months and glial cell inclusions (GCI) of the substantia nigra (SN) and pons at 16 months were assessed by stereology. Western blot analysis was used to assess aggregated alpha synuclein. The pole test was done at 11 and 16 months.

Results: At 11 and 16 months, PBT434 reduced alpha-synuclein aggregation (P=0.025 and 0.005, respectively) and preserved SN neurons (P=0.086 and 0.001, respectively). At 16 months, PBT434 reduced the number of GCI in SN and pons (P=0.0007 and 0.001, respectively). PBT434 improved motor function on the pole test at 16 months (P=0.049).

Conclusions: PBT434 reduced alpha-synuclein aggregation and glial cell inclusions, preserved SN neurons and improved motor function in an animal model of MSA. PBT434 is a small molecule iron chaperone with potential for treating MSA.

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Neurohormetic properties of mild physical stress against Parkinson's disease phenotype

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Objective: To test whether routine mild physical stress acts as a hormetic strategy against Parkinsonian symptoms among mice.

Background: Regular physical exercise is suggested to be enhancing the mitochondrial health among CNS neurons in terms of mitochondrial copy number and reduced oxidative stress. Mild sessions of exercise is thought improve the motor phenotype among Parkinson's disease patients. However, experimental evidence for this hypothesis is none. Hence, here we set out to study the possible beneficial effects of daily exercise on the motor symptoms and dopaminergic neurodegeneration in chemically induced Parkinson's disease among mice.

Methods: Male Swiss albino mice (12 wk old, n=3) were subjected to physical exercise by using a running wheel for 20 min (daily at 10 pm) for 4 weeks. The wheel running was voluntary for each mouse, however, the total period of 20 min was manually noted for each mouse. Mice were challenged with Rotenone (1mg/ kg bw/ d, ip) for the last 2 weeks. After total regimen period of four weeks, the mice were assessed for motor phenotype using stride length, locomotion in open field test and narrow beam test. Terminally the striatum and substantia nigra pars compacta were assessed for dopaminergic postive cells (Tyrosine hydroxylase immunoblot) and in situ oxidative stress markers.

Results: Mice subjected to routine exercise in the wheel manifested improved locomotion in open field test and demonstrated reduced stress in terms of increased rearing and exploratory behavior. Obvious phenotypic manifestations among Rotenone-mice like reduced stride length, increased beam walk latency were normalized with Routine Exercise. Further, Routine exercise significantly modulated Rotenone-induced complex I inhibition among mid brain mitochondrial fractions. Further, the Rotenone induced oxidative stress among striatum and substantia nigra were also ameliorated with routine exercise. In addition, the dopamine levels among Rotenone-mice striatum were also attenuated with Routine Exercise.

Conclusions: Our study, strongly supports routine mild exercise significantly reduced the Rotenone-induced Parkinson's disease among mice model. This line of treatment will be considered for further assessment by using molecular markers to substantiate this observation.

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The change of SIRT1 and HIF-1 in MPTP treated mouse

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Objective: To study the changes of the expression of silent information adjustment factor 1 (SIRT1) and hypoxia inducing factor 1 (HIF-1) and changes in behavior in mice of MPTP treatment.

Background: Silent information adjustment factor 1 (SIRT1) and hypoxia inducing factor 1 (HIF1) were found to involve in the pathogenesis of Parkinson's disease and plays an important role in disease progression.

Methods: To establish animal model of PD, MPTP was chose to treat C57BL6 mice, and then behavioral tests, HPLC and immunohistochemistry were conducted. Further, the levels of SIRT1 and HIF1- was detected.

Results: Mice in MPTP-treated group displayed a significant abnormal behavior , autonomic activity, gait and bradykinesia (all $P<0.001$). HPLC, which was used to investigate the levels of neurotransmitters, revealed decreased levels of DA and its metabolites ($P<0.001$) in MPTP-induced mice. MPTP reduced the levels of tyrosine hydroxylase (TH) and the dopamine transporter (DAT) remarkably ($P<0.01$), which are markers of substantia nigra dopaminergic neurons. At last, the expression levels of SIRT1 ($P<0.05$) was reduced and HIF1 ($P<0.05$) was increased in MPTP group compared with control group.

Conclusions: Abnormal behavior and decline of dopaminergic neurons markers prompted the successful establishment of PD model. The irregular expression of SIRT1/HIF - 1 in vivo supported our previous studies, and indicated that SIRT1 / HIF1 signaling may involve the disease process of PD.

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Development of a system to measure the kinetic responses of parkinsonian animal model

G.T. Kim (InCheon, Republic of Korea)

Objective: To develop a system for evaluating a parkinsonian animal model using the kinetic responses of foot pressures during normal walking.

Background: Animal model of Parkinson's disease plays a critical role in developing clinical treatments as well as understanding the disease itself. Once making an animal model, it is mandatory to evaluate the model based on the kinetic responses of models. Various devices and systems have been commercially available, but every researcher cannot afford these expensive products. Here, we designed an efficient and inexpensive system using a microcontroller (Arduino) to measure the kinetic responses of a model, which were the foot pressures during normal walking.

Methods: A rectangular space (100x10x20 cm) for a normal walk of an animal model was designed, and 12 rectangular pillars (4x4x8 cm) for force-sensitive resistors were placed with regular distance. The resistors were connected to Arduino board (Mega 2560), and again connected to a computer to monitor the real-time measurement of foot pressures. To test the reliability of the developed device, three rats were used. Animal models, which were trained to adjust the walking space, regularly allowed to walk on the resistors. The kinetic responses to the tests were measured and compared before and after model to evaluate the feasibility in the construction of models. Foot pressures were separately analyzed, and the results were compared before and after model. The pressures were presented in histograms, and the distribution in histograms was considered as the effects by Parkinson's disease.

Results: The foot pressures showed the unbalanced condition of animal models during normal walking, which was possibly related with the symptoms of Parkinson's disease. Also, the method avoided typical evaluation by separately investigating the foot pressures for each leg before and after model. Therefore, it allowed to assess the effects on each foot, helped to identify the affected foot after model.

Conclusions: A development of a system to measure the foot pressures of a parkinsonian animal model was proposed. Due to intuitive presentation of foot pressures, the results reflected the effects on foot pressures by Parkinson's disease as well as could be used as a tool for the evaluation of model. Also, the proposed system using foot pressures overcame the issues caused by the generalization in evaluating an animal model, and this would improve the reliability for performing any following experiments in vivo.

References: This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF- 2016R1D1A1B03930657).

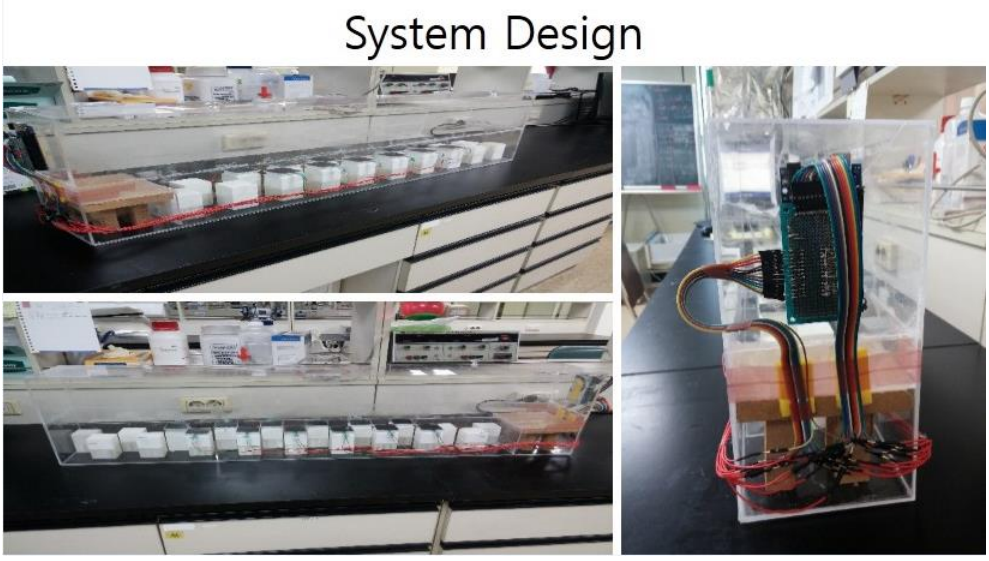

FIG. 1 (1129)

1178

Possible neurotoxic hazards of common adulterants in Tea among Drosophila; ascertainment of toxicity of Tea preparations from local shops of an Indian city

C. Ratnakaran, G. Chandran (Bengaluru, India)

Objective: The present study assesses the neurotoxic implications of common adulterants of Tea in drosophila system in terms of tremor behaviour and neurochemical changes. Also ascertains their presence in the tea beverages from the local shops.

Background: Tea, chief ingredient in the aromatic beverage of the same name, is the commercial name for dry/ cured dry leaves from the shrub, Camellia sinensis (Theaceae). There are different kinds of teas based on the post-harvest processing of the tea leaves viz., green tea, yellow tea, black tea. Black tea, the cure-dried Camellia leaves, are usually soaked in boiling water for a few minutes and consumed with or without sugar. Most people also add a dash of milk to black tea. Tea is the most consumed hot beverage across the globe and a proven stress buster and a neuromodulant. The beneficial effects of tea are attributed to the flavonoid variety reported from the genus. The scientific evidence continues to mount about the numerous health benefits of drinking tea. Hundreds of recent scientific research studies have found many potential health attributes associated with tea. Generally the color of the tea decides how strong the tea is. Hence, commercially interested elements find ways of increasing the tea color by additives leading to addition of unsafe adulterants. History of tea adulteration dates back to 1800s. The common adulterants of black tea are Prussian Blue, Indigo, coal tar etc.

Methods: Young (5d) and old male (40d) flies were independently exposed to adulterants of Tea (Prussian Blue, Indigo, coal tar) in DMSO vehicle at 0.01-0.05% in diet. The flies were monitored on alternate days during 21 days, for manifestation of tremors (climbing/speed). By end of the regimen, the fly brain was assessed for dopaminergic neuronal status. In addition, tea samples were collected from the local shops and tested for their tremor inducing properties among Adult Drosophila by following the similar protocol.

Results: Flies exposed to Tea adulterants significantly developed tremors at the lower concentrations of Prussian blue and Coal Tar (0.01%) and also demonstrated severe dopaminergic neuronal loss among the adult brain of Drosophila. The results were similar among the flies exposed to local tea samples which were suspected to be adulterated.

Conclusions: Our study confirms the neurotoxic effects of adulterants of Tea as well as the suspected tea samples among Drosophila. Further, studies are planned to elucidate molecular effects and gather more information before indicating the authorities.

1221

Fluvoxamine maleate effects on dopamine signaling in the prefrontal cortex of stressed parkinsonian rats: Implications for learning and memory

E. Dalle, W. Daniels, M. Mabandla (Durban, South Africa)

Objective: In the present study, we investigated the effects of reduced DA presence in the PFC on cognitive function and whether treatment with Fluvoxamine maleate (FM) attenuated these effects.

Background: Parkinson's disease (PD) affects extra-striatal midbrain cells resulting in reduced extrinsic supply of dopamine (DA) to the prefrontal cortex (PFC).

Methods: Maternal separation was used to develop an animal model for early life stress that has chronic effects on brain and behavior. Sprague-Dawley rats were treated with the antidepressant FM prior to 6-hydroxydopamine (6-OHDA) lesion to model motor deficits in rats. The Morris water maze (MWM) and the forelimb use asymmetry (cylinder) tests were used to assess learning and memory impairment and motor deficits respectively. Blood plasma was used to measure corticosterone concentration and prefrontal tissue was collected for lipid peroxidation, DA, and serotonin (5-HT) analysis.

Results: Our results show that animals exposed to early life stress displayed learning and memory impairment as well as elevated basal plasma corticosterone concentration which were attenuated by treatment

with FM. A 6-OHDA lesion effect was evidenced by impairment in the cylinder test as well as decreased DA and 5-HT concentration in the PFC. These effects were attenuated by FM treatment resulting in higher DA concentration in the PFC of treated animals than in non-treated animals.

Conclusions: This study suggests that FM may ameliorate cognitive impairment in PD by preserving DA and 5-HT transmission in the PFC.

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1234

Curcumin improves cognitive function in rotenone-induced rat model of Parkinson's disease (PD)

S. Madiha, S. Haider (Karachi, Pakistan)

Objective: In this study, we aimed to investigate the neuroprotective effect of curcumin against rotenone-induced memory impairments in rat PD model.

Background: In PD, oxidative stress has been stated to induce cognitive dysfunction. Rotenone-induced animal model mimicked characteristics features of PD including oxidative stress. So, therapeutic interventions that can prevent free radicals mediated neurodegeneration by inhibiting free radical production may be useful in preventing disease progression. Curcumin a natural occurring polyphenolic antioxidant and exhibits neuroprotection against different neurodegenerative diseases.

Methods: Adult male rats were administered subcutaneously with rotenone (1.5 mg/kg/day) for 8 days in order to develop PD-like symptoms. After the development of PD-like symptoms curcumin (100 mg/kg/day, p.o) was administered in respective group for two weeks. Cognitive functions were monitored by the morris water maze test (MWM) and novel object recognition task (NOR). Biochemical and neurochemical estimations were done in brain.

Results: Results showed that rotenone administration significantly ($p<0.01$) induced memory impairment in behavioral parameters and as well as significantly ($p<0.01$) produced oxidative stress as evident by increased lipid peroxidation (LPO) and decreased reduced glutathione (GSH) levels as compared to controls. Moreover, rotenone significantly ($p<0.01$) decreased dopamine (DA) and 5-hydroxytryptamine (5-HT) levels. Curcumin treatment significantly improved memory acquisition by significantly ($p<0.01$) decreased LPO and increased GSH levels. Curcumin significantly increased DA and 5-HT levels as compared to rotenone alone group ($p<0.01$).

Conclusions: Hence, our result indicated that curcumin exhibits antioxidant activity against rotenone-induced cognitive impairment. The findings of the present study strongly suggest that cognitive improving effect of curcumin might be attributed to its antioxidant properties.

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1253

Neuroprotective potential of curcumin in combination with piperine against 6-hydroxy dopamine induced motor deficit and neurochemical alterations in rats

S. Singh (Moga, India)

Objective: (1) Curcumin is well tolerated curcuminoid used as supplement for various disorders but problem is its low oral bioavailability. (2) Piperine is combined to enhance bioavailability of curcumin used as anti-inflammatory, neuromodulatory and neuroprotective in movement disorders like Parkinson's disease.

Background: 6-hydroxy dopamine (6-OHDA) is a neurotoxin which on intranigral administration produces severe nigrostriatal damage with motor and cognitive deficit in animals. Curcumin (CMN) in combination with bioenhancer piperine (PP) in 6-hydroxydopamine induced Parkinsonian rats was used to investigate the antioxidant, neuromodulatory and neuroprotective mechanisms.

Methods: Hemi-Parkinson's rat model was developed with intranigral infusion of 6-OHDA (8 µg/2 µl, once, unilaterally), treatment with CMN (25 and 50 mg/kg) and combination of PP (2.5 mg/kg) with CMN (25 mg/kg) was given daily for 21 days starting from the 7th day after 6-OHDA infusion. The behavioral (locomotor, grip strength and narrow beam walk) parameters were performed on weekly basis. On 22nd day isolated brain preparations were subjected to biochemical (lipid peroxidation, glutathione and nitrite), neuroinflammatory (IL-1β, IL-6 and TNF- α) and neurochemical (DA, NE, 5- HT, GABA, Glutamate) analysis.

Results: Oral administration of CMN had significantly prevented behavioral, neuroinflammatory and neurochemical changes and preserved the antioxidant potential of the nigrostriatum in rats treated with 6-OHDA.

Conclusions: In the present study PP and CMN had afforded a better neuroprotective effect compared to alone treatment on behavior, biochemical, neuroinflammatory and neurochemical parameters in rats.

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1257

A promising model for cognitive dysfunction in Parkinson's Disease by AAV-mediated alpha-synuclein overexpression in hippocampus

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Objective: Our aim is to model cognitive dysfunction of Parkinson's disease (PD) together with motor deficits by targeted overexpression of alpha-synuclein (a-syn) in bilateral dentate gyrus (DG) and substantia nigra (SN) of rats.

Background: Pathological aggregation of a-syn plays a key role in the neurodegenerative process of PD. Hippocampal a-syn pathology is accused to be responsible from different aspects of cognitive dysfunction seen in advancing stages of PD.

Methods: AAV-carrying a-syn (n=11) or saline (n=8) was injected bilaterally into DG and SN of female Sprague-Dawley rats (200-250g). Seven animals were used as naïve controls. All animals were tested for memory, spatial learning, anxiety, anhedonia, motor coordination and locomotion by novel object recognition (NOR), Morris water maze (MWM), elevated plus maze, sucrose preference, rotarod and locomotor activity tests respectively, 16-18 weeks following injection. Brain samples were analyzed by both Western blotting and immunohistochemistry for phosphorylated a-syn (p-a-syn), NeuN, tyrosine hydroxylase (TH) and synaptophysin. Neuronal loss in SN and hippocampus were evaluated with stereological quantification.

Results: Compared to controls, a-syn group spent shorter time with the novel object in NOR test and longer time to find the platform in MWM ($p<0.05$). Motor performance in rotarod and locomotor activity were lower in a-syn group, whereas increase in horizontal activity after apomorphine injection was more prominent in this group ($p<0.05$). P-a-syn overexpression in striatum and hippocampus was confirmed by Western blotting. Stereological count of TH-positive neurons in SN showed %43 loss ($p<0.05$) and synaptophysin expression levels decreased %28 in striatum, %46 in hippocampus in the a-syn group compared to naïve controls ($p<0.05$).

Conclusions: Previously, we did not detect significant behavioral or histological changes of bilateral DG a-syn overexpression (1). In this study, targeting both DG and SN caused behavioral deficits correlated with synaptic loss in both hippocampus and striatum while partially related to dopaminergic cell loss. We suggest

that the "dual targeting" may provide a better model for cognitive impairment of PD. A-syn and GFP viral vectors were kindly obtained from Michael J Fox Foundation.

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1288

Clinical phenotype analysis of Parkinson's disease associated with LRRK2 variants in Chinese Han population

C. Gu, K. Li, J. Zhang, H. Jin, Y. Ge, C. Liu (Suzhou, China)

Objective: Our study aimed to compare motor and non-motor symptoms, imaging features and molecular markers of patients with Parkinson's disease (PD) who are carriers of the leucine-rich repeat kinase 2 (LRRK2) gene variants with patients who are noncarriers.

Background: LRRK2 is commonly implicated in both familial and sporadic PD. The polymorphic variants G2385R and R1628P have been associated with a significantly increased risk of developing PD in various Asian populations. Different variants may have different phenotypes and it is important to understand the phenotype of LRRK2 variants.

Methods: Two hundred eighty-seven patients with PD were enrolled in our study. Demographic information was collected. Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr (H-Y) stage scale were also performed. The Mini-Mental State Examination(MMSE), Montreal Cognitive Assessment (MoCA), Hamilton Anxiety Scale(HAMA), Hamilton Rating Scale for Depression(HRSD) and the non-motor symptoms (NMS) questionnaire were applied to evaluate non-motor symptoms. Peripheral blood samples were collected and DNA was extracted for next generation sequencing. GBA mutation carriers were excluded. Among the 287 patients, 199 underwent transcranial ultrasound examination(TCS), and 147 admitted to the hospital underwent lipid pofile, uric acid, creatinine, homocysteine, folic acid and vitamin B12 tests.

Results: LRRK2 variants carriers(n=72) and non-carriers(n=203) were similar in age at onset, gender, course of disease, education, levodopa-equivalent daily dose(LEDD), UPDRS I, II, III, IV scores, MoCA score, HAMA score, HRSD score and NMSQ score. Carriers had higher H-Y stage and lower MMSE score(H-Y stage: 2.5 (2.0,3.0) vs 2.0 (1.5,2.5), Z = -2.098, P = 0.034; MMSE score: 26 (25,28) vs 27 (25, 29), Z = -2.437, P = 0.014) and were more likely to have motor fluctuation (49.2% vs 31.0%, P = 0.011). There was no significant difference between the two groups in terms of TCS parameters. Laboratory markers did not differ by mutation status either.

Conclusions: In our data, PD with LRRK2 variants in Chinese Han population is similar to idiopathic PD as to motor and non-motor symptoms, TCS profiles, common biochemical and hematological indicators, but characterized by more advanced disease stage and worse cognition.

1291

α-Synuclein induced dopaminergic neurons mitochondrial dysfunction via cytochrome c oxidase subunit 2

D. Lin (Guangzhou, China)

Objective: To investigate the mechanism of how α-Syn injure the the dopaminergic neurons.

Background: The misfolded α-Synuclein(α-Syn) transferring from cells to cells as prion protein is an important pathogenesis of Parkinson's disease(PD). It had been reported that the extraneous α-synuclein could induced the dopaminergic neurons apoptosis by causing mitochondrial dysfunction. However, the mechanism of how α-Syn injure the mitochondrial function is still unclear.

Methods: The microarray analysis was performed on the base of Arraystar Human Microarray V4.0. The intracellular reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) were detected by flow cytometry after dyed with DCFH-DA and JC-10, respectively. The gene expression was determined by qPCR and protein expression was measured by Western Blot. Furthermore,

Results: The results of gene microarray and western blot showed that the expression of cytochrome c oxidase subunit 2(MT-CO2,COXII) had increased significantly in SY-SH5Y cells stimulated by α-Syn for 24h. Furthermore, the decreased mitochondrial membrane potential (MMP) and enhanced ROS in cells treated by α-Syn had been reversed by inhibiting MT-CO2 gene expression. The following data have indicated that the up-regulation of MT-CO2 contribute to the abnormal expression of some apoptosis factor such as Bim, Bad and so on.

Conclusions: So we suggested that after being transferred into the dopaminergic neurons, α-synuclein result in mitochondrial injury via activating COXII. This discovery might reveal the initial step of the process by which α-Syn injures the dopaminergic neurons and provide new therapeutic targets of PD.

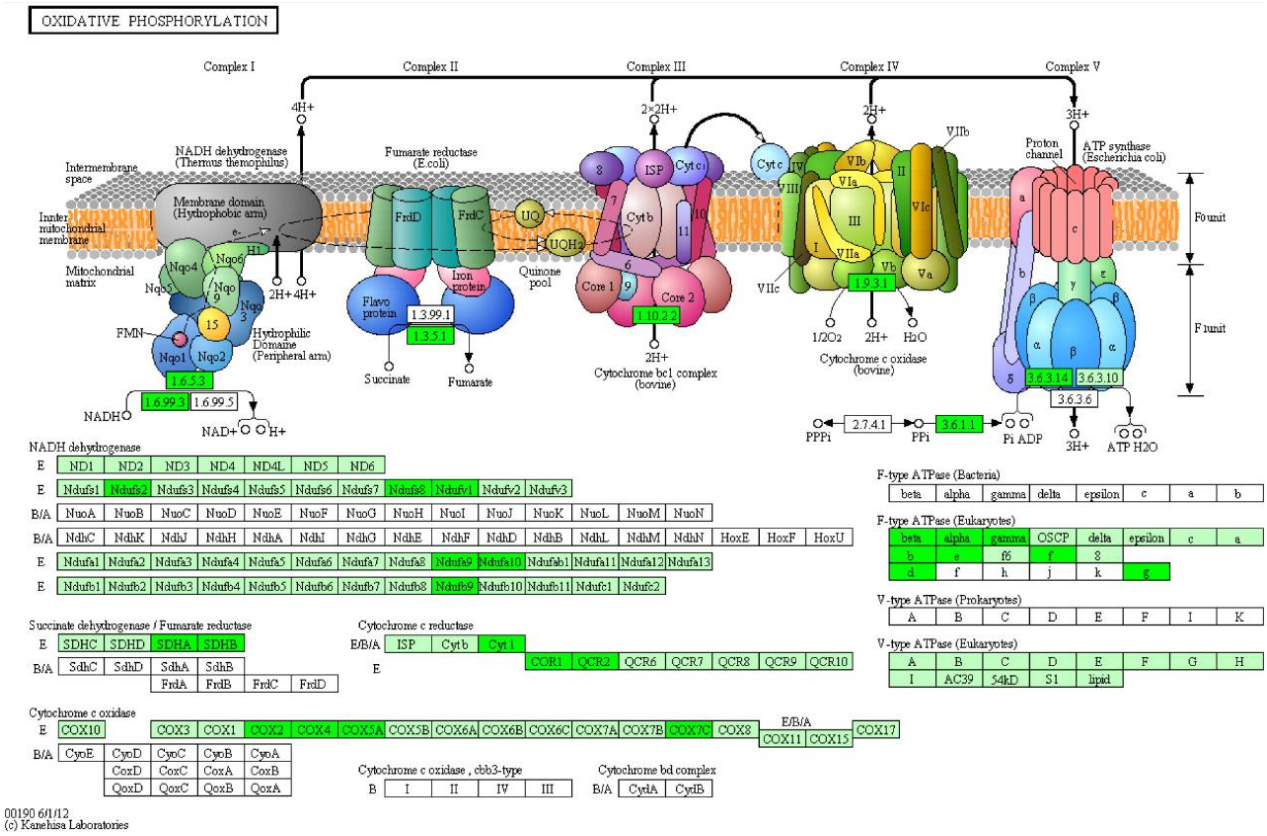

FIG. 1 (1291)

1294

Early synaptic loss and synaptic instability in a mouse model of prodromal Parkinson's disease

Y. Hatanaka, R. Takahashi (Kyoto, Japan)

Objective: In order to elucidate very early pathologis of Parkinson's disease (PD), we focused on synaptic pathologies in a newly developed mouse model of prodromal PD.

Background: Recent evidence implicates that the impairment of synapses and neuronal circuitry rewiring as important factors of pathogenesis in neuropsychiatric disorders. The synaptic mechanisms in PD pathogenesis remain to be elucidated, because of the absence of an animal model which replicates neuronal circuitry pathology. We generated BAC transgenic mice harboring human A53T α-synuclein gene SNCA with PD risk SNPs and its expression regulatory regions. A53T SNCA-BAC mice showed progressive

neuronal loss of dopaminergic neurons in the substantia nigra and accumulation of phosphorylated α-synuclein in the cerebral cortex. A53T SNCA-BAC mice did not show obvious PD-like motor dysfunctions, however, they exhibited RBD-like behavior at the age of 10 weeks and smell disturbance at 9-month-old. In this study, we focused on presymptomatic synapse pathologies in the primary motor cortex by using A53T SNCA-BAC mice as a model of prodromal PD.

Methods: By crossing A53T SNCA-BAC mice with Thy1-EGFP mice, layer 5 pyramidal neurons in the cortex of the offspring were labeled. A cranial window was implanted 4 weeks prior to imaging. In vivo two-photon imaging was performed weekly for 4 weeks.

Results: A53T SNCA-BAC mice showed a decreasing density of dendritic spines at the age of 12 weeks, and it reached the plateau at 24-week-old. This was due to the imbalance of enhanced spine formation and elimination at the age of 12 weeks, and the imbalance was equalized in 24-week-old. Although spine formation and elimination rate were balanced when they grow up, both formation and elimination rate of the spine remained to be higher than control mice. A53T SNCA-BAC mice also exhibited lower pre-existed spine stability and newly-formed spine survivability at the age of 24 and 48 weeks. Furthermore, colocalization of presynaptic protein marker and dendritic spine was lower in A53T SNCA-BAC mice.

Conclusions: These results suggest that a synaptic loss in the primary motor cortex is occurred even in a prodromal PD model, and this synaptic loss is caused by an excess elimination of dendritic spines. After the abnormal synaptic pruning is finished, the instability of dendritic spines still remains as a neuronal circuitry pathology.

1301

MicroRNA expression misregulation in iPSC-derived dopaminergic neurons from sporadic and LRRK2-associated Parkinson disease patients

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Objective: To investigate whether miRNA expression alterations occur in iPSC-derived DAn from sporadic PD (sPD) as well as monogenic LRRK2-associated PD patients.

Background: MicroRNA (miRNA) deregulation in blood has been associated with Parkinson disease (PD) but its role in the progression of disease is not known. We screened miRNAs expression in dopaminergic neurons (DAn) from PD patients generated by somatic cell reprogramming and induced pluripotent stem cells (iPSC) differentiation.

Methods: We quantified the expression of 377 miRNAs by using microRNA array cards in induced DAn from three sporadic PD patients (sPD), three monogenic LRRK2-associated PD patients (L2PD) (total six PD), and four healthy controls as to identify differentially expressed miRNA (DEmiR) associated with PD. We overlapped DEmiR expression data with transcriptomic data from the same samples as to evaluate potential regulatory implications of identified DEmiR.

Results: We found statistically significant differential expression of ten miRNA in PD of which five were up-regulated and five down-regulated. Expression changes were similar in sPD and L2PD. Identified DEmiR are involved among others in DAn survival and maduration. More specifically, up-regulation of two specific DEmiR is associated with the down-regulation of the transcription factors FOXA1 and NR3C1, which are related to epigenetic alterations in PD DAn, namely DNA hypermethylation of enhancer elements. Integrative analysis revealed significant correlations between miRNA/mRNA expression supporting a role of miRNAs in regulating gene expression.

Conclusions: In summary, our results indicate that the same miRNA changes are associated with both monogenic L2PD and sPD in our model of iPSC–DAn and co-occur with epigenetic changes in DAn from PD patients. Overall, this study shows that a systems biology-based approach can be suitable to investigate molecular changes of complex multifactorial neurodegenerative diseases such as PD.

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1302

Computational and Biochemical analysis of lipid raft proteins: A new perspective approach to understand the progression of late onset Parkinson's disease

L. Thangamani, S. Piramanayagam (Coimbatore, India)

Objective: In the current study we report mutations, particularly inPARK-7 gene and their impact on understanding the pathophysiology of Parkinson's disease (PD); it also lays down implications that this might solve various problems in therapeutic approaches.

Background: In the Human brain, astrocytes are the most populous glial subtype supporting cells of the brain and are critical for various brain functions. Classical PD is manifested as a complex motor disorder which results from the reduced dopaminergic input of the substantia nigra to the striatum, and the resultant altered basal ganglia modulation of motor control. PD is characterized mainly by the degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNc) due to the fact of neuro-inflammation in the SNc seen consistently in PD cases. Lipid rafts comprise a highly dynamic clustering of proteins and lipids playing a central role in signal transduction and intercellular communication, and their alterations have been associated with altered neuronal function, synaptic transmission and neurotransmitter signalling.

Methods: The study mainly concentrates on the screening procedures that evident the presence of PARK-7 mutations with respect to lipid raft fractions, in 27 late onset PD cohort's native to Coimbatore and surrounding regions.

Results: The current study reveals the functional roles of eight proteins in astrocyte biology involved in the onset of late onset PD, among them DJ-1 which is encoded by the PARK-7 gene is associated with the regulation of lipid rafts in astrocytes. We also focused on factors that show mutations in PARK-7 gene, resulting in the increased degradation of the lipid raft proteins flotillin-1 and caveolin-1.

Conclusions: Lipid rafts play central role in many cellular processes such as membrane sorting and trafficking, cell polarization, and signal transduction processes. Caveolae are small surface invaginations seen in many cell types. Numerous caveolae are seen in plasma membranes of cells such as adipocytes, endothelial cells, and smooth muscle cells, and these have been assumed to be formed by the clustering of raft proteins on the cell surface. Caveolar invagination is processes that is carried out by the polymerization of caveolins, and are of three variations namely: caveolin-1, -2, and - 3. Flotilin compounds are also a class of lipid raft proteins which are similar to that of the caveolins, their functions are unknown and the current study mainly reveals the main characteristic features of these proteins.

1306

MANF improves the MPP+/MPTP-induced Parkinson's disease via improvement of mitochondrial function and inhibition of oxidative stress

Y. Liu, J. Zhang, Q. Cai, H. Sun, L. Jin (Shanghai, China)

Objective: This study aimed to investigate the therapeutic effect of mesencephalic astrocyte-derived neurotrophic factor (MANF) on the MPTP/MPP+-induced model of Parkinson's disease (PD) and the potential mechanism.

Background: Currently, the most widely used treatment for PD is the dopamine replacement therapy, but long-term treatment can produce debilitating adverse effects and may not affect the progression of PD. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a novel neurotrophic factor and can selectively protect nigral dopaminergic neurons. It has been proven that MANF has neuroprotective and neurorestorative effects on PD in both in vitro and in vivo models.

Methods: Male C57BL/6 mice PD model with MPTP-induced were randomly injected bilaterally with MANF or PBS into the striatum. Two weeks later, Rotarod test, immunohistochemistry, and detection of dopamine (DA) and its metabolites, superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) were performed. A cell model of PD was established by incubating SH-SY5Y cells with MPP+, cells were pretreated for 2h with different concentrations of MANF before 24h incubation with MPP+. Cell viability, expression of Bax and Bcl-2, gene expression levels of Heme oxygenase 1 (HMOX1) and Superoxide dismutase 2 (SOD2), and mitochondrial transmembrane potential were detected.

Results: The latency reduction in PD mice was partially restored after MANF treatment ($P<0.05$); MANF significantly reduced the loss of tyrosine hydroxylase (TH)-positive dopaminergic neurons in the substantia nigra pars compacta (SNpc) ($P<0.01$); MANF significantly increased the striatal DA level in PD mice ($P<0.05$) and markedly increased the SOD activity ($P<0.01$) and GSH production ($P<0.01$). MANF pre-treatment significantly decreased the MPP+-induced reduction of cell viability ($P<0.01$), inhibited the ratio of Bax/Bcl-2 expression ($P<0.01$), activated gene expression levels of HMOX1 ($P<0.01$) and SOD2 ($P<0.05$), and reversed MPP+-induced loss of mitochondrial membrane potential ($P<0.01$).

Conclusions: MANF can attenuate the neuronal lesion in MPTP/MPP+-induced PD model, which may be related to the improvement of mitochondrial function and inhibition of oxidative stress.

1309

Increased Parkin expression in a PARK20 (SYNJ1 mutation) iPSCs-based model

W. Mandemakers, R. Masius, E. Berger, M. Grochowska, M. Quadri, M. Minneboo, M. Picillo, P. Barone, J. Schwamborn, V. Bonifati (Rotterdam, Netherlands)

Objective: To generate mechanistic insight into how the SYNJ1 p.Arg258Gln mutation leads to neurodegeneration in juvenile Parkinsonism.

Background: In humans, the SYNJ1 homozygous p.Arg258Gln missense mutation leads to juvenile Parkinsonism (PARK20). Strikingly, patients carrying mutations in the SYNJ1 gene that result in complete loss of SYNJ1 expression display more severe phenotypes, including early onset refractory seizures and juvenile lethality. These studies suggest a role for SYNJ1 mutations in neurodegenerative disease and indicate a phenotype-genotype correlation. The synaptojanin 1 protein (SYNJ1) plays an important role in synaptic vesicle cycling, and regulation of autophagic flux. A potential role for SYNJ1 in mitochondria has not been investigated.

Methods: Patient derived SYNJ1 homozygous p.Arg258Gln and control induced pluripotent stem cells (IPSCs) were exposed to small molecules to generate neural progenitor cells (NPCs) and subsequently differentiated for 3 weeks to induced dopaminergic (iDA) neurons. To investigate the role of SYNJ1 in autophagy, mitochondria and vesicle recycling, NPCs and iDA neurons were exposed to conditions that induce autophagy (starvation), mitophagy (CCCP and valinomycin treatment) and control conditions, and probed for markers of autophagy (i.e. WIPI2), mitophagy (i.e. Parkin) and synaptic vesicle recycling pathways (i.e. clathrin) by immunocytochemistry and Western blotting techniques. To analyze the functional impact of a SYNJ1 mutation on mitochondria in NPCs, the oxygen consumption rate was measured by extracellular flux analysis (Seahorse) including a mitochondrial stress test.

Results: Our data indicate that the SYNJ1 p.Arg258Gln mutation affects the autophagy pathway as indicated by increased levels of WIPI2 expression in patient derived NPCs and iDA neurons. Furthermore, increased number of Clathrin clusters and reduction of Clathrin cluster size, indicate that the SYNJ1 p.Arg258Gln mutation also disturbs proteins of the synaptic vesicle recycling pathway in iDA neurons. Finally, Parkin protein levels were increased in SYNJ1 p.Arg258Gln patient derived NPCs, and down

regulated during mitophagy. Strikingly, patient derived NPCs showed elevated mitochondrial activity in terms of basal respiration, ATP production, maximal respiration and energy-coupling efficiency.

Conclusions: Although further research is needed to clarify how the SYNJ1 p.Arg258Gln mutation leads to neurodegeneration, our data suggest a role for SYNJ1 in several cellular pathways, including autophagy, vesicle recycling and mitophagy.

1310

Knockdown of eEF1A2 reduced neuronal survival in a SH-SY5Y cellular model of Parkinson's disease through the PI3K/Akt/mTOR pathway

K. Khwanraj, P. Dharmasaroja (Bangkok, Thailand)

Objective: To investigate whether eEF1A2 promotes neuronal survival through PI3K/Akt/mTOR pathway in a toxin-induced Parkinson's disease model.

Background: Parkinson's disease is a common neurodegenerative disorder characterized by loss of dopaminergic neurons in substantia nigra pars compacta. Its molecular pathogenesis is not fully understood, which needs to be elucidated. Eukaryotic protein elongation factor 1 alpha 2 (eEF1A2) is a translation elongation factor involved in protein synthesis. It is only expressed in tissues of normal brain, heart, and skeletal muscle. Several studies revealed that eEF1A2 contributes cell protection against apoptotic cell death likely through the PI3K/Akt activation. Our previous study has also shown a correlated expression of eEF1A2 with the PI3K/Akt/mTOR pathway in a cellular model of Parkinson's disease.

Methods: The study was performed by using MPP+ to induce cell death in retinoic acid-differentiated neuroblastoma SH-SY5Y cells. To confirm whether eEF1A2 plays pro-survival role through PI3K/Akt/mTOR pathway, we conducted RNAi knockdown of eEF1A2. Expression of eEF1A2, PI3Ks, Akt, and mTOR was evaluated using real-time PCR and western blot analysis. PI3K inhibitors (LY294002 and wortmanin) were also used for studying the PI3K/Akt/mTOR signaling pathway.

Results: The result showed a significant positive correlation between the upregulation of both eEF1A2 and PI3K/Akt/mTOR pathway, suggesting that eEF1A2 might mediate cell survival and protect against toxin-induced apoptosis through Akt activation. Inactivation of eEF1A2 by siRNA reduced Akt activity and promoted apoptosis cell death by upregulation of cleaved-caspase-3 at both mRNA and protein levels. Inhibition of Akt activity by PI3K inhibitors induced upregulation of eEF1A2; however, it could not promote cell survival and protect against toxin-induced apoptosis in the Parkinson's disease model.

Conclusions: These findings suggested that reduced eEF1A2 expression may affect cell survival of MPP+-treated SH-SY5Y dopaminergic cells through the PI3K/Akt/mTOR pathway.

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1316

Mitochondrial phenotype related to the A30P alpha-synuclein mutation as a patient-derived cellular model of Parkinson's disease

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Objective: Our study aims to perform detailed phenotyping of the A30P alpha-synuclein familial case of PD, allowing to identify underlying mechanisms of the disease that may translate into novel therapies.

Background: Parkinson's disease (PD) is the second most common neurodegenerative disease. Approximately 20% of PD cases are known to be familial. From these, mutations in SNCA, the gene

encoding alpha-synuclein, are linked to an autosomal dominant inheritance of the disease. In 1998, our group discovered the second known point mutation within the SNCA gene, causing an A30P exchange of the peptide sequence.

Methods: We generated first patient-derived cellular model of the A30P alpha-synuclein mutation carrier, by obtaining fibroblasts from an affected sibling of the index patient, an unaffected sibling of the patient, and an age-matched gender-matched non-PD control. We reprogrammed these fibroblasts into induced pluripotent stem cells (iPSCs), and differentiated them into midbrain dopaminergic neurons.

Results: We obtained enriched cultures of ≥90% midbrain neurons (FoxA2+/Tuj1+), with approximately 20% dopaminergic (TH+), for which we observed electrophysiological activity and dopamine release. We detected a significant reduction of the protein level of mitochondria complexes II, IV, and V in the patient lines compared with the controls, additionally we found a significant impairment of mitochondrial respiration and an increased susceptibility of the cells to oxidative stress. Gene edited isogenic controls were generated to dissect mutation-specific effects. Furthermore, we investigated mitochondria morphology and dynamics, and how these processes contribute to the dopaminergic neurodegeneration. Additionally, we are implementing previously established readouts on our high-throughput automated screening platform that will allow us to identify FDA approved compounds with potential to be re-purposed and used as PD treatment.

Conclusions: We believe that detailed phenotyping of the A30P alpha-synuclein monogenic case may help to identify underlying mechanisms of the disease that may translate into novel therapies, which would also apply to the more common sporadic forms of PD.

1318

Evidence for dysregulation of inflammatory mechanisms involving the NF-κB complex in the living parkinsonian brain

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Objective: Sequence RNA in cortical samples taken from living PD patients to detect significant differentially expressed genes (DEGs) and altered biological pathways when compared to controls.

Background: The development of next-generation sequencing platforms has made sequencing the transcriptome feasible at a fraction of the time and cost. For Parkinson's disease (PD), this opens the possibility of capturing ongoing neurodegenerative processes at the transcriptional level. To date, examinations of the PD transcriptome have mostly been limited to non-CNS or cadaveric sources of tissue, which is likely at best a proxy of the disease process in the living brain. Small volume brain biopsies that can be safely obtained during deep-brain stimulation surgery for PD offer a feasible and novel alternative to these more traditional biological specimens (Xu et al., 2013).

Methods: Total RNA was extracted from cortical biopsies in 6 patients with PD and 6 controls, then sequenced on the Illumina HiSeq 2500 platform using a stranded paired-end protocol. This yielded approximately 90 million reads per sample, which we analyzed for differential expression using edgeR (v.3.8.6). Pathway enrichment and induced network analyses were also performed using the freely available analysis tools from the Gene Ontology Consortium and ConsensusPathDB.

Results: At a false discovery rate of <0.05, 763 DEGs were identified. Pathway analysis showed enrichment of genes responsible for regulating inflammatory response processes, tumor necrosis factor production and cellular response, the innate immune response and negative regulation of apoptotic processes. Disease relevant genes involved in these biological processes include CD44, Interleukin-10, GDNF, IL1β, and NF-kappa-B inhibitor alpha. Interestingly, induced network analysis revealed a robust network surrounding the NF-κB complex.

Conclusions: To our knowledge this is the first demonstration of differential CNS gene expression and enriched gene ontologies in cortical samples from living PD patients. The detected alterations offer a wealth of data that could facilitate identification of genetic biomarkers for diagnosis and treatment of PD. Significant results from pathway analysis and induced networks point to dysregulation of processes surrounding the NF-κB complex, lending further evidence to the growing body of literature relating neuroinflammation to PD and other neurodegenerative diseases.

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1320

Nurr1 gene: A new research target for Parkinson's disease

W. Le (Dalian, China)

Objective: The objective of our study is to determine if Nurr1 gene, a transcription factor essential for dopamine (DA) neuron development and survival, is a risk factor for Parkinson's disease (PD), and its potential role in the pathogenesis and therapy of this disease.

Background: PD is the second most common neurodegenerative disease in adult, mainly manifesting movement disorder symptoms. The loss of DA neurons in the substantia nigra (SN) is the pathological hallmark of PD. Although many PD related genes and environmental factors have been identified, the mechanisms underlying the relative specific loss of DA neurons in PD are still poorly understood. Previously we have documented that Nurr1 is a critical gene regulating DA neuron differentiation and survival. This study is a continue investigation to determine the role and mechanism of Nurr1 in PD.

Methods: (1) We established two conditional knock-out Nurr1 mice models in DA neurons and microglia, and determined the animal's movement behaviorals, nigral-striatal DA neuron pathologies, and quantitative measurements of Nurr1 down-stream genes/proteins expressions, Nurr1-α-synuclei interaction, and microglia activation and inflammation molecules in the SN. (2) We measured the expression levels of Nurr1 and several its down-stream genes in peripheral blood of over 700 PD patients' compared with 830 healthy controls and 580 various neurological disease controls using real-time PCR. In addition, we also assessed several inflammation molecules in patients' plasma using multi-ELISA assays.

Results: We have found that down-regulation of Nurr1 can cause DA neuron degeneration, decreased axonal guidance gene TopIIB, increase α-synuclein expression, and induce glia-mediated inflammation and neuronal injury. In addition, we documented that Nurr1 level was significantly lower, wherase inflammation molecules TNF-α, IL1β, IL-4, IL-6 and IL-10 were significantly higher in the peripheral blood of PD patients vs healthy and disease controls. The changes in Nurr1 expression and inflammation molecules were closely correlated with the disease progression. Moreover, we identified several small molecules acting on Nurr1 transcription site that can enhance DA synthesis and increase the survival of DA neurons in several in vitro and in vivo models of PD.

Conclusions: Nurr1 gene is a risk factor for PD, and we believe that Nurr1 may play an important role in the pathogenesis of PD and molecules targeting Nurr1 gene is a potential therapy for PD.

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1321

Acid sphingomyelinase deficiency rescues mitochondrial dysfunction in gba-/- zebrafish (Danio rerio)

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Objective: To determine the interaction between glucocerebrosidase deficiency and acid sphingomyelinase deficiency in a tractable vertebrate model system of Parkinson's disease.

Background: Heterozygous glucocerebrosidase (GBA) mutations are the strongest and most common risk factor for PD. An excessive burden of other lysosomal storage disorder gene variants, in particular for SMPD1 (encoding for acid sphingomyelinase) has also been reported [1]. However, it is unclear whether GBA and SMPD1 interact genetically. Zebrafish are an ideal vertebrate system to test for such interactions. We previously observed early microglial activation and alpha-synuclein independent neuronal cell death in gba1-/- zebrafish [2]. The aim of this study was to study the effect of gba_smpd1 gene-gene interaction in vivo.

Methods: An smpd1 mutant zebrafish line was generated using the Crispr/Cas9 technology. smpd-/- were crossed to our previously described gba-/- zebrafish line. A wide range of different parameters including survival, spontaneous motor behaviour, mass-spectrometry based glycolipid quantification and mitochondrial respiratory chain activity was assessed across four different genotypes (gba-/-, smpd-/-, gba-/-_smpd1-/-, wt).

Results: Sphingomyelinase activity was reduced by 93% in smpd-/- zebrafish which developed normally and did not have a shortened life span or altered spontaneous motor behaviour. A synergistic effect of combined glucocerebrosidase (GCase) and acid sphingomyelinase deficiency was observed for a range of different sphingolipid metabolites with highly significant increases ($p < 0.0001$) in gba-/-_smpd1-/- compared to gba-/- for sphingosine, sphinganine, ceramide, GM3 ganglioside and glucosylceramide. Unexpectedly, the characteristic "barrel-role" motor phenotype observed in adult gba-/- zebrafish was rescued in gba-/-_smpd1-/- double mutant zebrafish and survival of gba-/-_smpd1-/- double mutant zebrafish compared to gba-/- was significantly increased by ~ 25% from 102 days in gba-/- to 125 days in gba-/-_smpd1-/- ($p = 0.0055$). Mitochondrial respiratory chain complex IV activity was decreased in gba-/-, but normalized in gba-/-_smpd1 double mutant zebrafish.

Conclusions: Our work highlights the importance of a functional validation in tractable model systems for any gene-gene interaction rather than readily assuming an additive effect. Similar studies will be particularly important to study the biological interaction of PD risk genes identified in genome-wide association (GWA) studies.

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1324

Identification of gut microbial genes in Parkinson's disease by shotgun metagenomic analysis

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Objective: To carry out deep analysis on gut microbiome in patients with Parkinson's disease (PD) and to evaluate the potential application for diagnosing PD patients from gut metagenomes.

Background: Identification the roles of gut microbiome associated with diseases using metagenome-wide association studies has become a hot area worldwide. Accumulating evidences demonstrated that gut microbiota dysbiosis may play a key role in the progress of PD.

Methods: A case-control study based on deep next-generation shotgun sequencing of gut microbial DNA from 40 Chinese PD patients and their healthy spouses was devised and carried out using Illumina Hiseq Ten platform. Particularly, a fast and sensitive real-time PCR method was developed for specific detection of PD-associated gut microbial gene markers in the original 80 subjects and validated the results in an independent cohort of 70 patients and 64 controls.

Results: Significant differences were detected in the richness and communities of gut microbial gene between PD and healthy groups. A total of different 174,964 genes were identified and established the concept of a metagenomic linkage group, enabling taxonomic species-level analyses. 153 metagenomic species (MGS) were clustered to be differentially abundant between PD and healthy groups. Strain Alistipes indistinctus YIT 12060 and Alistipes finegoldii DSM 17242 were enriched in the PD group, and Bacteroides vulgatus ATCC 8482 was enriched in the healthy group. The most enriched functional of the gut metagenome associated with PD were involving metabolisms. 25 microbial gene markers were identified to distinguish PD from healthy group with areas under the receiver-operating curve (AUC) of 0.895 and a highly accurate index of PD patient discrimination was created. Detecting the 25 gene markers by method of real-time PCR could classify PD and healthy groups using shotgun sequencing with AUC of 0.922, and validated on an independent larger cohort with AUC of 0.869.

Conclusions: We present firstly the metagenome-wide association study in Chinese PD patients. Alterations of specific species and functional components of the gut metagenome were associated with PD. Particularly, gut microbial gene markers may be a powerful tool for diagnosis of PD, providing new leads for the development of new diagnostic tools and potential treatments.

1327

Ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) -mediated ubiquitination attributed to localization of Mortalin to mitochondria

L. Wu, W. Yang, Y. Tan, J. Ding, S. Chen (Shanghai, China)

Objective: The pathogenic mechanism of UCHL1 in PD remains unclear. In this study, we aimed to investigate the role of UCHL1 in the pathogenesis of PD.

Background: Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. The ubiquitin carboxy-terminal hydrolase L1 (UCHL1), a member of deubiquitinating enzymes, is primarily abundant in neurons. Down-regulation and dysfunction of UCHL1 have been detected in the brains of PD patients. In addition, UCHL1 gene mutants are linked to PD.

Methods: To study the mechanism of UCHL1 in the pathogenesis of PD, we used MPP+ to reproduce a cellular model of PD in SH-SY5Y cells. The effects of UCHL1 knockdown and overexpression in MPP+-induced cellular model of PD were determined. Immunoprecipitation and mass spectrometric analysis (IP-MS) were used to screen UCHL1 interacting proteins. Among these proteins, we focused on Mortalin. The interaction between UCHL1 and Mortalin was confirmed by Co-immunoprecipitation (Co-IP) in vivo and vitro using mice brain and HEK293T cells lysates. Co-IP was also applied to detect whether UCHL1 affects Mortalin ubiquitination. We used MG132 to block proteasome-dependent degradation and tested whether UCHL1 would influence Mortalin protein level in SH-SY5Y cells. UCHL1-siRNA transfected SH-SY5Y cells were fractionated into cytoplasmic and mitochondrial fractions, followed by western blotting to detect Mortalin localization change.

Results: UCHL1 knockdown enhanced MPP+-induced cytotoxicity in SH-SY5Y cells, as reflected by decreased cell viability, increased apoptosis protein level and declined mitochondrial membrane potential, while UCHL1 overexpression could rescue MPP+-induced cytotoxicity in SH-SY5Y cells. In total, 234 UCHL1 interacting proteins were screened through IP-MS. According to the findings, Mortalin was chosen as a target protein due to its association with PD reported previously. Mortalin co-immunoprecipitated with Mortalin in vivo and vitro was from rat brains and HEK293T cells lysates respectively. Furthermore, UCHL1 ubiquitinated Mortalin as ubiquitin ligase. Instead of affecting the protein level of Mortalin, UCHL1-mediated ubiquitination facilitated the localization of Mortalin to mitochondria.

Conclusions: The present data suggested that UCHL1 could mediate Mortalin to mitochondrial localization by ubiquitinating Mortalin as an ubiquitin ligase.

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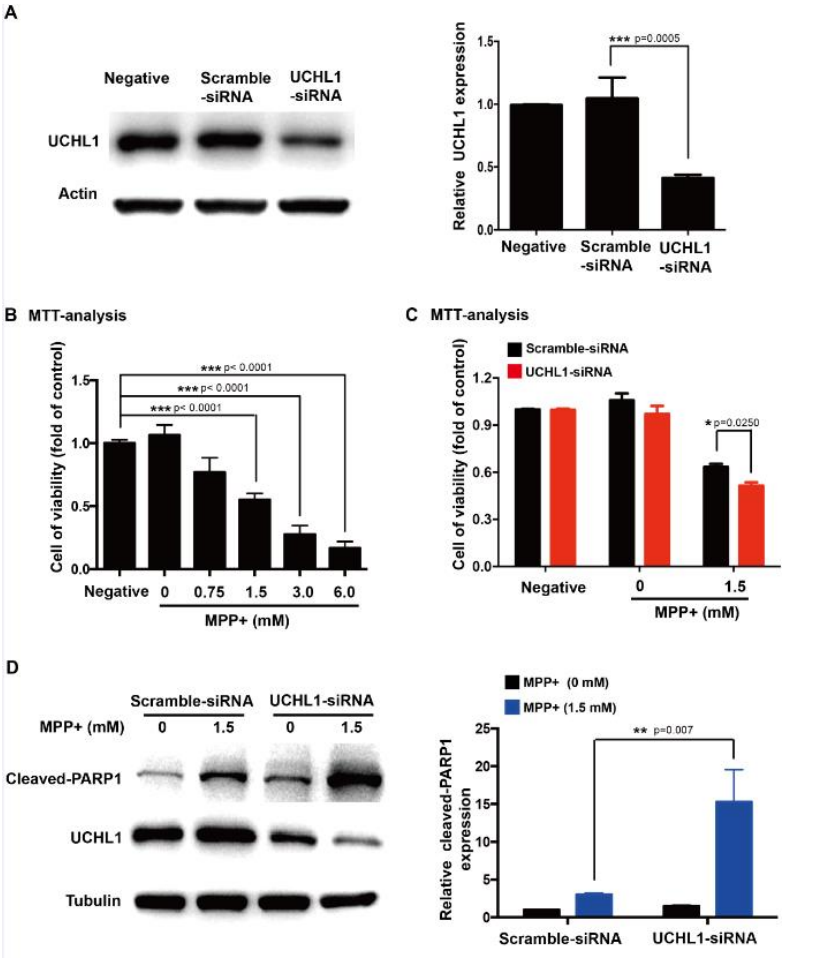
A
Negative
Scramble -siRNA
UCHL1 -siRNA
UCHL1
Actin
Relative UCHL1 expression
*** p=0.0005
Negative
Scramble -siRNA
UCHL1 -siRNA
B MTT-analysis
Cell of viability (fold of control)
*** p< 0.0001
*** p< 0.0001
*** p< 0.0001
Negative 0 0.75 1.5 3.0 6.0
MPP+ (mM)
C MTT-analysis
Scramble-siRNA
UCHL1-siRNA
Cell of viability (fold of control)
* p=0.0250
Negative
0
1.5
MPP+ (mM)
D
Scramble-siRNA
UCHL1-siRNA
MPP+ (mM) 0 1.5 0 1.5
Cleaved-PARP1
UCHL1
Tubulin
MPP+ (0 mM)
MPP+ (1.5 mM)
Relative cleaved-PARP1 expression
** p=0.007
Scramble-siRNA
UCHL1-siRNA

FIG. 1 (1327)

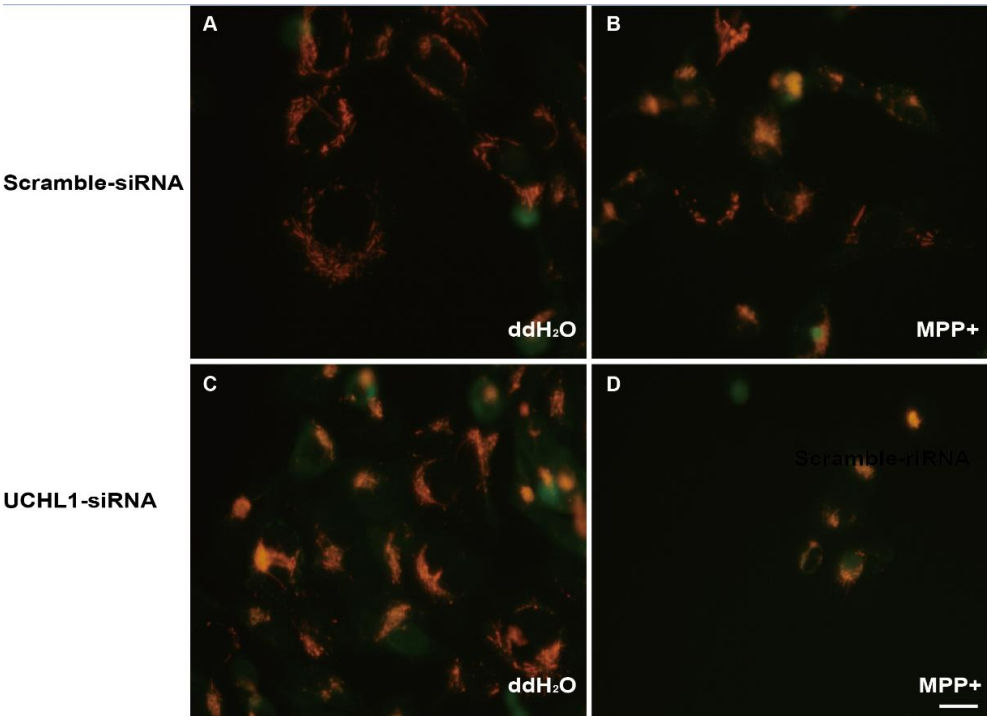
Scramble-siRNA
UCHL1-siRNA
A
ddH₂O
B
MPP+
C
ddH₂O
D
MPP+

FIG. 2 (1327)

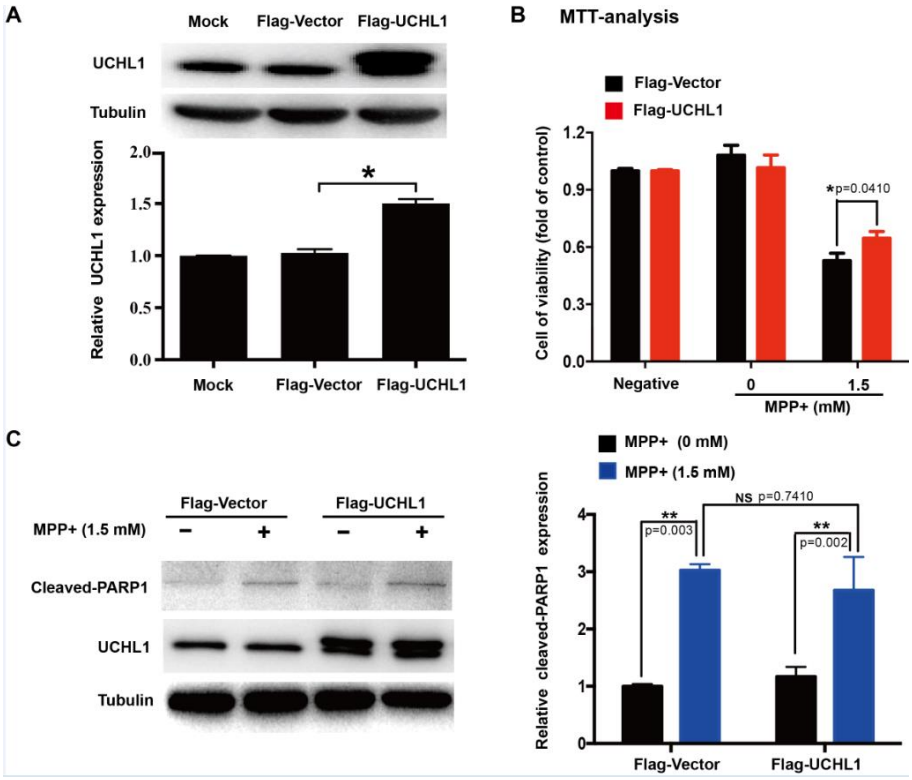

FIG. 3 (1327)

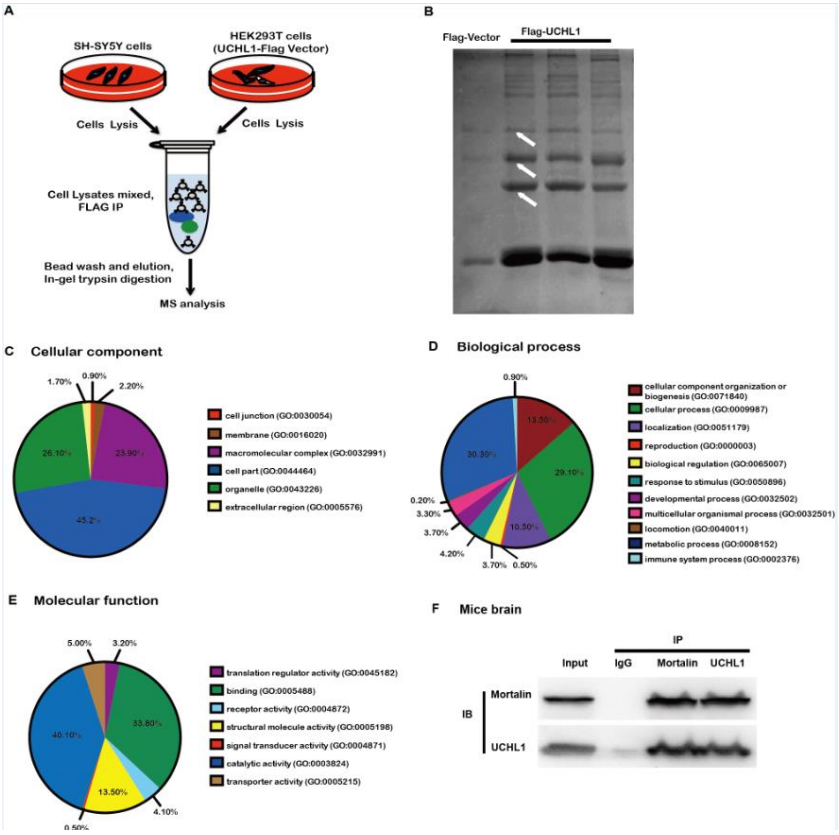

FIG. 4 (1327)

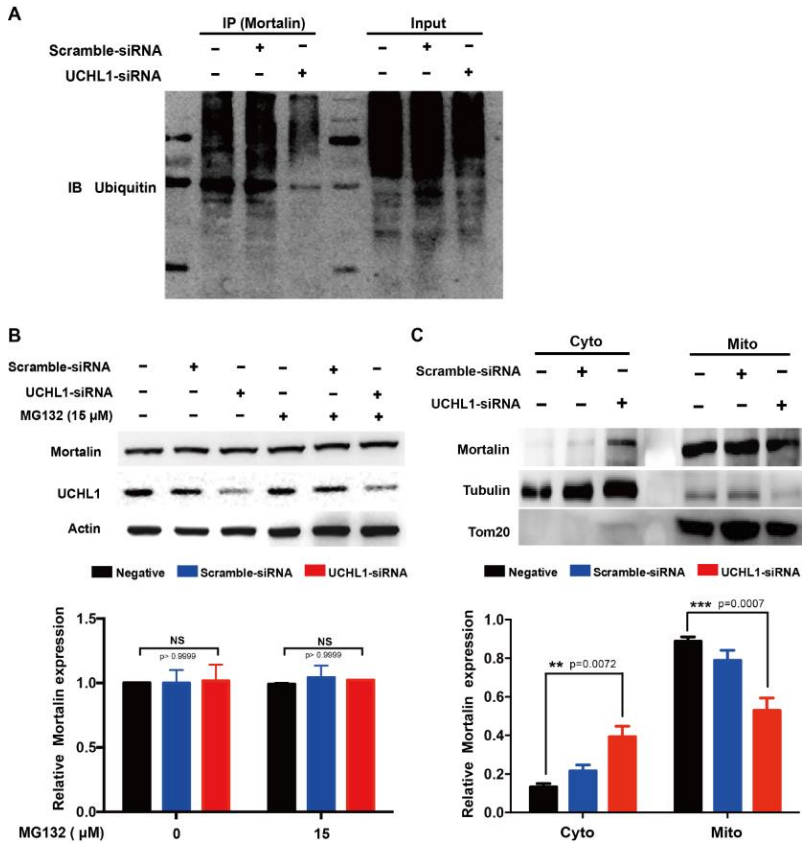

FIG. 5 (1327)]

1331

Neuronal KIF5b deletion induces striatum-dependent locomotor impairments and defects in membrane presentation of dopamine D2 receptors

T. Falzone, L. Cromberg, M. Alloatti, I. Fernandez Bessone, T. Saez (Buenos Aires, Argentina)

Objective: In this study we aim to elucidate the role of Kif5b motor subunit in the nigrostriatal pathway. We have generated different conditional knockout mice for the Kif5b subunit of Kinesin-1 to unravel its contribution to locomotion.

Background: Locomotion is an intricate process controlled by the basal ganglia and regulated by fine-tuned dopaminergic innervations from neurons in the substantia nigra pars-compacta (SNpc). Dysfunctions in dopaminergic neurotransmission leads to movement initiation impairments, gaiting defects and hypolocomotion in a variety of collective diseases so called Parkinsonism. Due to its high polarity and extreme axonal arborization, neurons depend on molecular motor proteins and microtubule-based transport for their normal function. Intracellular transport defects have been associated with Parkinsonism since axonopathies, axonal clogging, microtubule destabilization and lower levels of motor proteins were described in patient brains. However, the contribution of specific molecular motors to the regulation of the nigrostriatal network remains unclear.

Methods: Conditional mutant mice lacking Kif5b from neurons or specifically from dopaminergic neurons were generated and evaluated using a battery of behavioral, pharmacological, neurochemical and cellular experiments.

Results: Mice with neuronal deletion of Kif5b showed hypolocomotion with movement initiation deficits and motor coordination impairments. High pressure liquid chromatography experiments determined that dopamine (DA) metabolism is impaired in neuronal Kif5b-KO; however, no dopaminergic cell loss was observed. Contrarily, deletion of Kif5b only in dopaminergic neurons is not sufficient to induce locomotor

defects. Noteworthy, pharmacological stimulation of DA release together with agonist or antagonist of DA receptors revealed selective D2-dependent movement initiation defects in neuronal Kif5b-KO. Finally, subcellular fractionation from striatum, showed that Kif5b deletion reduced the amount of D2R in synaptic plasma membranes.

Conclusions: All this evidence strongly suggest the relevant contribution of Kif5b molecular motor subunit in the intracellular mechanisms necessary for nigrostriatal neuronal communication, which when impaired may lead to significant defects in motor coordination and overall locomotion. These results are important for understanding how molecular motor protein defects may contribute to the induction of locomotor impairments associated with parkinsonism.

1332

SIRT1/AMPK pathway is involved in neuroprotective effects of resveratrol on MPTP-induced neuron loss

Y.J. Guo, S.Y. Dong, W.J. Zhao, Y.C. Wu (Shanghai, China)

Objective: The present study was carried out to observe the neuroprotective effects of RV on MPTP-induced mouse model of PD, and explore its potential neuroprotective mechanism.

Background: Resveratrol (RV), a polyphenolic compound derived from grapes and red wine, can exert a wide range of beneficial effects. Parkinson's disease (PD) is a progressive neurodegenerative disorder primarily resulting from degeneration of dopaminergic neurons.

Methods: MPTP selectively damages dopaminergic neurons and has been proposed as an experimental animal model to study PD for many decades. Subacute intoxication regimen, which involves one injection of 30 mg/kg/free base MPTP daily for five consecutive days, was applied in our study. RV (100 mg/kg/day) was administrated by intragastric gavage (i.g.). EX527 (10 mg/kg/day), a specific SIRT1 inhibitor, was injected intraperitoneally. Male C57BL/6 mice were divided into four groups (n = 12): CON group, MPTP group, RV+MPTP group, and EX527+RV+MPTP group. Tyrosine hydroxylase (TH) positive cells, protein level of TH, SIRT1, AMPK, p-AMPK, Caspase 3 and Cleaved caspase 3 were determined by immunofluorescence staining and western blot.

Results: MPTP-induced mouse model of PD exhibited loss of dopaminergic neurons, decreased levels of TH ($P < 0.001$), SIRT1 ($P < 0.01$), p-AMPK ($P < 0.05$) and increased level of Cleaved caspase 3 ($P < 0.001$), in the present study. Administration of RV prevented MPTP-induced dopaminergic neuronal loss, caused up-regulation of SIRT1 ($P < 0.001$), TH ($P < 0.01$), p-AMPK ($P < 0.01$) and down-regulation of Cleaved caspase 3 ($P < 0.01$). Meanwhile, administration of EX527 reversed the effects of RV stated above, did not prevent MPTP-induced dopaminergic neuronal loss, caused down-regulation of TH ($P < 0.001$), SIRT1 ($P < 0.001$), p-AMPK ($P < 0.01$) and up-regulation of Cleaved caspase 3 ($P < 0.01$).

Conclusions: SIRT1/AMPK pathway was involved in the neuroprotective effects of RV on MPTP-induced neuron loss.

1339

Tef polymorphism predicts the decline of sleep disturbances in Parkinson's disease

P. Hua, W. Liu (Nanjing, China)

Objective: Circadian dysfunction may contribute to the etiology of motor and non-motor symptoms of Parkinson's disease (PD). The influence of polymorphisms of Cry1 rs2287161, Cry2 rs10838524, and Tef rs738499 in sub-group PD patients have been investigated in our previous studies. Here, we explore whether the there single nucleotide polymorphisms (SNPs) influence the rate of clinical symptoms' decline in PD patients.

Background: Recent years, increasing attention has been paid to the circadian rhythm system which may be a new diagnostic and therapeutic target in PD.As being inspired by the clock genes' involvement in the

etiology of major depression and sleep disorders, we have conducted two studies to analyze single-nucleotide polymorphisms (SNPs) of there clock genes (Cry1 rs2287161, Cry2 rs10838524, and Tef rs738499) in PD patients. The previous studies have shown that the TT genotype in Tef rs738499 was associated with poorer sleep quality and much severe depression of PD patients. The present study conducted in a longitudinal PD patient cohort aimed to explore whether the above genes contributed to the decline of clinical symptoms in this PD patient cohort, followed on average 3.3 years into disease course.

Methods: Gene-PD susceptibility associations were analyzed between 186 PD patients and 196 healthy controls, and 170 PD patients completed a longitudinal study with the follow-up period on average 3.36±1.03 years. The study re-assessed motor and non-motor symptom scales, then, stepwise linear regression analysis adjusted by related factors was used to assess longitudinal associations between genotypes and clinical progression scores.

Results: Faster annual decline rate of the PDSS scores and progression rate of H-Y were found in carriers of the TT genotypes of Tef rs738499. Stepwise linear regression on the decline of PDSS adjusted by the progression rate of HAMD, HAMA, PDNMS showed that Tef rs738499 alone account for 4.5% of the variance. decline rate of PDSS. Tef rs738499 played a relatively marginal effects on the progression of H-Y as it was excluded through the stepwise linear regression adjusted by clinical factors.

Conclusions: The study supports previous research identifying circadian dysfunction may be a key etiology of the non-motor symptoms of PD. The TT genotypes of Tef rs738499 can be used as predictors of faster sleep decline in PD.

1342

Lysosphingolipids accumulation in macrophage model of Gaucher disease

M. Nikolaev, A. Kopytova, K. Senkevich, A. Emelyanov, G. Baydakova, E. Zakharova, G. Salogub, S. Pchelina (Gatchina, Russian Federation)

Objective: The aim of this study was to investigate if GCase deficiency in macrophage model of GD lead to lysosphingolipids accumulation.

Background: Mutations in the GBA gene lead to a deficiency of glucocerebrosidase (GCase) enzymatic activity and to the development of Gaucher disease (GD) which belongs to lysosomal storage diseases. At the same time GBA mutations increase the risk of Parkinson's disease (PD) in 7-8 times. Developing of the appropriate cellular models of GD are crucial for the new therapeutic strategies for GD and PD treatment.

Methods: Lysosphingolipid (hexosylsphingosine HexSph (glucosylsphingosine GlcSph+ galactosylsphingosine GalSph)) levels and GCase enzymatic activity were measured by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in dry blood spots (DBS) and dry macrophage cell spots with the concentration of 2x10^6 cells/ml in GD patients (n=4) and healthy controls (n=10)The effect of dose-dependent inhibition of GCase enzymatic activity with conduritol-B-epoxide (CBE) (150 and 450 ng/ml) on HexSph level was estimated in macrophages of 9 healthy individuals. Mononuclear fraction was isolated using the Ficoll gradient centrifugation. The cells were differentiated into macrophages using RPMI supplemented with 10% bovine serum, 1% streptomycin-penicillin and 10 ng/ml M-CSF for 4 days, with daily media changes. GCase enzymatic activity was inhibited with CBE (0, 150 and 450 ng/ml) for 4 days.

Results: We showed a significant GCase enzymatic activity decrease in patients with GD compared to control group both in DBS and in cultivated macrophages ($p<0.001$ and $p<0.001$, respectively) as well as increase in HexSph levels ($p<0.001$ and $p<0.001$, respectively). A dose-dependent increase in macrophage HexSph level was shown: CBE concentrations: 150 and 450 ng/ml compared to cells without CBE ($p<0.001$ and $p<0.001$, respectively), and in cell with 450 ng/ml CBE concentration compared to 150 ng/ml CBE ($p=0.036$) [figure1].

Conclusions: We showed that GCase enzymatic activity and HexSph levels in cultured macrophage estimated by LC-MC/MC from GD patents reflect the same parameters in blood. A dose-dependent GCase activity inhibition with CBE in macrophages of healthy individuals leads to dose dependent HexSph accumulation. We showed that macrophages, showing GCase dysfunction, accumulate lysospingolipids in

vitro that supports the possibility of using macrophage model of GD in exploring new therapies for GD and related disorders.

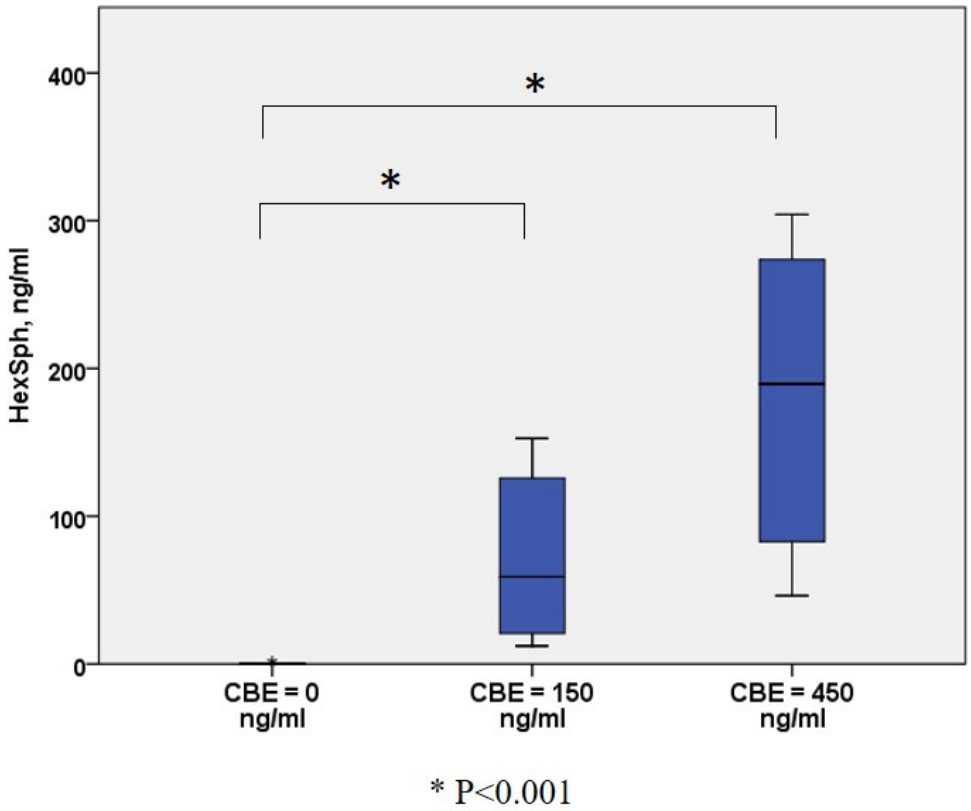

FIG. 1 (1342) A dose-dependent increase levels in macrophage cells cultivated with different CBE concentrations.

1347

Microarray analysis upon an synthetic α-synuclein induced model reveals some susceptibility genes in Parkinson's disease

E. Tao (Guangzhou, China)

Objective: To uncover new disease-associated genes and their relevant mechanisms in the pathogenetic process of neurodegenerative disorders, we carried out a gene microarray analysis based on a Parkinson's disease (PD) in vitro model induced by α-synuclein oligomers.

Background: The gene microarray was performed upon the cellular model induced by 25 mol/L α-synuclein oligomers, which has been confirmed to show the stable, transmissible neurotoxicity of α-synuclein, a typical PD pathological marker. And the different expressed long non-coding RNA(lncRNA) was screened out for the reseach of its potential influence upon the pathogenetic process of neurodegenerative disorders.

Methods: Immumofluorescence and electron microscope were used to make sure α-synuclein have already entered the cells and led to α-synuclein lesions. The expression of lncRNAs and mRNAs is analyzed by gene microarray and GeneSpring GX v12.1 software package. Differently-expressed genes(DEGs) were verified by real time qPCR and the selected genes were studied by ceRNA(competing endogenous RNAs) analysis.

Results: A significant differentially expressed lncRNAs, G069488, were chosen as a breakthrough point for the further research because it was located to the gene NEED9, which is relative to the axon growth and

neurodevelopment closely. Subsequent verified qPCR experiment determined the same variation trend as the result of microarray analysis showed.

Conclusions: The results of the present study widen our horizon of PD susceptibility genes and provide new pathways towards efficient diagnostic biomarkers and therapeutic targets for PD.

A

GeneSymbol	G069488
seqname	T297427
P-value - LncRNAs	0.033696068
Fold change - LncRNAs	2.5192302
Regulation - LncRNAs	up
Chrom	chr6
txStart	11391981
txEnd	11395142
RNAlength	2173

B

NearbyGeneSymbol	NEDD9
NearbyGene	NM_006403
P-value - mRNAs	0.040789689
Fold change - LncRNAs	2.5345612
Regulation - LncRNAs	down
Chrom	chr6
txStart	11183530
txEnd	11232915
NearbyProteinName	neural precursor cell expressed, developmentally down-regulated 9

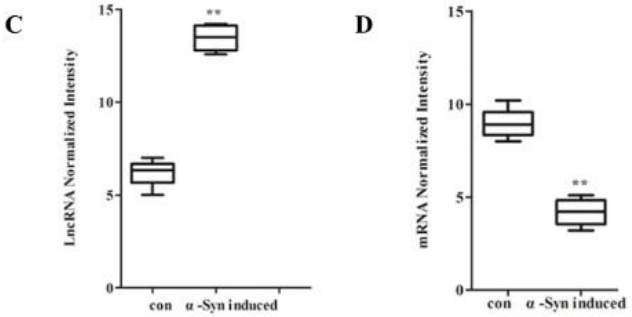

FIG. 1 (1347)

1355

eEF1A2 and Prdx1 as possible new targets for Parkinson's disease therapeutics

W. Wirakiat, P. Dharmasaroja (Bangkok, Thailand)

Objective: We investigated expression of eEF1A2 and Prdx1 in differentiated SH-SY5Y cells in searching for possible new targets for Parkinson's disease treatment.

Background: Parkinson's disease (PD) is a progressive disorder of the nervous system. Development of disease-modifying drugs has been a remarkable advancement in recent years, based on proteins involved in the pathogenesis. Eukaryotic translation elongation factor 1 alpha 2 (eEF1A2) is one of the two isoforms of elongation factor eEF1A. eEF1A2 has a very restricted pattern of expression and they are found in neurons, cardiomyocytes and myotubes, while eEF1A1 extensively expresses. A previous study showed that exposure of SH-SY5Y cells to MPP+ significantly increased the expression of eEF1A2 in accordance with the PI3K/Akt/mTOR signaling proteins. Moreover, the investigation in yeast two-hybrid system showed eEF1A2 interacts with peroxiredoxin type 1 (Prdx1) but not with eEF1A1 to defense stress conditions. Prdx1 is a typical 2-Cys peroxiredoxin, ubiquitously expressed in all mammalian cells, and plays role in cell protection by reducing reactive oxygen species. Its expression in mouse dopaminergic neurons of the substantia nigra is very low, suggesting that Prdx1 may affect neuronal sensitivity to oxidative stress and may contribute to the development of PD.

Methods: We induced SH-SY5Y cells differentiation with 10 µM retinoic acid for 3, 5, 7 and 10 days. Tyrosine hydroxyl expression was measured by Western blotting to ensure the dopaminergic phenotype.

Gene and protein expression of eEF1A2 and Prdx1 were investigated by real time PCR and western blot analysis, respectively. The localizations of eEF1A2 and Prdx1 were visualized by immunofluorescence.

Results: The real time PCR and western blot analysis showed that differentiated cells had a significant increase of eEF1A2 mRNA and protein expression ($p<0.05$), compared to undifferentiated cells. Conversely, Prdx1 protein was progressively decreased during differentiation. These results were consistent with the immunofluorescent study.

Conclusions: eEF1A2 and Prdx1 have a reverse expression during neuronal differentiation. Further investigation on manipulation of these two protein expression in cellular and animal models of PD may shed light for development of PD therapeutic strategies.

References: Khwanraj K, Madlah S, Grataitong K, Dharmasaroja P. Comparative mRNA Expression of eEF1A Isoforms and a PI3K/Akt/mTOR Pathway in a Cellular Model of Parkinson's Disease. Parkinsons Dis. 2016;2016:8716016. Goemaere J, Knoops B. Peroxiredoxin distribution in the mouse brain with emphasis on neuronal populations affected in neurodegenerative disorders. J Comp Neurol. 2012 Feb 1;520(2):258-80.

1356

Diagnostic utility of a targeted resequencing technique of next generation sequencing in detecting copy number changes in PARK2

NY. Kim, SK. Hong, YE. Kim, HI. Ma, YJ. Kim (Anyang, Republic of Korea)

Objective: We investigate feasibility of Next Generation Sequencing (NGS) targeted sequencing technique using Ampliseq® technology by Ion PGM® to detect copy number variation mutation in PARK2 gene.

Background: Among causative genes for familial Parkinson's disease (PD), PARK2 is the most common autosomal recessive gene for familial Parkinson's disease. In East Asian, while 10% of mutations in PARK2 are single nucleotide variants, 90% of PARK2 mutations are copy number variation (CNV) mutation (i.e., deletion/duplication) caused by exonic rearrangement. Targeted resequencing of a panel of genes using NGS technique has been known to capture single nucleotide variant accurately. However, whether this technique can detect CNV mutation precisely enough to be used in clinical genetics has not been systemically studied.

Methods: Targeted resequencing of five PD-causing genes (PARK2, ATP13A, PLA2G6, PINK1, SNCA) using Ion PGM® was performed in a group of 32 PD patients with early onset PD. Results of copy number change analyses in PARK2 based on depth-based algorithm was compared to those obtained by a gold standard method, real-time PCR (RT-PCR).

Results: An average of 144,767 mapping reads per a sample were on target (99.28%) were obtained with an average coverage depth 1,273X and coverage uniformity of 95.40 %. In unsupervised analyses, the concordances as determined by Cohen's kappa between depth-based CNV detection and RT-PCR was poor to good (kappa=0.43, 95% CI 0.24-0.63). In a supervised analysis, kappa was higher (kappa=0.77, CI 0.63-0.91), however, results of CNV analyzed by Ampliseq® technology by Ion PGM® using coverage depth-based algorithm were significantly different from those by a gold standard method, RT-PCR (McNemar test, $p<0.05$).

Conclusions: Our results suggest that depth-based CNV analysis algorithm using data obtained by Ampliseq® technique of NGS is not comparable to RT-PCR in detecting PARK2 exonic rearrangement.

1361

Loss of VPS29 disrupts retromer function and synaptic transmission, leading to neurodegeneration in Drosophila

H. Ye, D. Li-Kroeger, Y. Li, H. Bellen, J. Shulman (Houston, TX, USA)

Objective: To understand the role of the retromer complex in the central nervous system(CNS), and its link to Parkinson's disease (PD) susceptibility.

Background: Mutations in VPS35 cause autosomal dominant, late-onset PD. However, how retromer dysfunction contributes to PD pathogenesis remains elusive. The retromer core consists of a conserved, heterotrimeric complex including VPS35, VPS26, and VPS29, that mediates the recycling of cargo proteins from the endosome. Tissue-specific loss of Vps35 or Vps26 in the Drosophila eye causes progressive retinal degeneration, but embryonic lethality has hindered systematic studies of retromer dysfunction in the CNS.

Methods: Using CRISPR/Cas9 technology, we generated a Vps29 null allele, in which the entire coding sequence is deleted. Since Vps29 null homozygotes are adult viable, we evaluated age-dependent survival, startle-induced negative geotaxis, electroretinograms (ERG), and CNS histology analysis to reveal evidence of potential neurodegeneration. Western blotting and immunofluorescence were also performed to evaluate other retromer components and the integrity/function of the endolysosomal system.

Results: Vps29 null animals exhibit reduced survival and age-dependent locomotor defects, and these phenotypes are dominantly enhanced in a Vps35 heterozygous genetic background. Based on ERG, loss of Vps29 causes progressive loss of photoreceptor depolarization and transient potentials, similar to the Vps35-mutant phenotype. Consistent with this, histology revealed extensive retinal vacuolar degeneration in aged Vps29 mutants. Using a rapid stimulation paradigm, ERG transients are progressively diminished in Vps29 null mutants, suggestive of defects in synaptic vesicle recycling. All observed phenotypes were rescued by a Vps29 genomic transgenic construct or via targeted expression in neurons. Interestingly, while loss of Vps29 did not significantly affect Vps35 protein level, Vps35 showed an aberrant perinuclear localization co-localizing with Rab7 GTPase, potentially consistent with impaired retromer turnover and endolysosomal dysfunction.

Conclusions: Loss of Vps29 in Drosophila causes progressive synaptic dysfunction, impaired locomotor behavior, and neurodegeneration, thus recapitulating salient features of PD, and establishing a valuable model for studying the role of the retromer and associated endolysosomal system in the aging, adult nervous system.

1364

A pilot study of plasma ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) as a potential blood-based biomarker in Parkinson's disease

A. Ng, YJ. Tan, ZH. Lu, S. Ng, E. Ng, F. Setiawan, N. Keong, WL. Au, EK. Tan, L. Tan (Singapore, Singapore)

Objective: To determine plasma levels of UCH-L1 in patients with PD, and to investigate the association with both genetic (leucine-rich repeat kinase 2, LRRK2 and alpha-synuclein, SNCA) and clinical measures.

Background: Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), a pivotal component of the ubiquitin-proteasome system, is highly expressed in neurons and shown to be involved in regulation of the ubiquitin pool, apoptosis, learning and memory. Reduced expression of UCH-L1 has been reported in post-mortem brains with Lewy body dementia, while suppression of UCH-L1 activity in vivo resulted in accumulation of presynaptic alpha-synuclein in neurons. CSF levels of UCH-L1 are reported to be lower in PD than controls, but UCH-L1 levels in peripheral blood remain unknown.

Methods: Plasma levels of UCH-L1 and alpha-synuclein were measured using Single Molecule Array (Simoa) technology in 80 subjects (73 patients with PD and 7 healthy controls). All participants underwent tests of global cognition (mini-mental state examination, MMSE), motor function on the Unified Parkinson's Disease Rating Scale (UPDRS), as well as disability scoring on the Hoehn & Yahr (H&Y) scale. All subjects were genotyped for reported Asian LRRK2 risk (S1647T, G2385P and R1628P) and protective variants (N551K and R1398H), and SNCA promoter region (Rep1) allele length (short-0/1, medium-1/2, long-2/3). The association of longer Rep1 allele length with increased risk of PD has been well-reported in multiple studies.

Results: Plasma UCH-L1 levels were significantly lower in PD patients than in controls (7.68 pg/ml vs 13.82, p=0.02). In the PD group, UCH-L1 levels did not correlate significantly with age, disease duration, MMSE, UPDRS motor and H&Y scores. However, PD patients with lower MMSE scores (MMSE≤25) showed lower UCH-L1 than controls (4.26 pg/ml vs 13.82, Bonferroni adjusted p=0.02). Notably in both PD

patients and controls, subjects with longest Rep1 alleles (allele 2/3) had lower UCH-L1 levels than carriers of short- and medium-length alleles.

Conclusions: The results of this pilot study suggests that plasma UCH-L1 may be a potential biomarker for PD, with lower levels seen in PD vs controls, particularly in PD patients with lower cognitive scores, or with longest SNCA Rep1 alleles. More samples will be included in ongoing studies to validate these findings.

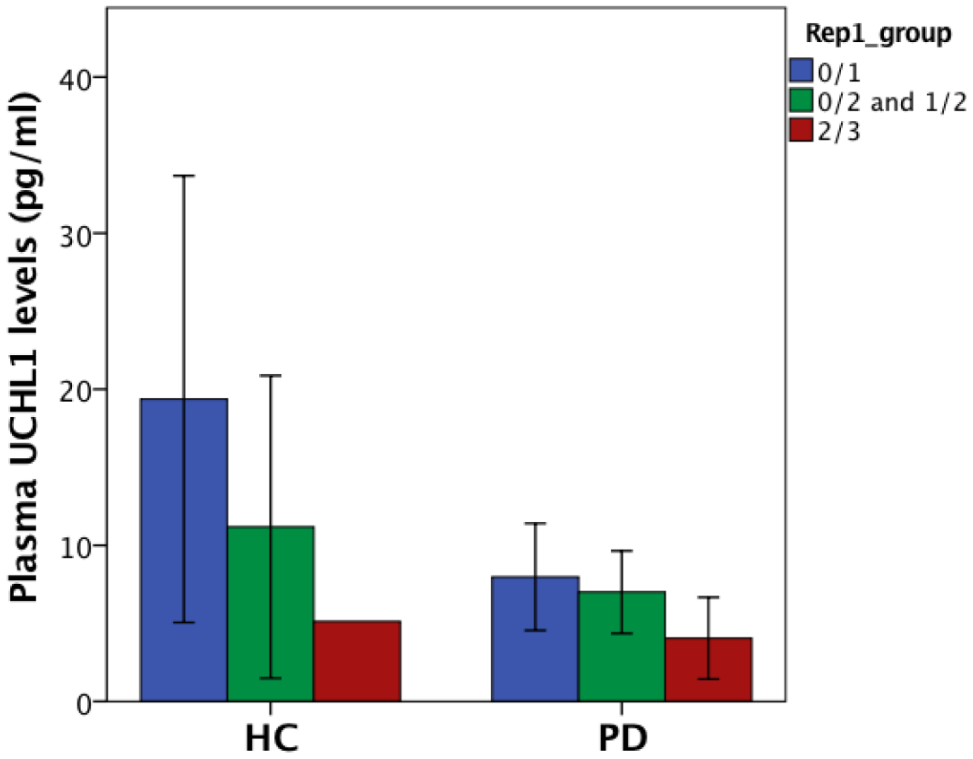

FIG. 1 (1364)

1367

Identification and analysis of differential miRNA in exosomes of dopaminergic neurons overexpressing α-synuclein

Y. Liang, D. Lin, T. Zhou, D. Zheng (Guangzhou, China)

Objective: The aim of this study was to compare the miRNA expression in secreted exosomes from dopaminergic neurons overexpressing α-synuclein with healthy neurons.

Background: Exosomes, nano-sized membrane vesicles, are released into the extracellular environment by most cell types. Recent studies have demonstrated that exosomes are capable of delivering their content (mainly RNAs, proteins, and lipids) to target cells, which is a new way of intercellular communication, removal of cellular debris, and transfer of pathogens between cells. In Parkinson's disease (PD), exosomes are considered as protective carriers of toxic α-synuclein that contribute to the spread of α-synuclein aggregates in CNS and the dissemination of pathology.**Methods:** We first transfected SH-SY5Y cells with either lentivirus expressing wild-type human SNCA or control lentivirus and then collected their exosomes. Microarray was used to detect differential miRNAs of these exosomes. Real-time PCR was used to verify the expression of miRNAs. Target genes of these miRNAs were predicted by TargetScan and miRanda, and then further analyzed by Gene Ontology and KEGG pathway enrichment.

Results: According to the fold change and P value, 10 distinct miRNAs were selected for verification using real-time PCR, and their expression were consistent with the results of microarray. Our analysis revealed the top enriched GO terms of the target genes of differential miRNAs include G-protein coupled

photoreceptor activity, voltage-gated ion channel activity involved in regulation of postsynaptic membrane potential, and photoreceptor activity. While TGF-beta signaling pathway, axon guidance, and mTOR signaling pathway are involved in the top enriched KEGG pathways.

Conclusions: In conclusion, the results of the present study showed that exosomes from neurons overexpressing α-synuclein are characterized by a specific miRNA signature, which will have crucial influence on discovery of biomarkers and therapeutic targets for neurodegenerative diseases including PD.

1369

Parkinson-related CHCHD2 is necessary for oligomerization of ALS/FTD-related CHCHD10

D. Narendra, X. Huang, B. Wu, Y. Liu, D. Nguyen, M. Marani (Bethesda, MD, USA)

Objective: Characterization of isogenic CHCHD2, CHCHD10, and CHCHD2/10 double knockout out cell lines with assays of mitochondrial function, mitochondrial sublocalization, and homo- and heterodimerization.

Background: Mutations in paralogous mitochondrial proteins CHCHD2 and CHCHD10 were recently found to cause autosomal dominant Parkinson Disease (PD) and ALS/FTD, respectively. The proteins exhibit 58% amino acid sequence identity and are thought to have resulted from a gene duplication event. Additionally, most pathogenically proven missense mutations in CHCHD2 (including T61I) and CHCHD10 (including G58R, S59L, and G66V) cluster in the same highly conserved region. The extent to which the proteins have retained a common function in their divergent evolution, however, is not known. To this end, we generated CHCHD2 KO, CHCHD10 KO, and CHCHD2/10 double KO cells on an isogenic background to allow direct comparison of CHCHD2 and CHCHD10 localization and function.

Methods: Isogenic CHCHD2, CHCHD10, and CHCHD2/10 double knockout cell lines were generated on a HEK293 and HeLa cell background by Crispr/Cas9. Localization was assessed using confocal, stimulated emission depletion (STED), and immuno-electron microscopy. Mitochondrial protein expression was assessed by immunoblotting and mitochondrial function was assessed using the Seahorse oxygen consumption assay. Homo- and heterodimerization of CHCHD2 and CHCHD10 was assessed in co-immunoprecipitation and crosslinking studies.

Results: We find that CHCHD2 and CHCHD10 are partially functionally redundant, share localization throughout mitochondrial cristae, and form heterodimers. CHCHD2 is strictly required for oligomerization of CHCHD10. CHCHD2, in contrast to CHCHD10, readily forms homodimers in the absence of CHCHD10, which may account for the more severe phenotype resulting from loss of CHCHD2. We exploit the dependence of CHCHD10 oligomerization on CHCHD2 to develop a CHCHD2/CHCHD10 heterodimer incorporation assay and demonstrate that CHCHD2 and CHCHD10 with disease-causing mutations readily incorporate into heterodimers.

Conclusions: CHCHD2 and CHCHD10 have retained a similar function and directly interact. Notably, CHCHD10 is dependent on CHCHD2 for oligomerization. Disease-causing mutations do not disrupt the direct interaction of CHCHD2 and CHCHD10 and suggest a mechanism by which pathogenic mutations in CHCHD2 could directly affect functioning of wildtype CHCHD10 and vice versa.

1370

Molecular mechanisms of GCH1-associated Parkinson's disease

J. Terbeek, W. Vandenberghe (Leuven, Belgium)

Objective: To unravel the molecular mechanisms by which loss of GCH1 function enhances the risk of Parkinson´s disease (PD).

Background: Loss-of-function mutations in GCH1 are the most common cause of autosomal dominant DOPA-responsive dystonia (DRD), a non-neurodegenerative movement disorder. GCH1 encodes GTP cyclohydrolase 1, the rate-limiting enzyme in the biosynthesis of tetrahydrobiopterin (BH4). BH4 is an essential cofactor for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of dopamine. Recent

genetic evidence indicates that GCH1 mutations also increase the risk of PD. The mechanisms by which GCH1 mutations predispose to nigrostriatal cell death, are unknown.

Methods: We cultured skin fibroblasts from 2 patients (Patient 1, Patient 2) with the same heterozygous missense mutation in GCH1 (p.Y75S; c. 224A>C) and from 2 healthy age-matched controls (Control 1, Control 2). Patient 1 was a 42-year-old male who developed clinically typical DRD at the age of 8 years and had severely abnormal dopamine transporter (DAT) imaging at the age of 37 years. Patient 2, the 63-year-old mother of Patient 1, developed clinically typical DRD at the age of 8 years and had normal DAT imaging at the age of 60. We induced GCH1 expression in the fibroblasts by 24-hour treatment with interferon-gamma. We assessed apoptosis using TUNEL staining, western blotting for PARP and immunostaining for cleaved caspase 3.

Results: GCH1 abundance after IFN-gamma treatment was lower in the mutant fibroblasts than in their respective controls. Moreover, GCH1 abundance was lower in Patient 1 than in Patient 2. Fibroblasts from Patient 1 were more susceptible to staurosporine- and H2O2-induced apoptosis than those of Control 1. By contrast, staurosporine- and H2O2-induced apoptosis did not differ between Patient 2 and Control 2. The survival defect of fibroblasts of Patient 1 was rescued by incubation with sepiapterin, a precursor of BH4 via the GCH1-independent salvage pathway, indicating that the survival defect was caused by BH4 deficiency.

Conclusions: The clinical phenotypes of the 2 GCH1 mutant patients correlated with the susceptibility of their skin fibroblasts to apoptosis in vitro. As skin fibroblasts do not produce dopamine, our findings suggest that GCH1 mutations can impair cellular survival via mechanisms unrelated to dopamine synthesis defects.

1373

Levels of plasma alpha-synuclein as measured using Single Molecule Array technology is higher in Parkinson's disease compared to controls and is not influenced by LRRK2 genotype

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Objective: To investigate plasma alpha-synuclein levels in PD using ultra-sensitive protein detection technology, and to determine the association of plasma alpha-synuclein with cognitive, motor and disability scores.

Background: Discriminating between healthy subjects and Parkinson disease (PD) patients using blood-based biomarkers has been limited by the very low concentrations of circulating alpha-synuclein in blood that remain difficult to accurately detect and quantify using existing methods.

Methods: 224 subjects were included in this study (52 gender-matched controls and 172 PD patients). Plasma alpha-synuclein was measured using Quanterix's Single Molecule Array (Simoa) technology. All subjects underwent tests of global cognition (mini-mental state examination, MMSE), motor evaluation using the Unified Parkinson's Disease Rating Scale (UPDRS) and disability scoring on the Hoehn & Yahr (H&Y) scale.

Results: Plasma alpha-synuclein levels were significantly higher in PD than controls (15556.2 vs 13122.8 pg/ml, p=0.042), after adjusting for age and gender. In PD patients, plasma alpha-synuclein levels did not vary significantly by disease stage (H&Y 1-2 vs H&Y 2.5-5, p=0.26), nor by UPDRS motor scores (p=0.875). Synuclein levels in PD with lower cognitive scores (MMSE<=25) were significantly higher than in controls (p=0.015, Bonferroni adjusted p=0.044); while levels in PD with MMSE>25 were not significantly different compared to controls (p=0.063, Bonferroni adjusted p=0.190). ROC analysis revealed that plasma alpha-synuclein levels could differentiate PD from controls (AUC= 0.597, 95% CI =0.507 – 0.687) and PD with MMSE<=25 from controls (AUC= 0.627, 95% CI =0.519 – 0.735). In PD patients carrying the reported Asian leucine-rich repeat kinase 2 (LRRK2) risk variants S1647T, G2385P and R1628P, plasma alpha-synuclein levels did not differ significantly between carriers and non-carriers. Notably, in controls, carriers of the reported Asian LRRK2 protective variants (N551K and R1398H in linkage disequilibrium) demonstrated a non-significant trend towards lower plasma synuclein levels than in non-carriers; whereas carriers of LRRK2 risk variants (S1647T, R1628P and G2385P) showed higher alpha-synuclein levels than in non-carriers (p=0.421, after controlling for age and gender).

Conclusions: The single molecular array method of quantifying levels of plasma alpha-synuclein may act as a potential biomarker for Parkinson's disease, particularly in patients with lower cognitive scores. Further studies are required to validate these findings.

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Olfactory dysfunction, sleep disturbances, neuronal loss, and regional brain atrophy in an inducible mouse model of Alpha-Synucleinopathy

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Objective: The objective of this work is to develop a unique mouse model of spreading α-synucleinopathy to accelerate the development of novel disease-modifying treatments for Parkinson's disease (PD) and other synucleinopathies.

Background: In PD, non-motor deficits, such as olfactory impairment and sleep disturbances, typically precede the cardinal motor symptoms by several years. In this study, we characterized functional, structural, and pathologic changes associated with the olfactory system in an inducible mouse model of α-synucleinopathy using behavioral testing, in vivo magnetic resonance imaging (MRI), and quantitative immunohistochemistry (qIHC).

Methods: The mouse model of α-synucleinopathy was induced in M83 transgenic (Tg) mice by stereotaxic inoculation of human preformed α-synuclein fibrils (PFFs) into the anterior olfactory nucleus (AON). Animals were tested for olfactory deficits using the buried pellet test and for sleep dysfunctions using the PiezoSleep Mouse Behavior Tracking System. Anatomical MRI scans were acquired at baseline and follow-up (at 2, 3, and 4 months post-inoculation of PFFs), and images were processed using Biospective's fully-automated NIGHTWING™ software. Anatomical regional volumes and cortical thickness measures were assessed. Finally, qIHC studies were performed to assess α-synucleinopathy, neurodegeneration, and neuroinflammation using Biospective's PERMITS™ software.

Results: Injection of PFFs into the AON induced statistically significant olfactory deficits, as measured by the latency in the buried pellet test in Tg mice compared to control animals. Injection of PFFs into the AON also had an impact on the quality of sleep of Tg mice compared to control animals. The α-synuclein pathology was observed in anatomically-connected olfactory regions in Tg mice. Quantitative analysis of the MRI data revealed that injection of PFFs into the AON resulted in significant decreases in regional neuroanatomical volumes associated with loss of neurons in those regions in Tg mice.

Conclusions: We have developed a unique mouse model of spreading α-synucleinopathy that demonstrates olfactory dysfunction and structural brain changes. This model is well-suited for preclinical efficacy studies to accelerate the development of novel disease-modifying treatments for PD and other synucleinopathies.

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Closed loop spinal cord stimulation restores locomotion and desynchronizes corticostriatal beta oscillations

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Objective: To test if closed loop Spinal Cord Stimulation (SCS) could be used to restore locomotion in a 6-hydroxydopamine (6-OHDA) rodent model of Parkinson's Disease (PD) and to investigate if closed loop stimulation is better than open loop stimulation in alleviating PD symptoms and its neurophysiological correlates.

Background: Spinal cord stimulation has been recently proposed as an alternative therapeutic option for PD patients suffering from gait disorders and postural abnormalities [1,2]. Optimizing stimulation patterns of SCS is necessary for maximizing its therapeutic potential [3]. Closed loop deep brain stimulation (DBS) has shown improvements over traditional DBS in ameliorating symptoms of PD [4,5].

Methods: Rats were implanted with bilateral microelectrode recording arrays in motor cortex (M1) and dorsolateral striatum (DLS), along with bilateral injection cannulae in the dorsolateral striatum. Stimulation electrodes were implanted on the dorsal surface of the spinal cord [6]. One week post surgery, 6-OHDA was injected in the striatum to induce bilateral intrastriatal lesions. Motor activity was recorded as rats explored an open field chamber before and after the lesion along with spiking activity of M1 and DLS neurons and local field potential (LFP) signals. Spinal cord stimulation was administered in a closed loop fashion driven by either M1 or DLS activity. Stimulation was triggered either by 1) M1 or DLS neuron firing within beta (10-25 Hz) oscillatory frequency range or 2) Filtered beta band LFP signal from DLS crossing a baseline threshold.

Results: Bilateral 6-OHDA lesioning in rats resulted in severe akinesia and corresponding increase in corticostriatal beta oscillations. Closed loop SCS increased the magnitude of distance traveled and reduced the amount of time spent in rest by the rats. Improvement in akinesia symptoms was corroborated by a decrease in beta (10-25) Hz corticostriatal oscillations. Improvement of akinesia and reduction of beta oscillations was higher in closed loop mode as compared to open loop mode. Closed loop SCS resulted in 80-90% reduction in stimulation pulses delivered.

Conclusions: Our results show that closed loop SCS triggered by corticostriatal oscillatory activity in rats is effective in ameliorating motor symptoms associated with PD and that stimulation delivered in a closed loop fashion might be more effective than continuous open loop stimulation.

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Behavior and PET Imaging Study in Parkinsonian Rat Models Induced by Injection of 6-OHDA in Medial Forebrain Bundle

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Objective: To employ behavioral test, positron emission tomography (PET) imaging and immunohistochemical staining to assess the changes of brain function and dopaminergic system in rat models of Parkinson's disease (PD) induced by unilateral injection of 6-hydroxydopamine (6-OHDA) in MFB (medial forebrain bundle).

Background: Rats unilaterally lesioned with 6-OHDA have been used as a useful hemi-Parkinson model for studying dopamine (DA) -related functions and assessing treat ment of PD. PET is capable of repeated and quantitative measurements in Parkinsonian animal models.

Methods: Thirty healthy male Sprague-Dawley (SD) rats were randomly divided into PD modeling group (N = 22) and normal control group (N = 8). Rats in modeling group received stereotactically and unilaterally administered 6-OHDA in the right MFB with two points. Behavioral studies were carried out by apomorphine rotational test (>7 turns/min is a successful PD model) and Cylinder test (assessing forelimb function) in all lesioned rats 3-5 weeks after the surgical procedure. [18F]fluoro-deoxyglucose ([18F]FDG) and [11C]CFT PET were performed to assess brain function and dopamine transporter in both PD model and normal rats. Tyrosine hydroxylase (TH) immunohistochernical staining was used to detect the change of dopaminergic neurons in substantia nigra in PD rat models.

Results: There were 14 successful PD rat models (63.6% success rate). Apomorphine test induced remarkable left rotations in PD rat models, with an average number of 12.0 ± 2.7 turns / min. Cylinder test indicated total forelimb activities were significantly reduced in PD rat models (0.5 ± 0.3 rears/min) than control group (2.8 ± 0.8 rears/min). Asymmetric forelimb activities were significantly increased in PD rat models of (90.0%) than control group (10.7%). [18F]FDG PET imaging showed decreased glucose metabolism in the right sensorimotor cortex, striatum and hippocampus area of PD rat models, compared with that in normal rats. [11C]CFT PET imaging showed significantly decreased DAT binding in the lesion side of striatum of PD rat models. TH immunohistochemistry showed significantly reduced TH-positive cells in the lesion side of substantia nigra.

Conclusions: Unilateral injection of 6-OHDA in MFB can establish PD rat models with high success rate. [18F]FDG and [11C]CFT PET imaging, combining with behavioral observations, can be served as one of standards for PD model verification and a useful molecular imaging tool for basic and clinical research of PD.

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Study on feasibility to develop a central nervous system (CNS) Leucine-rich repeat kinase 2 (LRRK2) PET tracer

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Objective: To assess feasibility of developing a CNS LRRK2 PET tracer.

Background: LRRK2 enzyme belongs to the leucine-rich repeat kinase family. Pathogenic mutations in the LRRK2 gene alter enzyme level, activity, and susceptibility to Parkinson's disease (PD). So, inhibition of LRRK2 may be beneficial to treatment of PD. Positron Emission Tomography (PET) is a non-invasive imaging technique, wildly used for assessing target occupancy/efficacy of drug in vivo. A CNS LRRK2 PET tracer supports development of LRRK2 inhibitor. But, is it feasible to develop a CNS LRRK2 PET tracer?

Methods: Hot saturation binding assay was done with human LRRK2 enzyme, and tissue homogenates prepared from various tissues, including brain and Kidney. In vitro autoradiography (ARG) was done with frozen tissue slices. [3H]A* and [3H]B* were synthesized in house. Non-displaceable binding (NDB) was defined using self-block.

Results: [3H]A and [3H]B showed displaceable and saturable binding to one site with very high affinities (Kd = 56pM and 57pM respectively), and large displaceable binding window to human WT full-length LRRK2 enzyme. However, in tissue homogenates, [3H]A showed minimal or no displaceable binding (rat, rhesus monkey and human CPu), while only very modest specific binding of [3H]B was observed. [3H]B bound to more than one site in rhesus CPu and cortex. The Kd values of high affinity binding site of [3H]B were 90pM (CPu) and 50pM (cortex) with the Bmax values of 0.3nM (wet tissue weight) for both tissues. [3H]B also showed modest binding in WT rat kidney homogenates, but not in LRRK2 KO rat kidney. In ARG study, [3H]A showed no displaceable binding in brain slices of rat, rhesus monkey, and human.

Conclusions: The data show [3H]A and [3H]B are radioligands with very high binding affinity to human WT full-length LRRK2. But in brain CPu homogenates of rat, rhesus monkey and human, [3H]A shows no specific binding, consistent with [3H]A ARG, which shows minimal specific binding in brain slices of rat, rhesus monkey and human. Only very modest specific binding of [3H]B is presented in CPu and cortex of rhesus monkey brain. The data demonstrate low binding site densities of [3H]A and [3H]B in brain tissues examined, indicating low feasibility to develop a CNS PET tracer for LRRK2.

References: *: the structures of compounds will be disclosed at presentation.

1502

Beta burst coupling across the motor circuit in patients with Parkinson's disease

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Objective: Here, we test the hypothesis that beta bursts not only involve dynamically elevated local synchronisation but also distributed coupling across circuit nodes, further restricting information coding capacity in patients with Parkinson's disease (PD).

Background: Exaggerated activity in the beta band (13-35Hz) is a hallmark of basal ganglia signals in patients with Parkinson's disease (PD). Beta activity however is not constantly elevated, but comes in bursts. In previous work we showed that the longer beta bursts are maintained, the more the local oscillatory synchronisation within the subthalamic nucleus increases, which is believed to limit the information coding capacity of the circuits. And indeed, a higher incidence of longer bursts is positively related with clinical impairment, while the opposite is true for short, probably more physiological bursts.

Methods: Local field potentials from the subthalamic nucleus and EEG from the motor cortex area were recorded in eight PD patients during temporary lead externalization during surgery for deep brain stimulation and overnight withdrawal of levodopa. Beta bursts were defined as periods exceeding the 75th percentile of signal amplitude and the coupling between bursts was considered on two temporal scales: % overlapping (OVL) of beta bursts over the order of hundreds of milliseconds and the synchronisation of successive beta cycles over shorter time periods, termed phase synchrony index (PSI).

Results: %OVL between STN and cortex and between the two STN was strikingly higher than expected by chance. Similarly, PSI was higher during bursts as opposed to non-bursts periods. In addition, %OVL was greater for long compared to short bursts. The increase in PSI during long compared to short burst was more subtle, although synchronisation on this time scale was clearly more sustained during long duration bursts.

Conclusions: Our results support the hypothesis of long range coupling of beta bursts between nodes in the basal ganglia-cortical network, which is greater during long as opposed to short duration beta bursts. Accordingly, we posit that episodes of simultaneously elevated coupling at multiple nodes in the basal ganglia-cortical circuit further limit information coding capacity and have a key impact upon motor function.

1515

Neuroprotective effect of exogenous melatonin on apoptosis of neuroadernergic neurons in a rat model of Parkinson's disease

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Objective: To investigate melatonin therapeutic potential effects in Parkinson's disease.

Background: Sleep disorders constitute major nonmotor features of Parkinson's disease (PD) that have a substantial effect on patients' quality of life and can be related to the progression of the neurodegenerative disease[1]. the etiology of sleep disorders in PD remains undefined, so, the assessment of the components of the circadian system, including melatonin, could give therapeutically valuable insight[2]. Our work focus on melatonin as a regulator of the sleep/wake cycle and also as an effective antioxidant and mitochondrial function protector. We investigate melatonin therapeutic potential effects in PD. The answer to this question impact deeply on sleep disorders treatment and also neuroprotection in PD.

Methods: In this study, we administered melatonin as an antioxidant factor and neuroprotective agent to prevent neural death. A Parkinson's disease model was induced in rats by subcutaneous injection of rotenone at the back of their necks. Melatonin was administered for 7 days, the count and the volume of the LC neurons examined due to stereology methods. The enzymatic test for GSH measurement of Caspase-3, C-Fos was done to assess the antioxidant property and apoptosis process and neural activity respectively. Immunohistochemistry of Anti-TH factors was done to assess the noradrenergic neurons and the Iba-1 test was also done to show the microglial migration.

Results: Melatonin leads to a reduction in the level of apoptotic factor Caspase-3 expression followed by PD. According to stereology analysis, the count of adrenergic neurons and the volume of the nucleus reduces in the administered-melatonin group. The apoptotic protein Caspase-3 reduces to prevent neural death.

Microglial migration to the LC occurs after neural death and the melatonin increases GSH levels in this group.

Conclusions: Melatonin with neuroprotective property can be used to treatment of adrenergic-depended sleep disorders and prevention of neural apoptosis.

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1518

Circadian modifications in Parkinson disease

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Objective: To observe the expression of circadian rhythm related genes Bmal1 and melatonin receptors in different brain regions of 6-OHDA-induced acute Parkinson's disease rats.

Background: More and more studies have found that there is a close relationship between dopamine system and circadian rhythm system. On the one hand, the key rate-limiting enzyme tyrosine hydroxylase that synthesizes dopamine is controlled by circadian rhythms, resulting in diurnal fluctuations in dopamine levels in the striatum. On the other hand, dopamine can pass through Dependent pathways regulate the expression of the clock gene and regulate the activity of BMAL1 / CLOCK dimers that play an important role in circadian rhythm mechanisms. These findings mean that the dopaminergic system and the circadian rhythm system play a role in the regulation of each other, both in the occurrence and development of Parkinson's disease plays a role, is a very interesting research direction.

Methods: Taking western blot to test Bmal1 and MT2 expression in both substantia nigra and corpus striatum of the rats. Using immunohistochemistry to test MT1 and MT2 expression in pineal gland of the rats.

Results: A significant circadian rhythm of Bmal1 and MT2 is found in the striatum of normal rats. Bmal1 is also found to be rhythmic in substantia nigra but with smaller amplitude. Comparing with the control group, the normal circadian rhythm of Bmal1 and MT2 expression is lost in Parkinson disease rats. A general decrease of Bmal1 and MT2 is found in Parkinson disease rats. MT2 expression is not rhythmic in pineal gland but MT1 is expressed rhythmically. MT1 is significantly increased in pineal gland in Parkinson disease rats in contrast with control group.

Conclusions: 1. A much larger amplitude of circadian rhythm is found in stratum comparing to substantia nigra. 2. A decrease of circadian amplitude is found in both substantia nigra and corpus striatum in Parkinson disease model rats comparing to that in the control group rats. A general decrease of Bmal1 and MT2 are found in both substantia nigra and corpus striatum in Parkinson model rats, indicating that circadian rhythm dysfunction is closely related to Parkinson’s disease. MT1 is increased in pineal gland in Parkinson disease rats. It may be due to the compensatory response of pineal gland to decrease circadian amplitude.

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1522

Total CSF alpha-synuclein is inversely associated with non-motor symptoms in a population of patients with Parkinson's Disease

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Objective: To evaluate the association between PD non-motor symptoms (NMS) and CSF levels of neurodegeneration-related proteins in a population of PD patients, aimed at identifying reliable biomarkers for NMS.

Background: NMS often precede motor signs (MS) and progress along the course of PD, leading to severe disability and poor quality of life in patients. Despite such clinical relevance, NMS still lack of effective therapies and reliable predictors. CSF proteins mirror brain pathological changes, thus representing useful biomarkers for diagnosis and prognostic clustering of patients with neurodegenerative diseases.

Methods: Demographic, biochemical and clinical data were prospectively collected from 46 PD patients and compared to 37 age/sex matched healthy controls (CTL). CSF quantitative analysis included total alpha-synuclein (a-syn), amyloid-beta-42 (Ab42), total and phosphorylated tau (t-tau, p-tau), Ab42/p-tau, p-tau/t-

tau, t-tau/a-syn+Ab42, p-tau/a-syn+Ab42 ratios. In PD group, clinical assessment consisted of MMSE, UPDRS pars 2-3, NMS scale (NMSS, total and subitems scores), levodopa equivalent dose (LED) calculation. Parametric and non-parametric tests were conducted to evaluate differences between the groups. The ROC curve analysis with cut-off point calculation was further run. Spearman' test and subsequent linear regression analysis (using age, disease duration and LED as covariates) were applied to test the association between biomarkers and clinical scores.

Results: a-syn, t-tau and p-tau/t-tau ratio levels were lower in PD than CTL, independently from age and gender. a-syn was inversely related to NMSS, either total or items 3-9 scores, independently from age, disease duration and LED.

Conclusions: In this cohort of PD patients, total a-syn CSF levels were inversely and independently related to NMS, in the absence of significant correlations with MS. We thus preliminarily suggest that CSF a-syn may reflect synuclein-related degeneration of non-dopaminergic neuronal networks, supporting the use as a biomarker to predict and/or monitor NMS burden.

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Effect of CDNF on non-motor symptoms like motivation, fear, stress and depression-like behavior

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Objective: The goal of this study was to characterize the effect of CDNF on motivation, stress and depression-like behavior, because most of the non-motor symptoms, which are related to Parkinson`s disease, are thought not to respond to dopaminergic treatment.

Background: Parkinson's disease (PD) is progressive neurodegenerative disorder characterized by the motor symptoms as tremor, muscular rigidity and slowness of movements. In addition to motor symptoms, there are non-motor symptoms, which are common in early PD. Non-motor features include mild cognitive impairment, loss of memory, sleep disturbances and pain. Non-motor symptoms can debilitate patients` activities and quality of life. Currently, no effective treatment for PD is yet available. We have discovered a protein and named it Cerebral Dopamine Neurotrophic Factor (CDNF) (1). CDNF is a secreted protein and a member of a novel conserved NTFs family, protecting and repairing mammalian dopaminergic neurons in vivo. CDNF regulates ER stress and UPR intracellularly, secreting from the cells to extracellular space during ER-stress. CDNF has a therapeutic potential in rodent and non-human primates models of PD. CDNF is a potent molecule to protect and restore function of DA neurons. In addition to the prevention of apoptosis, CDNF-treatment also reduces ER stress markers in experimental model of PD (2). It was observed that CDNF affects stressed neurons. Recent data demonstrate that CDNF is possibly able to reduce α-synuclein aggregates. Importantly, CDNF is a stable protein, which diffuses in brain tissue better than any other neurotrophic factor.

Methods: Adult female and male C57BL/6JRccHsd mice received intracerebral injection of CDNF of different doses or vehicle. Forced swim test is applied for assessment of depressive-like behavior. Fear Conditioning assessed contextual and cued memory formation. Moreover, Intellicage system was used for measurements of the motivation, memory and overall activity of animals.

Results: We show that single intracerebral injection of CDNF has an effect on expression of fear by decreasing freezing time in fear condition test. CDNF shows dose-depending decreasing tendency of depression-like behavior in Porsolt forced swimming test. Moreover, CDNF increases the level of motivation is increased. We see no positive effect on learning of näive animals. Interestingly that CDNF decreased the dopamine level in prefrontal cortex and in VTA. CDNF increases the number of c-Fos positive cells in comparison with vehicle.

Conclusions: CDNF has effects on non-motor symptoms and can be a potential new drug candidate for treating these symptoms, which are related to Parkinsonian patients.

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Temporal evolution of biomarkers from healthy ageing, isolated REM sleep behavior to early Parkinson's disease

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Objective: To study the temporal evolution of biomarkers of various modalities in healthy ageing controls (HC), the prodromal condition of isolated REM sleep behavior disorder (iRBD) and the overt motor Parkinson's disease (PD).

Background: At the time of PD diagnosis the neurodegenerative process of aggregating alpha-synuclein (aSyn) and neuronal loss is already advanced and the prodromal phase needs to be studied. iRBD represents a highly specific prodromal condition for the development of aSyn aggregation disorders with annual conversion rates to disease of about 6%. Biomarkers to understand the early progressing neurodegeneration and to objectively reflect the evolution of overt disease in the early phase are needed to support clinical trials with putative neuroprotective agents.

Methods: DeNoPa is a single center, longitudinal observational study to evaluate progressing neurodegeneration. Assessments at baseline and follow-up after 24 months included questionnaires on non-motor signs (NMS), cognitive testing, video-polysomnography (PSG), electrocardiogram (ECG), olfactory testing, magnetic resonance imaging with voxel based morphometry (VBM) and cerebrospinal fluid (CSF) measures (1).

Results: We investigated previously identified biomarkers for early PD in 34 iRBD subjects and compared these to 88 HC and 91 PD patients. Most biomarkers in the iRBD group feature between HC and the PD group. ECG frequency was elevated in PD, but normal in iRBD and HC. Other biomarkers already show abnormalities similar to PD such as the high NMS burden, decreased beta-amyloid 1-42 and total tau protein in CSF. There was also a trend towards more marked abnormalities in iRBD compared to the PD group, such as more severe hippocampal atrophy by VBM, more pronounced cognitive decline, visuospatial deficits and lower CSF aSyn.

Conclusions: In prodromal PD abnormalities in NMS, imaging and fluidic markers are already obviously pointing towards the development of overt disease. Based on these results iRBD may represent a prodromal state of various aSyn aggregation disorders and suggests development of a specific phenotype with more rapid hippocampal atrophy, lower CSF aSyn and early cognitive decline.

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1531

Characterization of the gastrointestinal dysfunction in mice overexpressing the human mutation (A53T) of alpha-synuclein

S. Diwakarla, R. Constable, O. Artaiz, D. Finkelstein, J. Berger, J. Furness (Melbourne, Australia)

Objective: The aim of this study was to determine if mice overexpressing the human mutation of alpha-synuclein develop gastrointestinal (GI) dysfunction, and to understand the time course of events in relation to central nervous system (CNS) dysfunction.

Background: GI dysfunction, such as prolonged GI transit time and constipation, has been associated with the pathological changes seen in the brain, and often precedes the onset of motor symptoms in Parkinson's disease (PD) by decades. A hallmark of PD is the aggregation of the protein alpha-synuclein in various regions of the brain, which has been strongly linked to nerve cell death. Aggregates of alpha-synuclein are also found in the enteric nervous system of PD patients and may be a cause of enteric neuron damage and subsequent GI dysfunction.

Methods: We performed a complete characterization of the GI phenotype in the A53T mouse model of PD, which overexpresses the human mutation of alpha-synuclein driven by the mouse prion promoter. GI

and motor function were monitored at one month intervals from 18-80 weeks. Constipation, colon motility, and GI transit were assessed using the fecal pellet output test, bead expulsion test, and whole-gut transit test, respectively. Motor deficits were monitored using the beam traversal test.

Results: When compared with WT mice, A53T mice developed progressive GI dysfunction from 58 weeks of age, which included reduced colon motility and whole gut transit ($P<0.05$). Interestingly, motor deficits were observed prior to the onset of GI symptoms in A53T mice from 32 weeks of age, indicating that in this mouse model CNS dysfunction precedes the manifestation of GI symptoms.

Conclusions: Mice overexpressing the human mutation of alpha-synuclein, driven by the mouse prion promoter, develop GI dysfunction after the onset of motor deficits. Although this model may not fully reproduce the human condition, it continues to serve as a useful tool to understand both the CNS and GI dysfunction associated with PD.

1544

α-synuclein propagation via olfactory pathway in non-human primate model

M. Sawamura, H. Onoe, N. Uemura, T. Isa, R. Takahashi (Kyoto, Japan)

Objective: Recent studies have revealed that intracerebral injection of synthetic α-synuclein (α-syn) fibrils into olfactory bulb (OB) of mice induced prion-like propagation of α-syn aggregates pathology. Here we sought to reveal the propagation fashion in non-human primates (NHP).

Background: Parkinson's disease (PD) is the neurodegenerative disease characterized by α-synuclein (α-syn) aggregates, called Lewy bodies. The α-syn aggregates are believed to propagate in brains in a prion-like fashion via two major pathways: the olfactory and vagal pathways. Recently, the common marmoset (Callithrix jacchus) has gathered a lot of attention in the field of neuroscience because of its useful characteristics as a non-human primate model. In this study, we inoculated α-syn fibrils in the olfactory bulb of a common marmoset and analyzed its pathological progression.

Methods: Recombinant full-length marmoset α-syn was purified and incubated with agitation for a week to generate α-syn fibrils. A two-year-old female common marmoset was anesthetized with ketamine and isoflurane/oxygen mixture. Then, 0.8ul of fibrils solution (4mg/ml in sterile PBS) was stereotaxically injected using glass capillary at two sites in the unilateral olfactory bulb (OB). Three months after α-syn fibrils injection, the marmoset was sacrificed and perfused with PBS followed by 4% PFA in PBS. Eight-μm coronal sections were made and immunostained using phosphorylated α-syn (p-α-syn), anti-ubiquitin (Ub) and anti-p62 antibodies.

Results: Widespread p-α-syn positive cells were observed in the ipsilateral OB, piriform cortex and amygdala, suggesting the spreading along with anatomically connected neurons. These pre-Lewy body-like aggregation were also positive for Ub and p62. Importantly, we observed very few p-α-syn pathology in the contralateral side. In addition, Mn-contrasted magnetic resonance imaging (MRI) revealed mild atrophy of ipsilateral OB.

Conclusions: We created a new non-human primate PD model triggered in olfactory bulb.

1560

The Effect of Sleep Deprivation on Short-Term Memory and Emotion of Rotenone Treated Zebrafish Model of Parkinson Disease

Y. Xiong, D. Lv, S. Wei, F. Wang, C. Liu (Suzhou, China)

Objective: We investigated the long-term consequences of sleep deprivation on short-term memory and emotion using a rotenone treated zebrafish model of Parkinson disease.

Background: Parkinson disease (PD) is the second most common neurodegenerative disorder in the world. Except for motor deficits, daily life of the patients is seriously affected by disturbances, cognitive impairment and emotional disorders. However, the effects of bad life habits such as stay up late on the patient's cognition and mood are not clear.

Methods: Wild-type male zebrafish aged at 4 months were treated with rotenone (2□g/L) for 4 weeks. Then rotenone treated zebrafish and the control fish were sleep deprived for 24 h. We evaluated the influence of sleep deprivation on motor function, short-term memory and emotional changes through locomotor activity, object discrimination task and light-dark box respectively. The effect of rotenone was verified through Western Blot. Dopaminergic systems were assessed using HPLC and Q-PCR.

Results: Our locomotor activity test showed no obvious difference between rotenone treated and control fish in the duration of freezing and swimming activity at a slow speed. However, the rotenone treated fish showed a reduction in swimming duration and distance traveled at a fast speed compared with control fish. But sleep deprivation had no effect on activity ability. Meanwhile, we found that TH expression reduced by 30% in rotenone treated fish. The object discrimination task exhibited that the short-term cognitive deficits of rotenone treated fish are more serious than the control fish after sleep deprivation. Light-dark box test showed that rotenone treated fish are more dysphoric than the control fish after sleep deprivation. Dopamine and DOPAC significantly reduced in rotenone treated fish compared with the control fish. However, the content of DOPAC recovered after sleep deprivation. The expression of DRD 2a, DRD 2b, DRD 2c and DRD 3 in rotenone treated zebrafish elevated compared with control group and sleep deprivation group. However, the rotenone treated zebrafish manifested a decrease level after sleep deprivation. DRD 1a and DRD 1b did not show any significant changes among the four groups.

Conclusions: Our findings suggest that after sleep deprivation, rotenone treated zebrafish may have a more severe damage on memory and emotional function, which may be related to the changes in the DA systems.

1581

Smooth muscle dysfunction in the gastrointestinal system in the 6-OHDA model of Parkinson´s Disease

P. Murillo, P. Aronsson, M. Winder, T. Carlsson (Gothenburg, Sweden)

Objective: To explore the connection between the central dopamine system and the autonomous nervous system in GI dysfunction in PD, using animal models.

Background: Gastrointestinal (GI) dysfunction in Parkinson´s disease (PD) is characterized by motility imbalance at different levels of the GI tract often producing symptoms such as constipation, which affects the PD patient´s quality of life. Denervation of the nigrostriatal dopaminergic system, using the 6-OHDA PD rat model, suggests that the GI impairment is affected by neurotransmitter alteration. However, the pathophysiological mechanisms linking PD to enteric dysmotility needs more investigation in order to find new treatment targets for this non-motor symptom.

Methods: Rats received 6-OHDA in the median forebrain bundle (MFB). Sham-operated animals and healthy animals were used as controls. Four weeks post-lesion, proximal colon were excised and brains were collected. Circular and longitudinal smooth muscle contraction was investigated in an organ bath setup using electrical field stimulation, direct receptor stimulation with cholinergic and purinergic agents.

Results: Preliminary results suggest that the magnitude of the circular and longitudinal smooth muscle contraction were reduced in the 6-OHDA animals in comparison with controls. However, when a combination of these two muscles is studied, an intact piece of the tube, the magnitude of the contraction seems to be enhanced.

Conclusions: These data suggests a discrepancy between the excitation-contraction mechanisms within the dissected tissues which may play a role in GI dysmotility in PD.

1600

Gastrointestinal Symptoms and Enteric Nerve Dysfunction in A53T Mice

R. McQuade, P. Rajasekhar, S. Diwakarla, R. Constable, D. Poole, J. Berger, D. Finkelstein, J. Furness (Melbourne, Australia)

Objective: To investigate the relationship between α synucleinopathy-induced enteric neuropathy and gastrointestinal dysfunction in A53T mice.

Background: Approximately 80-90% of PD patients suffer from GI symptoms including dysphagia (swallowing difficulty), gastroparesis (slowed stomach emptying) and chronic constipation. Importantly, these GI symptoms often precede the onset of motor deficits by decades (1). GI function is primarily controlled by the enteric nervous system (ENS), a subdivision of the autonomic nervous system, and its central nervous system (CNS) connections. Beginning in the oesophagus and extending down to the anus, the ENS is embedded in the lining of the GI tract and interacts with the CNS through parasympathetic (vagus and pelvic nerves) and sympathetic (prevertebral ganglia) connections to regulate contraction, relaxation, secretion and absorption throughout the GI tract. It is well established that changes at the level of the ENS have functional GI consequence (2). Whilst the identification of abnormalities in the ENS of PD sufferers was discovered over 30 years ago and these changes have been acknowledged as a key contributor in the manifestation of PD-induced GI dysfunction, the relationship between α synuclein aggregation, ENS deterioration and the onset of GI dysfunction remains unclear.

Methods: Male and female human α synuclein overexpressing transgenic (A53T) mice and WT littermate controls aged 7 months were euthanised by cervical dislocation and the entire colon was removed. Colons were arranged horizontally in organ-bath chambers and the contractile activity of each colon was video recorded and used to construct spatiotemporal maps using in-house edge detection software. Separate segments of colon tissue were loaded with a high-affinity Ca2+ indicator, Fluo-4 and optically probed via in situ calcium imaging to evaluate electrically excitable in the ENS.

Results: α-synuclein overexpressing transgenic mice (A53T) had significantly reduced colonic motor activity compared to WT mice, this loss was primarily due to a reduction in the number and proportion of short propagating contractions. Changes in colonic motor activity demonstrated in A53T mice corresponded with altered Ca2+ signalling at the level of the myenteric plexus.

Conclusions: Our research investigating motor patterns in isolated colon indicate that GI dysfunction in A53T human α synuclein overexpressing transgenic mice is intrinsically mediated by the ENS.

References: 1. Fasano A, Visanji NP, Liu LW, Lang AE, Pfeiffer RF. Gastrointestinal dysfunction in Parkinson's disease. The Lancet Neurology. 2015:625-39. 2. Wood JD. Enteric Nervous System: Neuropathic Gastrointestinal Motility. Digestive diseases and sciences. 2016:1-14.

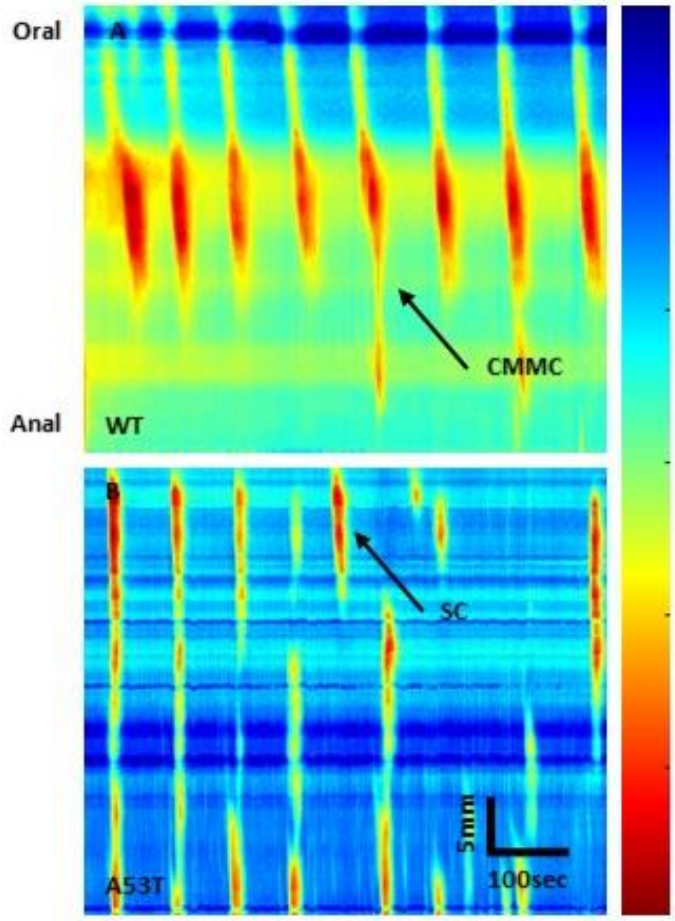

FIG. 1 (1600)

1607

Blockade D1-like and D2-like dopamine receptors within nucleus accumbens attenuate hyperalgesia responses in a 6-OHDA induced parkinson's disease rat model

L. Li, CJ. Mao, XQ. Zhang, F. Wang, CF. Liu (Su Zhou, China)

Objective: To investigate the role of blockade D1-like and D2-like dopamine receptors within nucleus accumbens attenuate hyperalgesia responses in a 6-OHDA induced parkinson's disease rat model.

Background: Pain is one of the most common non-motor symptoms in patients with Parkinson's disease (PD). It can occur before motor symptoms and is also one of the major causes of misdiagnosis of early PD. But the underlying mechanisms of pain in Parkinson's disease remain elusive.

Methods: Rotarod test and open field test were used to evaluate motor function. The von Frey test and radiant heat test were conducted to evaluate mechanical and thermal pain thresholds, respectively. Western blotting was used to evaluate the D1-like and D2-like dopamine receptors expression in the NAc. Immunohistochemistry was used to examine dopamine D1 receptor and D2 receptor NAc. HPLC was used to determine DA and DOPAC levels in NAc. D1 receptor and D2 receptor agonist or antagonist were microinjected into the NAc while their controls just received intraaccumbal saline at 0.5ul, respectively.

Results: The time on the rod was significantly reduced in 6-OHDA-treated rats in rotarod test. Both of mechanical and thremal nociceptive thresholds were reduced compared to saline-treated rats. The expression of the DA D1R and DA D2R were increased while DA D3R were no changes. DA concentration was reduced in the NAc. Furthermore, the administration of D1 receptor and D2 receptor antagonis (SCH-23390 and raclopride) significantly attenuated pain hypersensitivity, but the administration of agonist (SKF-38393

or quinpirole) or the selective dopamine reuptake inhibitor GBR-12090 had no effect on the pain hypersensitivity.

Conclusions: Our data suggested that the decreased DA contents, D1-like receptors and D2-like receptors within the NAc may be involved in hyperalgesia in the 6-OHDA-induced model of PD.

1612

Preclinical cardiovascular sympathoexcitatory effects of TD-9855, a novel norepinephrine transporter (NET) inhibitor in development for the treatment of symptomatic neurogenic orthostatic hypotension (nOH) in patients with primary autonomic failure

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Objective: TD-9855 is a novel NET inhibitor (1) being studied for the treatment of symptomatic nOH. The objective of the present study was to characterize the cardiovascular (CV) effects of TD-9855 in rats and relate them to plasma drug exposure and peripheral NET target engagement assessed through inhibition of tyramine pressor responses.

Background: nOH and its symptoms originate from sudden reduction in blood pressure that occurs upon standing and is caused by impaired activation of post-ganglionic sympathetic neurons innervating the vasculature. Augmentation of norepinephrine release at the vascular sympathetic neuroeffector junction, through inhibition of NET, is a potential approach for treating the symptoms of nOH in patients with primary autonomic failure (2).

Methods: Normotensive male Sprague Dawley rats were anesthetized with thiobutabarbital and instrumented to enable intravenous (IV) dosing and direct measurement of mean arterial pressure (MAP) and heart rate (HR). Following a stabilization period of 60 minutes, animals were dosed intraperitoneally with either vehicle or a single dose of TD-9855 (ranging from 0.01 – 30 mg/kg). The intrinsic CV effects of vehicle or TD-9855 were recorded for 25 mins. Each animal was subsequently challenged IV with bolus ascending doses of tyramine (ranging from 0.03 - 1 mg/kg) at 5 minute intervals. In a separate group of animals, blood samples were collected at 15 and 60 minutes post-TD-9855 dosing to assess drug exposure.

Results: TD-9855 produced dose-dependent increases in MAP and HR in anesthetized rats (peak changes from baseline were 13.2 mmHg and 28.1 bpm, respectively). At doses which evoked CV effects, TD-9855 also produced dose-dependent and complete inhibition of tyramine-induced pressor responses, consistent with NET target engagement. The plasma unbound TD-9855 concentration, at 60 mins post-dose, producing 50% inhibition of tyramine pressor responses was 0.86 nM (95% CI of 0.58 – 1.27) which is similar to the molecule's potency for rat native NET (IC50 = 1.3 nM) and human recombinant NET (IC50 =2.5 nM).

Conclusions: The findings of this preclinical study are consistent with TD-9855 facilitating sympathetic neurotransmission in cardiovascular tissues via a NET inhibitory mechanism and support clinical investigation of this molecule as a potential therapy to treat symptomatic nOH.

References: 1. Smith et al. (2015). Int. J. Neuropsychopharmacology, 18: 1-11. 2. Ramirez et al. (2014). Hypertension, 64:1235-40.

1652

The subthalamic stimulation inhibit bladder contraction by modulating local field potential and monoamine in the medial prefrontal cortex

T. Yamamoto, R. Sakakibara, T. Uchiyama, S. Kuwabara (Chiba, Japan)

Objective: We aimed to clarify the changes in neuronal activity (local field potential and levels of monoamine) of mPFC induced by STN-DBS with relation to bladder contraction using normal and PD model rats.

Background: The subthalamic nucleus deep brain stimulation (STN-DBS) is widely used for alleviating motor complications in the advanced stage of patients with Parkinson's disease (PD). Although lower urinary

tract symptoms (LUTS) such as overactive bladder (OAB) are also prevalent in advanced stage of PD, the efficacy of STN-DBS on LUTS are not well elucidated. The medial prefrontal cortex (mPFC) is known as higher micturition centre and receives output signal of basal ganglia which is highly modulated by STN-DBS. Therefore, STN-DBS might regulate bladder contraction by changing the activity of mPFC.

Methods: Experiments were performed under urethane anesthesia in normal and 6-hydroxydopamine hemi-lesioned PD model rats. STN-DBS was applied to the left STN, and bladder contractions were monitored simultaneously. Local field potential (LFP) in mPFC was recorded before, during and after STN-DBS (n=6: normal rats, n=6: PD rats). Extracellular fluid in mPFC was collected before, during, and after STN-DBS (n=5: normal rats, n=6: PD rats).Each experiments were performed separately. Spectral analysis of LFP for calculating beta power was performed, and the levels of monoamine were measured.

Results: STN-DBS significantly increased inter bladder contraction interval in normal and PD rats (Fig.1). The beta power in mPFC was significantly decreased during and after STN-DBS in normal and PD rats (Fig.2). The levels of levodopa, dopamine, serotonin and their metabolites in mPFC were significantly decreased during and after STN-DBS in PD rats, whereas the levels of serotonin and its metabolite and homovanillic acid (HVA) were significantly decreased after STN-DBS in normal rats (Fig.3).

Conclusions: STN-DBS could increase inter bladder contraction interval in normal and PD rats probably by changing the neural activity as evaluated by the beta power and monoamine levels in mPFC. The effect of STN-DBS on the levels of monoamine in mPFC was different between normal and PD rats.

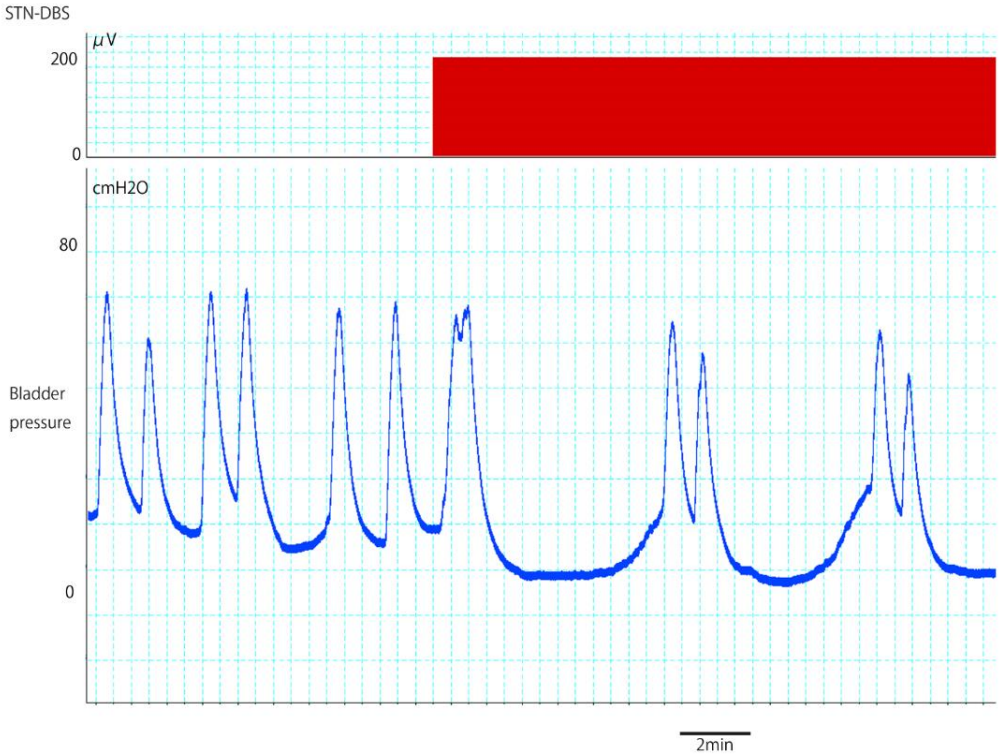


FIG. 1 (1652)

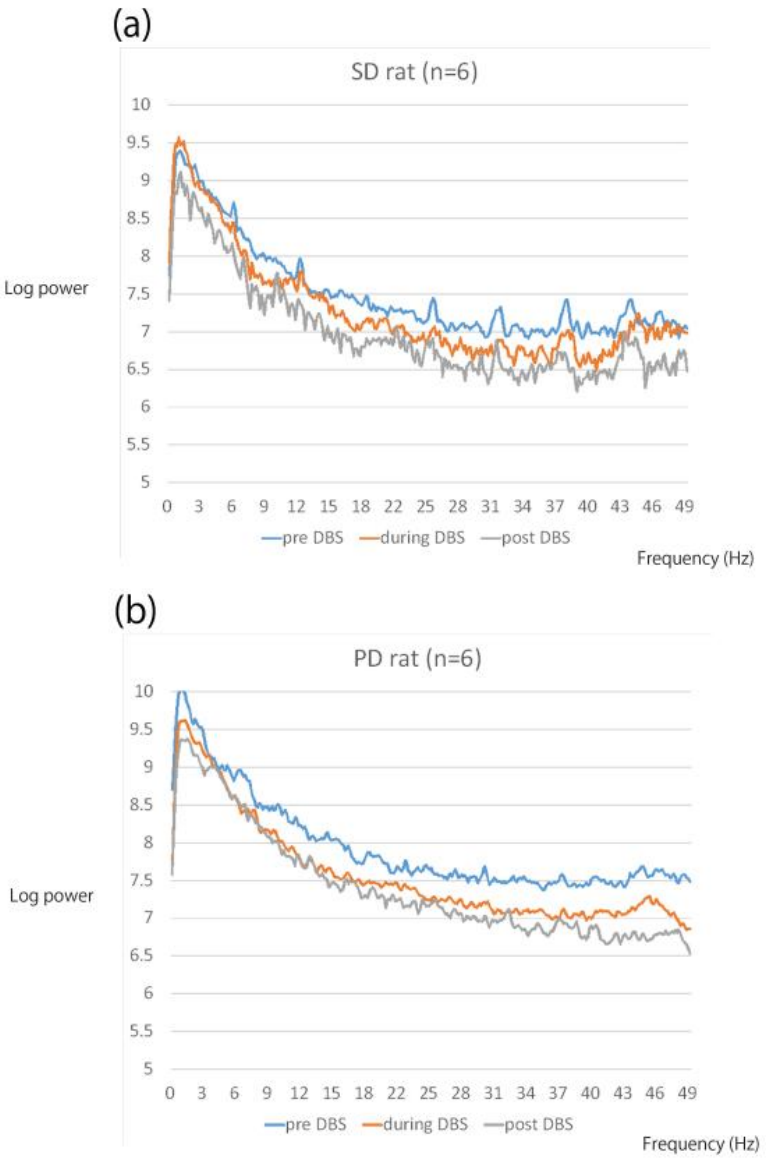
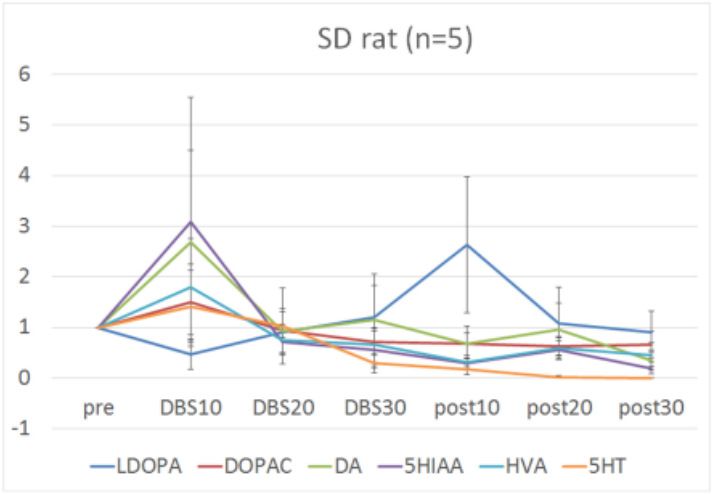

(a)
SD rat (n=6)
Log power
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
0 3 6 9 12 15 18 22 25 28 31 34 37 40 43 46 49
pre DBS
during DBS
post DBS
Frequency (Hz)
(b)
PD rat (n=6)
Log power
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
0 3 6 9 12 15 18 22 25 28 31 34 37 40 43 46 49
pre DBS
during DBS
post DBS
Frequency (Hz)

FIG. 2 (1652)

(a)



(b)

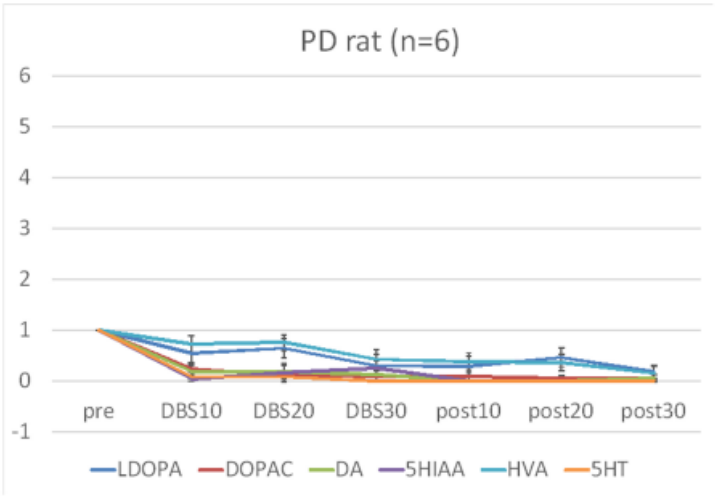


FIG. 3 (1652)

1657

Salivary Alpha-Synuclein and tau in Parkinson's Disease and Progressive Supranuclear Palsy

G. Vivacqua, A. Fabbrini, R. Mancinelli, D. Belvisi, G. Fabbrini, A. Suppa, A. Berardelli (Cambridge, United Kingdom)

Objective: The aim of this study is to measure alpha-synuclein (a-syn) total, a-syn oligomers (a-syn olig) and total tau protein concentration in the saliva of patients affected by PD and PSP in order to test whether salivary analysis can be used for differential diagnosis between PD and PSP.

Background: A-syn aggregation and tau deposition are respectively the pathological hallmarks of PD and PSP. In PD patients, we have detected lower salivary a-syn total and higher a-syn oligomers than healthy subjects and we have found that salivary a-syn total correlates with patients 'clinical scores (Vivacqua et al., 2016).

Methods: 100 PD patients and 20 patients affected by PSP were admitted to the study, together with 80 age and sex matched healthy subjects. Samples of saliva were collected following the protocol of previous study (Vivacqua et al., 2016). ELISA analysis was performed using three specific ELISA kits: SensoLyte 55550 for a-syn total, MyBioSource MBS043824 for a-syn olig and Life Technologies KHB0041 for total tau. Statistical significance was evaluated by Mann-Whitney U test. Spearmann Rank correlation coefficient was used for clinical correlations and Receiving Operating Analysis (ROC) was applied to determine sensitivity and specificity.

Results: A-syn total is significantly lower in PD patients confronting to healthy subjects, whereas a-syn olig is significantly higher ($p<0.05$). Conversely, in PSP patients salivary a-syn total is comparable to healthy subjects. Salivary tau total is significantly higher in both PD and PSP patients confronting to healthy subjects ($p<0.05$) but no significant differences were detected between PD and PSP patients ($p>0.05$). ROC analysis revealed that salivary a-syn total is able to differentiate PD patients from PSP patients with a sensitivity of 100% and a specificity of 96,51%. Conversely salivary tau total is able to distinguish PD and PSP patients from healthy subjects, but it cannot differentiate PD and PSP patients.

Conclusions: Decreased salivary a-syn total is likely to be specific of PD, whereas salivary tau total is significantly increased in both PD and PSP patients may leading to the occurrence of peripheral neuropathy involving the small autonomic fibers. Our data support salivary a-syn detection as a promising biomarker for differential diagnosis between PD and PSP.

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1658

Alteration of the fecal microbiota in Chinese patients with Parkinson's disease

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Objective: Emerging evidences suggest that gut microbiota dysbiosis plays a role in Parkinson's disease (PD). However, the alterations in fecal microbiome in Chinese PD patients remains unknown. This case-control study was conducted to explore fecal microbiota compositions in Chinese PD patients.

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor and non-motor symptoms that currently affects approximately 1.7% of people over 65 years of age in China. Emerging studies from North America and Europe using 16S ribosomal RNA (rRNA) gene sequencing have shown that patients with PD exhibit gut microbiota dysbiosis during the past three years. However, there are clearly major differences in the bacterial profiles of gut dysbiosis that have been reported to be associated with PD in different world populations. Increasing evidence indicates that the key factor in determining gut microbiota composition is diet, the spouses of PD patients could serve as controls to minimize variation caused by diets.

Methods: Microbiota communities in the feces of 45 patients and their healthy spouses were investigated using high-throughput Illumina Miseq sequencing targeting the V3-V4 region of 16S ribosomal RNA (rRNA) gene. The relationships between fecal microbiota and PD clinical characteristics were analyzed.

Results: The structure and richness of th fecal microbiota differed between PD patients and healthy controls [figure 1]. Genera Clostridium IV, Aquabacterium, Holdemania, Sphingomonas, Clostridium XVIII, Butyricicoccus and Anaerotruncus were enriched in the feces of PD patients after adjusting for age, gender, body mass index (BMI),
and constipation [table 1]. Furthermore, genera Escherichia/Shigella were negatively associated with disease duration. Genera Dorea and Phascolarctobacterium were negatively associated with Levodopa equivalent doses (LED). Among the non-motor symptoms (NMSs), genera Butyricicoccus and Clostridium XlVb were associated with cognitive impairment [figure 2].

Conclusions: Overall, we confirmed that gut microbiota dysbiosis occurs in Chinese patients with PD. The fecal microbiota was closely related to PD clinical characteristics.

References: 1. Yiwei Qian#, Xiaodong Yang#, Shaoqing Xu, Chunyan Wu, Yanyan Song, Nan Qin*, Shengdi Chen*, Qin Xiao*, Alteration of the fecal microbiota in Chinese patients with Parkinson's disease, Brain Behav Immun, 2018, doi: 10.1016/j.bbi.2018.02.016.

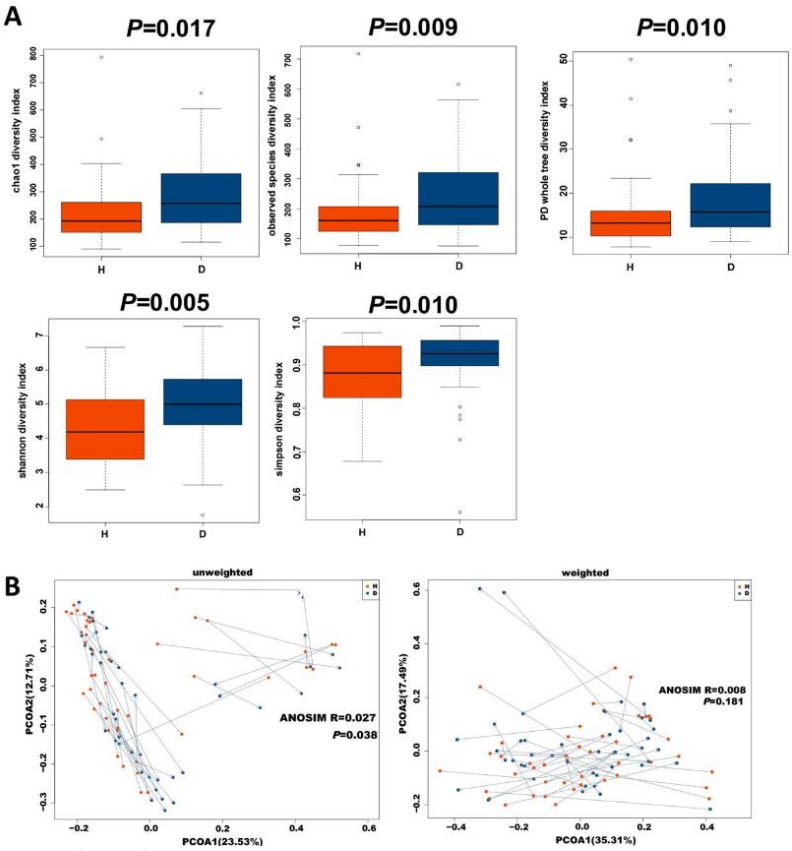

FIG. 1 (1658)

TABLE 1 (1658)

GLMs for fecal genera based on differences between the PD and healthy groups.

	Study group	Genus	b value	95% CI	*P* value
Feces	PD vs. Healthy	*Clostridium IV*	1.813	1.156–2.471	<0.0001
		Aquabacterium	1.515	0.811–2.219	<0.0001
		Holdemania	2.336	0.725–3.948	0.004
		Sphingomonas	0.770	0.236–1.304	0.005
		Clostridium_XVIII	1.082	0.152–2.012	0.03
		Butyricicoccus	0.600	0.044–1.156	0.034
		Anaerotruncus	1.013	0.013–2.013	0.047

Result of the GLMs for significant genera (sequence counts) based on the group factors (PD and healthy group) and possible confounding factors (age, gender, BMI and constipation) of 90 individuals.

The b value (positive number) indicated the taxa were associated with PD patients.

GLM, general linear model; CI, confidence interval; BMI, body mass index.

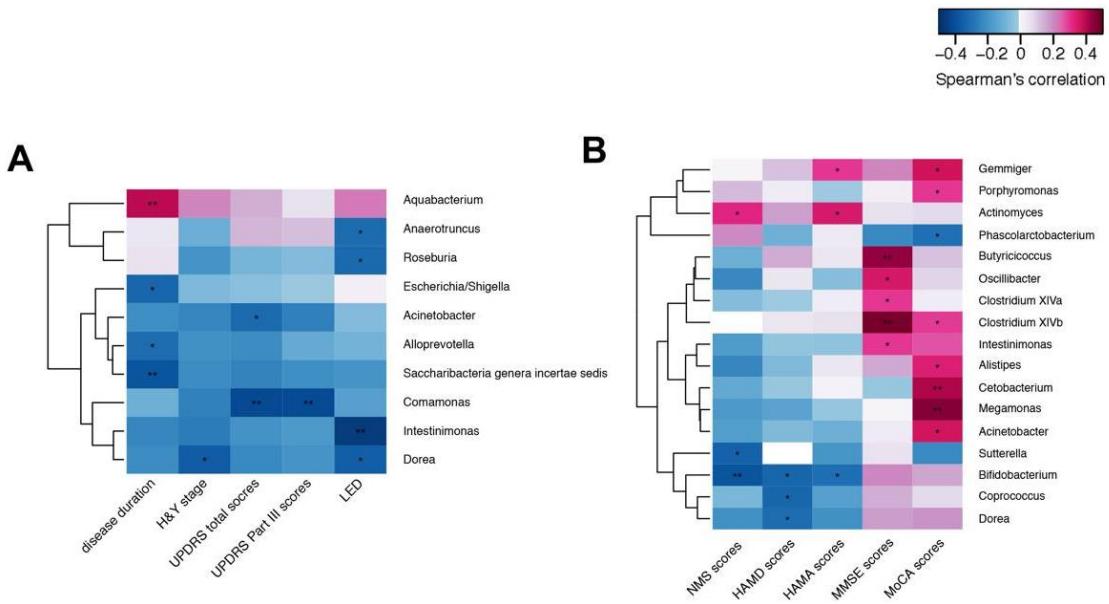

FIG. 2 (1658)

1659

Validation of protein phosphatases that regulate the LRRK2 phosphorylation cycle

M. Drouyer, M. Emanuele, R. Lefebvre, K. Cailliau-Maggio, A. Martoriati, T. Comptdaer, P. Semaille, E. Mutez, W. Sibran, N. Wolkoff, E. Lobbestael, M. Bolliger, V. Baekelandt, J. Nichols, MC. Chartier-Harlin, JM. Taymans (Lille, France)

Objective: To identify and validate candidate phosphatases regulating the LRRK2 phosphorylation cycle.

Background: Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) are one of the most common genetic causes of Parkinson's disease (PD). LRRK2 is a highly phosphorylated protein and one particular phosphosite cluster (S910-S935-S955-S973) is dephosphorylated in several disease mutant forms of the protein, in sporadic PD brain and after pharmacological LRRK2 kinase inhibition. This indicates that phosphatases play an important role in LRRK2 cellular regulation.

Methods: We first analyzed functional interaction of LRRK2 with candidate phosphatases by immunofluorescence microscopy in HEK293T cells, under basal and dephosphorylation conditions. We also injected Xenopus oocytes with combinations of PP2A phosphatase for a kinetic analysis of LRRK2 dephosphorylation. Expression of selected candidate LRRK2 phosphatases was modulated using lentiviral vector and CRISPR/Cas9 technology in SH-SY5Y neuroblastoma cells.

Results: A close association of LRRK2 with a subset of candidate catalytic and regulatory subunits of PP1 and PP2A was observed. LRRK2 preferentially interacts with PPP1CA as well as PPP2CA and PPP2CB. Our kinetic analysis in a physiological system shows an increase of dephosphorylation of LRRK2 at S935 when both catalytic and regulatory subunits of PP2A were injected compared to the injection of the catalytic subunit alone. CRISPR/Cas9 constructs used to alter expression of PP1 and PP2A subunits will be used to confirm phosphatase holoenzymes regulating LRRK2 phosphorylation.

Conclusions: Based on our previous work identifying several phosphatases in LRRK2 phosphoregulation and the present validation experiments, we confirm the importance of PP1 and PP2A for regulating LRRK2 phosphorylation. This study will guide future work exploring LRRK2 phosphorylation as both a disease and pharmacodynamics biomarker.

1660

New evidence for thalamic dysfunction in Parkinsonian rats

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Objective: Abnormal oscillatory activity within the cortico-basal ganglia (BG)-thalamo-cortical circuit has been observed in Parkinsonian state, especially of β and γ bands (βB and γB). Nevertheless, there is lack of information concerning a crucial BG-cortex interface, i.e. the motor thalamus (MTh)-nucleus reticularis thalami (NRT) loop. Therefore, in the present study we investigated MTh and NRT βB (SSN2018) and γB in animal model of Parkinson's disease (PD).

Background: Peculiar PD motor symptoms, as tremor or bradykinesia, are results of aberrant βB and γB in the cortico-BG-thalamo-cortical circuit. Since these bands could be considered valuable PD biomarkers, it is fundamental to better understand their dysfunction. βB is anti-kinetic, associated with rigidity and bradykinesia (13-30 Hz) and tremor (31-45 Hz), whilst γB is supposed to be mostly pro-kinetic, associated with dyskinesia. Even the opposite role in motor control, both are exaggerated in BG circuit. Surprisingly, there is no evidence of their activity in MTh-NRT loop, despite its key role as hub station for encoding motor information between BG and cortex.

Methods: We investigated the cortical, MTh and NRT low β (Lβ, 13-25 Hz), high β (Hβ, 25-40 Hz) and γ (60-90 Hz). In order to disentangle mechanisms belong to initial and late dopaminergic lack, we performed experiments in acute and chronic dopamine(DA)-denervated rats, by means of tetrodotoxin (TTX) or 6-hydroxydopamine (6-OHDA) infusion into the medial forebrain bundle (MFB), respectively.

Results: We observed that cortical βB and γB are strongly affected by chronic DA depletion, suggesting its involvement in all peculiar motor symptoms, whilst thalamic activities are affected also by acute DA depletion (TTX), meaning for the onset of early thalamic mechanisms basis for later cortical changes. Hence, the MTh βB decreased in acute state, whilst the γB changed in both acute and chronic states. In NRT, LβB decreased in both conditions, whilst Hβ and γB decreased in acute state, increasing then in chronic denervation.

Conclusions: The present results represent the first evidence underlying the crucial role of the MTh-NRT loop in PD. In particular, changes in MTh-NRT underlie thalamic role in acute state, thus leading to later cortical increase. The increase of γB could be considered as a basis for develop of dyskinesia triggered then by L-DOPA. Overall, we posit the MTh-NRT role to potentially consider it as target for new therapies.

References: SSN2018. 20th Swiss Society for Neuroscience Meeting. February 2018, Zurich (Switzerland). Poster H7.

1662

Effects of neural stem cell transplantation on the differentiation of dopaminergic neurons in the substantia nigra and changes to rotational behavior of Parkinson's disease rats

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Objective: To explore the effects of neural stem cells transplantation on the differentiation of substantia nigra dopaminergic neurons and on the rotational behavior in a rat model of Parkinson's disease.

Background: Parkinson's disease (PD) is second only to Alzheimer's disease as the most common progressive neurodegenerative disease. It is characterized by the degeneration of dopaminergic neurons in the substantia nigra coincident with progressive depletion of dopamine in the striatum. At present, the main treatments for PD are medication and functional surgery , however, the efficacy reduces sharply with increased complications as the period of treatment increases. The current treatments neither prevent the diffuse loss of neurons in the substantia nigra area of the brain area nor stop the progression of PD. The causes of PD are not fully elucidated. Patients are commonly diagnosed in the middle to late stages and already have severe symptoms when seeking medical help. Consequently, the currently symptom-targeting or modifying treatments available do not result in long-term optimal outcomes.

Methods: Adult rats were randomly split into three groups of five: control, sham and transplanted groups. 6-OHDA (2 μg/μl) was microinjected (8 μl) into the right medial forebrain bundle of sham and transplanted rats. The right substantia nigra of rats in the transplanted group were injected with 5 μl neural stem cells suspension (5×104 cells/μl), while an equal volume of PBS solution was injected in the sham group.

Results: Eight weeks after transplantation, Tyrosine hydroxylase-ir neurons presented slight somata and few dendrites, the cell counts, protein synthesis and mRNA expression were significantly decreased in both the sham and transplanted groups ($p<0.05$). However, compared with the sham group, the levels in the transplanted group increased ($p<0.05$). Two weeks after transplantation, the rotational behavior in the transplanted group significantly was improved compared with pre-transplantation; this was also significantly different in the eighth week ($p<0.05$). There were no significant improvements in the rotational behavior of the sham group.

Conclusions: Transplanted neural stem cells are able to differentiate into substantia nigral dopaminergic neurons and attenuate characteristic behaviors in rat model of Parkinson's disease.

1663

Bidirectional gut-to-brain and brain-to-gut propagation of α-synuclein pathology in non-human primates

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Objective: The prototypic synucleinopathy Parkinson's disease (PD) is hypothesized to spread out from the enteric nervous system (i.e. the gut) via the vagal nerve up to the central nervous system (Lionnet et al., 2017). Such popular hypothesis is supported by indirect clinical evidences and by experimental data showing gut-to-brain transfer of synucleinopathy using either viral vector delivery of synuclein or recombinant synuclein preformed fibrils.

Background: The aim of this study was to test the alternate hypothesis that synucleinopathy can indeed develop upward but also downward, i.e. from the gut to the brain but also from the brain to the gut.

Methods: To this end, we used our primate model of synucleinopathy obtained with administration of α-synuclein species contained in PD-derived Lewy bodies (LB) (Recasens et al., 2014). We examined in non-human primates, (i) if LB administration in the ventral wall of the stomach (n=5) leads to central nervous α-synuclein aggregation and possibly nigrostriatal degeneration and (ii) if LB administration in the striatum (n=6) might lead to synucleinopathy into the enteric nervous system. Two years after injection, extensive analysis was performed to assess qualitatively, quantitatively and spatially in the whole brain and in the enteric nervous system the extent and pattern of lesion as well as the occurrence of synucleinopathy using both biochemical and histochemical procedures.

Results: Enteric injection of LB in non-human primates results in enteric nervous system pathology and nigrostriatal lesion in keeping with the well-accepted hypothesis. However, striatum LB-injected animals, in addition to the expected nigrostriatal degeneration, showed also enteric ner¬vous system pathology at the stomach level.

Conclusions: This study establishes that α-synuclein species might move up and down the neural axis in non-human primates questioning (i) the hypothesis of a peripheral origin of synucleinopathies (ii) and the specificity of enteric nervous system as biomarker of early/presymptomatic PD.

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1667

Motivational effects on action selection and memory

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Objective: Eye movements provide important insights into the pathophysiology of neurological disorders. Here two paradigms were devised to quantify effects of motivation on motor control and decision making.

Background: The speed-accuracy trade-off can be overcome by reward, invigorating movements and improving response precision. In PD, characterised by a dopaminergic deficit, reduced reward sensitivity suggests a higher cost for controlling intrinsic neuronal noise(1). We asked whether motivational control costs extend to two tasks, limited by decision- and memory noise. Task1: Choosing between a larger number of options, responses become slower (Hick's law). If there is a cost for improving the effective signal-to-noise-ratio (SNR), motivation should result in reaction time (RT) scaling(2). Task2: Sequences held in working memory; Latencies increase and spatial precision falls with the length of the sequence keeping with lower SNR(3). If motivation reduces noise in spatial memory, an improvement should be observed with longer sequences of saccades, or longer memory retention intervals.

Methods: 22 healthy subjects performed both saccadic tasks recorded by an infrared eye tracker. Both involved three incentive levels (-50p, 0p, 50p). Reward/loss was calculated depending on the performance compared to their running average. Task1: Participants fixated centrally while either 2, 4 or 8 placeholders indicated possible target locations. A central arrow indicated the target location for the eye movement. Task2: While participants fixated centrally, either a single target or a sequence of 4 was shown. Participants were required to make the saccade or sequence of saccades to the remembered locations.

Results: Task1: RTs were faster with reward and loss. Amplitudes decreased with larger numbers of possible targets, and peak velocity was correspondingly slower. Reward increased velocity, but this was strongest when just two potential targets were shown. Task2:RTs were faster with reward. Latencies were longer, and error was larger with longer sequences. For single memory-guided saccades, accuracy was improved by both incentives. For the sequence of 4 saccades, both incentives increased speed but at the cost of diminished accuracy.

Conclusions: We demonstrate that motivation has characteristic effects on action selection and memory. Asymmetrical effects are observed where reward but not penalty invigorated saccade velocity, when choices were simple. RTs for choice and memory were improved by both incentives. Energisation of motor commands may depend on neural reward signals, such as dopamine, whereas noise reduction in decision-making may be valence-independent.

References: 1 Manohar et al 2015. 2 Holmes et al 2006. 3 Zingale et al 1987.

1672

No MPTP-induced behavioral deficits or Striatal field potential alterations in crossbreds of MPTP-susceptible and MPTP-resistant mice strains

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Objective: To evaluate the electrophysiological and motor behavioural correlates of strain-dependent differential susceptibility of mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the role of admixing thereon.

Background: Asian-Indians are less vulnerable to Parkinson's disease (PD) than the Caucasians. Interestingly their admixed population, the Anglo-Indians are at much lesser risk. We studied this phenomenon using MPTP-susceptible C57BL/6J mice, MPTP-resistant CD-1 and their crossbreds. The latter groups show differences in nigrostriatal cytomolecular features that may assign resistance to MPTP; which is likely in humans too.

Methods: Local field potentials (LFPs) from dorsal striatum were recorded from C57BL/6J, CD-1 and their F1 crossbred mice using deep steel electrodes. Saline controls and MPTP groups (15 mg/kg MPTP-HCl, intraperitoneal, 4 injections, 2h interval) were compared pre and post injection. Expression of nigral dopaminergic (DA) calbindin D-28K, a modulator of striatal activity through nigral projections was

evaluated using immunohistochemistry. Motor co-ordination was assessed using rotarod and grip strength parameters.

Results: Basal striatal LFPs were significantly higher in crossbreds than the parent strains. MPTP injection enhanced the activity in delta (0.5-4 Hz) and low beta (12-16 Hz) ranges in C57BL/6J. It caused a significant increase in CD-1 across all frequency bands up to high beta (0.5-30 Hz). Interestingly, in the crossbreds it remained unaltered in response to MPTP. Basal calbindin expression in nigral DA neurons of C57BL/6J was low, which depleted further upon MPTP injection, but the crossbreds showed upregulation of the already higher levels. MPTP impaired the rotarod performance and grip strength of only the C57BL/6J.

Conclusions: We provide the first evidence for increased striatal β-oscillations in MPTP mice model (C57BL/6J), as seen in PD patients. Higher power in CD-1 might be compensatory; as noted by others in monkeys during pre-symptomatic PD. Augmented basal striatal firing and maintenance in toxic conditions in crossbreds, both agree with our earlier cytomolecular observations of superior neuroprotection. Upregulated nigral calbindin might help maintain striatal activity through sustained dopamine release, by buffering MPTP mediated calcium overload in nigra. Thus, preserved motor behaviour despite MPTP-toxicity in CD-1 and the crossbreds may result from compensated striatal LFPs. We envisage similar mechanisms in human phenomenon of differential PD prevalence and its modulation in the admixed, as seen in the Anglo-Indians.

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Molecular Genetics and Functional Studies of LRRK2 Gene Variations in Parkinson's Disease

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Objective: To evaluate the function role of ROC domain in the brains of LRRK2 transgenic mice model and normal control mice.

Background: Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common genetic causes of Parkinson's disease (PD). LRRK2 gene encodes a large protein belongs to the family of ROCO proteins. LRRK2 mutations exhibit a clinical and pathological phenotype indistinguishable from idiopathic PD. Recent documents have shown the pathologic mutants of LRRK2 could reduce the rate of GTP hydrolysis and increase the kinase activity and GTP-binding activity and then cause cell death in vitro and in vivo. Notably, the process of cell death may be involved in several signalling pathway containing the ubiquitin-proteasome system, the autophagic-lysosomal pathway, intracellular trafficking, and mitochondrial dysfunction.

Methods: Mice overexpressing the mutated form of hLRRK2 (R1441G) and their age-matched controls were cardiac perfused and fixed for examination of mitochondria and lysosome morphology in dorsal striatum using electron microscopy. Protein-protein interaction was determined by immunoprecipitation and Western blotting. The GTPase activity was determined using an ATPase/GTPase ELIPA Biochem Kit.

Results: We shown the neurite degeneration was also investigated in substantia nigra of transgenic LRRK2 R144G homozygous mice. In addition, we found the enhancement of GTPase activity in the brain of LRRK2 R144G homozygous mice. Drp1 was increased in the mitochondrial fraction of transgenic LRRK2 R144G homozygous mice brain. Levels of proteins related to mitochondrial fusion, including Mfn1, Mfn2, and Opa1, were not altered. Furthermore, p62 and LC3II were also increased in brain tissue.

Conclusions: Our findings indicated that aberrant mitochondrial fission is causally associated with mitochondrial dysfunction and mitophage in brain. Thus, disruption of mitochondrial dynamics and mitophage may underlie the pathogenesis of LRRK2 R1441G mutation in PD.

1675

Role of microglia in the transmission of α-syn-containing exosomes

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Objective: we investigated the role of microglia in the transmission of α-syn-containing exosomes and offer a new insight into the mechanism of PD pathogenesis.

Background: Current understanding of the pathophysiology of Parkinson's disease (PD) suggests a key player of "prion like" propagation of alpha-synuclein (α-syn) in the pathogenesis.The idea that exosomes may be involved in the spreading of α-syn has recently gained considerable attention. Previous studies have shown that the activation of microglia is associated with the aggregation of α-syn in PD, suggesting an important role of microglia in clearing α-syn. In this study, we investigated the role of microglia in the transmission of α-syn-containing exosomes and offer a new insight into the mechanism of PD pathogenesis.

Methods: The exosomes-rich fraction was purified from the plasma of PD patients treated BV2 cells and healthy controls using a standard protocol of differential ultracentrifugation. Twenty sporadic mild-late stage PD patients were rolled. The concentrations of α-syn in the plasma-derived exosomes were determined by enzyme-linked immunosorbent assay (ELISA), thioflavin T-assay and western blot analysis. PKH26-labeled exosomes were stereotaxically injected into the unilateral striatum of 8-month-old mice. After 1 week of exposure, brain sections were double stained with the cell marker antibodies of brain cells. Plasma exosomes was added to the microglial cell lineBV2; then expression levels of α-syn, IBA1, LC3, P62 and Beclin1 were analyzed by western blotting(WB). Detection of TNF-α and IL-6 by ELISA were performed.

Results: The concentrations of total, oligomeric and monomeric α-syn in the plasma-derived exosomes were higher in patients with PD versus healthy controls.Intrastriatally infused exogenous exosomes are taken up by microglia and transported to substantianigra and cortex. Plasma exosomes preferentially target microglia and BV2 cells in vivo, rather than neuron or astrocyte. Plasma exosomes increased IBA1, Beclin1and P62 expression, the NO production ,TNF-α and IL-6 secretion. the ratio of the LC3 II/I .A significant elevation of oligomeric and monomeric α-syn protein level in exosomes-treated cells as compared to non-treated BV2 cells was observed.

Conclusions: Plasma exosomes derived from PD patients were highly selectively uptake by microglia in vivo and vitro.Moreover, exosomes with toxic a-syn can activate microglia cells, thereby inhibiting autophagy activity, reducing the scavenging activity of microglia cells, and further promoting the transmission of a-syn.

References: 1. Li, J.Y., et al., Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med, 2008. 14(5): p. 501-3. 2. Wang, S., et al., alpha-Synuclein, a chemoattractant, directs microglial migration via H2O2-dependent Lyn phosphorylation. Proc Natl Acad Sci USA, 2015. 112(15): p. E1926-35. 3. Stuendl, A., M. Kunadt, N. Kruse, C. Bartels, W. Moebius, K.M. Danzer, B. Mollenhauer, and A. Schneider, Induction of alpha-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain, 2016. 139(Pt 2): p. 481-94.

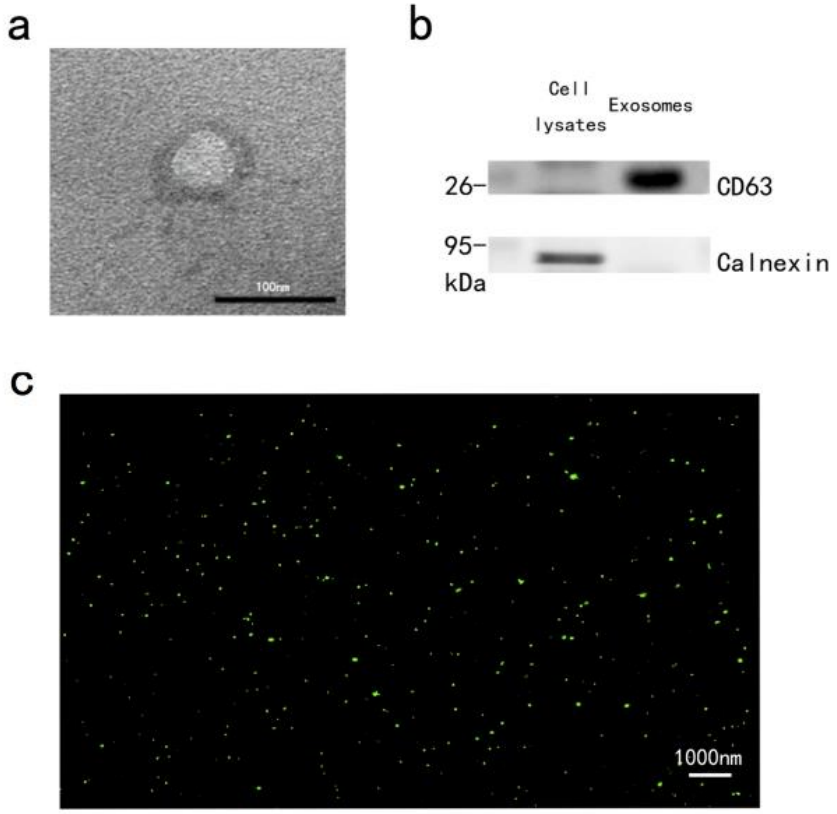

Figure 1. Characterization of exosomes isolated from human plasma. (a) Representative transmission electron microscopy observation of exosomes isolated from human plasma, Scale bar = 100 nm. (b) Western blot shows the presence of exosomal marker CD63 and the absence of negative marker Calnexin in plasma-derived exosomes. (c) Super-resolution imaging was performed to visualize the density of labeled-exosomes (green) in the exosome preparations.

FIG. 1 (1675)

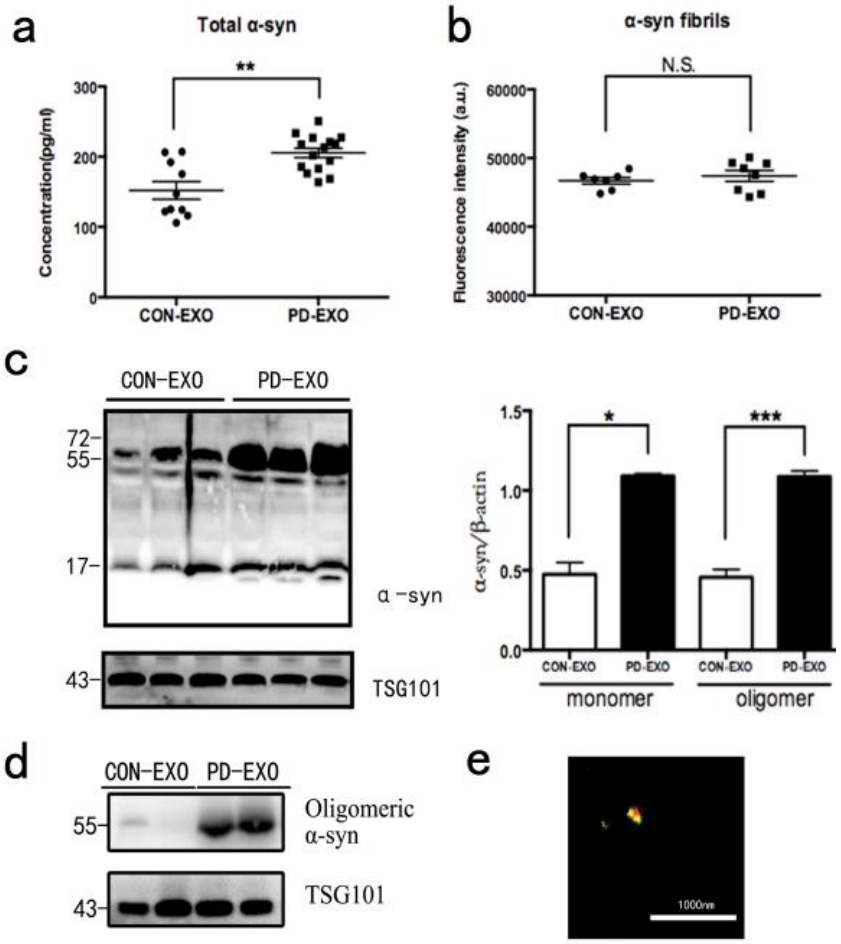

Figure 2. Levels of α-syn in plasma-derived exosomes of patients with PD (PD-EXO) and normal controls (CON-EXO). (a) Concentrations of exosomal total α-syn in plasma determined by ELISA. (b) ThT-assay of the tested samples containing exosomes. (c) Immunoblots of exosome proteins of PD patients and normal controls show the levels of oligomeric and monomeric α-syn expression. Bolts were probed for TSG101 as a loading control. (d) Representative immunoblots show oligomeric α-syn expression in the exosomes derived from plasma. Oligomeric human α-syn was probed using the Oligomer A11 Antibody. This antibody recognizes amino acid sequence-independent oligomers of α-syn. This antibody does not recognize monomers or mature fibers of α-syn. TSG101 is shown as a loading control. (e) Representative image of double immunolabeling analysis for α-syn (red) with either lipophilic dye DiD (green) in exosomes. DiD-labeled exosomes showed immune-reactivity against α-syn antibody. Images were captured on a super-resolution microscopy. Scale bar = 1000nm.

FIG. 2 (1675)

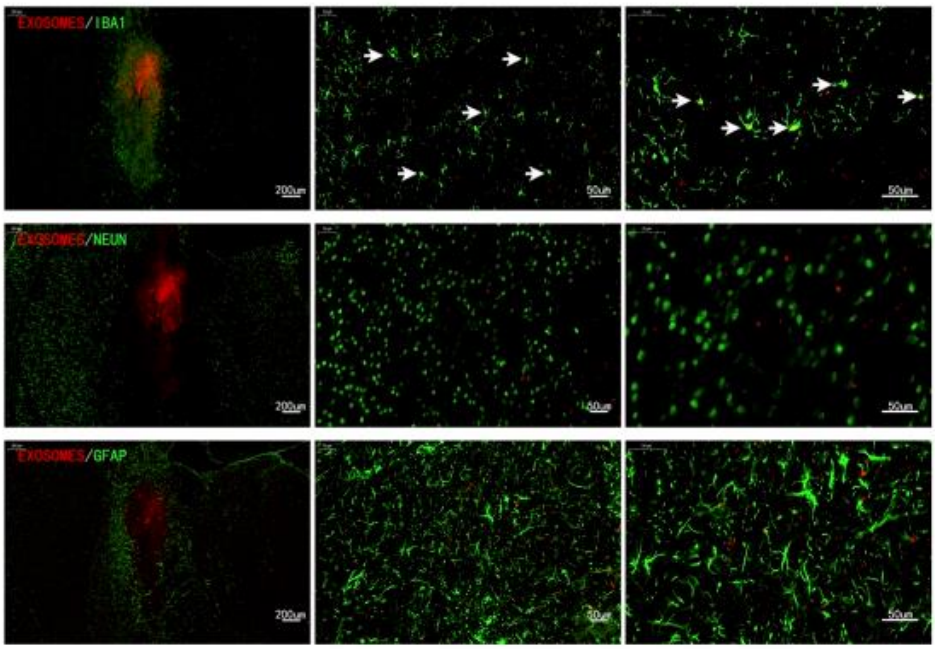

Figure 3. Plasma exosomes derived from PD patients preferentially target microglia in vivo, rather than neuron or astrocyte. The brain tissues were harvested from mice 1 week after unilateral intrastriatal injection of PKH26-labeled exosomes. Representative immunofluorescent images showed exosomes were co-localized with the marker of microglia IBA1 in the wild-type mouse brain. Labeled-exosomes were taken up by microglia surrounding the injection site. Neuron labeled with the neuronal marker NEUN and astrocyte labeled with the marker of astrocyte GFAP show phagocytosis deficit of exosomes surrounding the injection site.

FIG. 3 (1675)

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B lymphocytes may be protective in early Parkinson's disease and in an animal model of dopaminergic cell death

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Objective: To investigate B lymphocyte phenotype and function in human PD and in an animal model of disease.

Background: The clinical course in Parkinson's disease (PD) is highly heterogeneous with approximately half of patients progressing to dementia at 10 years, leading to increased risk of nursing home admission and dependence. We hypothesised that immune factors may play a role in determining disease course and progression, and focused on the potential contribution of B lymphocytes. B cells produce antibodies but can also regulate immune responses.

Methods: We recruited a cohort of 41 early stage PD patients, risk stratified into three groups based on cognitive measures and MAPT genotype (i) a group at high risk of rapid progression to dementia (ii) an intermediate group and (iii) a 'benign' group at low dementia risk. Controls were matched for age, gender and MAPT genotype. Peripheral blood B lymphocytes were phenotyped using flow cytometry.
We also used the 6-OHDA lesioned mouse model of PD and examined disease course by longitudinally, testing motor capacity, in mice lacking B lymphocytes (MT) mice and in wild type controls (C57bl/6). Dopaminergic cell counts and staining were compared.

Results: B lymphocytes were decreased in PD patients at high risk of progressing to early dementia compared to matched controls. Mice lacking peripheral B lymphocytes developed a worse motor phenotype,

had a slower recovery, and showed more extensive loss of midbrain dopaminergic cells in the brain than wild type controls.

Conclusions: Our data suggest that B cells play a protective role in PD, potentially via regulation of immune responses to aberrant forms of alpha synuclein or cell death.

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α-synuclein aggregation and propagation in marmoset brains deteriorates its motor function

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Objective: To confirm that α-synuclein aggregation and propagation in marmoset brains deteriorates its motor function.

Background: Recently, the α-synuclein amyloid fibril is thought to be a main component of Lewy pathology. The propagation of α-synuclein aggregates has been reported. The α-synuclein aggregates and Lewy bodies extracted from brain samples of Parkinson's disease (PD) patients were administered to mice brains. The aggregates were found in ipsilateral brains. As a result, α-synuclein fibril is thought to be pathogenesis of PD.

Methods: We prepared α-synuclein fibrils using recombinant α-synuclein derived from Escherichia coli. We inoculated α-synuclein fibrils to the left striatum of marmoset brains and evaluated its motor function and pathological changes. To detect marmoset motor function, we developed a novel multimodal system, MarmoDetector, for the automated 3D analysis of marmoset behavior under freely moving conditions. To evaluate pathological changes of marmoset brains, we performed immunohistochemical studies including phosphorylated α-synuclein pathology.

Results: Inoculating α-synuclein fibrils to marmoset brains caused α-synuclein aggregation and propagation. Three months after inoculation, we assessed behavioural changes using MarmoDetector for over several days, and marmoset motor activity was decreased by about 30% compared with that before inoculation. In pathological analysis, we also confirmed α-synuclein aggregation and propagation in marmoset brains.

Conclusions: We confirmed α-synuclein aggregation and propagation in marmoset brains and its changes influence on marmoset behaviors. These results suggest that marmoset symptoms correspond to the onset of PD. Our marmoset model can be a new primate model recapitulating the pathology of human PD.

1682

Pharmacological modulation of lysosomal glucocerebrosidase activity in the Thy1-aSYN mouse model of Parkinson's disease

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Objective: To study the pharmacological modulation of glucocerebrosidase activity in the Thy1-aSYN mouse model of Parkinson's disease (PD).

Background: PD is a complex neurodegenerative disorder characterized by loss of dopaminergic neurons, pathological accumulation of alpha-synuclein and chronic neuroinflammation. Carrying a mutated allele of GBA1 is one of the most frequent risk factors for PD. GBA1 encodes the enzyme glucocerebrosidase that breaks down glucocerebrosides into glucose and ceramide inside lysosomes. How glucocerebrosidase defects lead to increased PD risk is not elucidated. A reduction in glucocerebrosidase activity has been reported in the brain of PD patients, carrying or not a GBA1 mutation.

Methods: In this study, we used conduritol-beta-epoxyde (CBE), an irreversible competitive inhibitor of glucocerebrosidase, to model a decrease of glucocerebrosidase activity in transgenic mice overexpressing human wild-type alpha-synuclein under the Thy1 promoter (Thy1-aSYN mice).

Results: Daily administration of CBE (intraperitoneal injection, 100mg/kg/day) decreased glucocerebrosidase enzymatic activity and caused an accumulation of glucocerebrosides in the brain of Thy1-aSYN mice and wild-type littermates, as measured by fluorogenic assay and mass spectrometry, respectively. CBE-treated transgenic animals showed decreased performance in the adhesive removal test. CBE-treated transgenic animals also presented an increased number of CD11b+/CD45low (e.g. microglia) in the brain as numbered by multicolor flow cytometry.

Conclusions: Altogether, our data support that the reduction of glucocerebrosidase activity worsens sensory-motor deficits and chronic neuroinflammation in the Thy1-aSYN mouse model. Analyses of key immune factors and alpha-synuclein proteins will further determine whether enhancing lysosomal glucocerebrosidase enzymatic activity is an interesting pharmacological strategy against Parkinson's disease.

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Variable selection using machine-learning to identify new signatures of patient-derived aggregated α-synuclein-induced neurodegeneration in non-human primates

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Objective: Emerging evidence strongly suggests that α-synuclein, a major protein component of LB, may be responsible for the spreading of the pathological process within affected individuals. Recently, through an innovative strategy based on the purification of Lewy bodies (LB) containing aggregated α-synuclein from the substantia nigra pars compacta of PD patients, we assessed the prion-like properties of endogenous α-synuclein assemblies in wild-type mice and non-human primates (Recasens et al., Ann. Neurol. 2014).

Background: The pilot nature of the demonstration however called for a properly powered demonstration in non-human primates, which was the aim of this study, achieving in a large group of baboons (n=49).

Methods: After in vitro and in vivo (in wild-type mice) LB-induced toxicity validation, α-synuclein-containing extracts were injected bilaterally into the striatum (either a mixture of LB fractions or no-LB fractions derived from the same 3 PD patients, which contains soluble or finely granular a-synuclein but lacks large LB-linked a-synuclein aggregates.

Results: After a live phase of 2 years, extensive analysis was performed using biochemical and histochemical techniques in the whole brain. This study collected over 180 variables in each monkey. To overcome the roadblock associated to the "p > n" problem that occurs when the number of variables measured is greater than the sample size, we developed a multiple layer perceptron (MLP), i.e. an artificial neural network commonly used in machine learning. The performance of a given combination of variables was measured to predict the level of degeneration and extract meaningful variables. Variables were then sorted according to their occurrence in the top 1% of the best combinations.

Conclusions: This MLP allowed to identify two types of variables: the ones that reflect the actual neurodegeneration - the variables that describe the phenomenon to be explained - and the ones that might contribute to the pathogenic mechanism - the variables that could be useful to explain the phenomenon. Overall, this study using this unbiased methodology, confirmed highly-expected variables but, more importantly, also identified unexpected variables that appear to be excellent predictors for dopaminergic neurodegeneration.

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1684

Experimental rotenone model

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Objective: To evaluat rotene model of parkinsonian syndrome in rats.

Background: Rotenone is of highly lipophilic substances that can freely enter hematoencephalic barrier through and biological membranes. The mechanism of action of rotenone as mechanism MPTP actions associated with mitochondrial respiratory chain. The advantage of the method rotenone modeling Parkinson's disease is ability rotenone to provoke dopaminergic neurodegeneration, most similar in its symptoms and molecular biological characteristics to those of Parkinson's disease.

Methods: In order to simulate Parkinson's disease, rotenone is used primarily on cellular structures and embryological studies. In adult animals, primarily rats, rotenone used less frequently because of its low chemical stability in animal tissues and body fluids. rotenone administered by infusion, by micropumps, preferably intravenously.

Results: Behavior of animals studied at 3, 6 and 9 days of the experiment. Used a series of tests for the detection of extrapyramidal disorders, as well as test apomorphine verticalization. Apomorphine is administered at a dose of 1 mg / kg subcutaneously 5 min assess vertical activity, and after 10 min - postural instability. Also used unilateral introduction of rotenone (12 .mu.g in 0.5 .mu.l with Dimexidum a rate of 0.1 .mu.l / min.) in «medial forebrain bundle» by coordinates (AP: +0,2; L: ± 1,8; DV: 8 mm) stereotactic atlas.

Conclusions: Intranigralnoe single unilateral introduction of rotenone in small doses on today is available as one of the adequate models of parcinsonian syndrome of rats, which reproduces lengthy neurochemical and neuropathological changes similar to the changes in Parkinson diseases in the nigrostriatal system. Application of this method rotenone administration (doses of 3, 6 and 12 .mu.g) caused no change in peripheral organs for 4 weeks follow-up.

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1686

Nanoscale imaging reveals disorganization of the brain extracellular space in Lewy body-injected mice

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Objective: To explore the brain extracellular space and its matrix at a nanometric scale in a context of α-synuclein-induced neurodegeneration.

Background: The extracellular space (ECS) is a system of interconnected compartments constituted by a dynamic scaffold known as the extracellular matrix (ECM). In the brain, the ECS plays a key role in intercellular communication and spreading and clearance of disease-related molecules. In pathological conditions, the ECS volume is affected in response to inflammation and tissue remodeling after neuronal death and glial cell adaptations. Changes in the highly-hygroscopic hyaluronan (HA) network can dramatically modify the ECS volume. Lewy Bodies (LB), the hallmark of Parkinson's disease, are intraneuronal proteinaceous inclusions mainly constituted by misfolded species of α-synuclein. The ECS/ECM participate both in the spreading of toxic conformers of α-synuclein and in the regulation of inflammation, key events leading to the loss of dopaminergic neurons in the substantia nigra (SN).

Methods: We sought to explore the ECS/ECM changes in LB-injected mice, a unique parkinsonian model where LB fractions isolated from PD patients are inoculated in the SN, inducing a progressive PD-like neurodegeneration in the substantia nigra. High-pressure cryo-fixation of ventral midbrain acute slices and electron microscopy provided data about the ECS volume. ECS local viscosity was assessed by single-molecule tracking of single-walled carbon nanotubes (SWCNTs) by near-infrared microscopy. Finally, the hyaluronan matrix and microglial cells were analyzed by immunofluorescence.

Results: We detected a local ECS disorganization at nanoscale level in LB-injected mice. Pockets of increased local thickness, as well as decreased local viscosity were identified into the SN by Cryo-EM and SWCNTs tracking. We observed a substantial decrease of the HA network in LB-injected mice compared to controls, and engulfment of HA structures by activated microglia.

Conclusions: These results reveal a disorganization of the extracellular space in the SN of parkinsonian mice, as a result of decreased levels of hyaluronan and microglial activation. Our results provide insight on a crucial and underexplored component of the brain and adds to our understanding of the pathophysiology of PD.

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1687

Cell and animal models lacking RAB39B show biochemical and behavioural phenotypes that model aspects of Parkinson's disease

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Objective: To determine the pathogenic mechanisms underlying RAB39B-mediated Parkinson's disease (PD).

Background: PD is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra and presence of α-synuclein (αSN) aggregates. We previously demonstrated loss of function mutations in Ras Analog in Brain 39B (RAB39B) cause early onset PD, with dysregulation of αSN observed in vitro and in vivo [1]. However, the normal cellular role(s) of RAB39B, and how loss of function causes neurological disease, are currently unknown.

Methods: We utilised CRISPR/CAS9 genome editing to generate isogenic pluripotent stem cell (PSC) lines with deletion/knockout of RAB39B, in addition to PSC from RAB39B deletion patient fibroblasts. We also utilised CRISPR/CAS9 to generate Rab39b knockout (KO) C57BL/6 mice and investigated the temporal and spatial distribution of Rab39b mRNA and protein in the mouse brain by real-time PCR, in situ hybridisation and western blot analysis of micro-dissected brain.

Results: We successfully differentiated cortical and dopaminergic neurons from the isogenic and patient stem cell lines. Western blot analysis demonstrated a significant increase in steady-state levels of αSN in the mature RAB39B knockout neurons compared to controls. By two weeks of age steady-state levels of Rab39b were high in the hippocampus, cerebral cortex and substantia nigra. Rab39b-KO mice displayed sustained hind-limb clasping and behavioural analysis at three time points (11, 18 and 22 months) identified motor deficits. On the balance beam, knockout mice performed significantly worse than littermate controls, slipping more than five times as often at 22 months (Wildtype 2.3±1.0, n=6; Knockout 12.8±3.3, n=6, p=0.006). Similarly, there was a significant deficit recorded by accelerating rotarod at 22 months (Wildtype 169.2±17.6, n=6; Knockout 83.2±21.4, n=6, p=0.010).

Conclusions: We have generated unique models that recapitulate aspects of the human disease; these will be useful tools to determine the neuropathological mechanisms underlying RAB39B-mediated PD.

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1689

The increased α-syn expression inhibits exosomal secretion

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Objective: Exosomes, nano-sized extracellular vesicles, play an important role in many physiological processes in the central nervous system. Recently, we and others have demonstrated that exosomes mediate α-syn secretion. While exosomes may contribute to propagation of pathogenic proteins in neurodegenerative disorders, exosomal α-syn secretion can mitigate its accumulation in neurons. We hypothesized that exosome-mediated α-syn secretion is linked to α-syn accumulation in neurons.

Background: Pathological feature of Parkinson's disease (PD) is the formation of Lewy bodies/neurites of which the main component is α-synuclein (α-syn). Although extensively studied, the precise mechanism of α-syn accumulation remains elucidated.

Methods: We used α-syn-inducible human neuroglioma (H4) cells and dopaminergic (DA) neurons differentiated from induced pluripotent stem cells (iPSCs) taken from the patients with SNCA triplication. The number of exosomes was measured by nanoparticle tracking analysis. The formation of intraluminal vesicles (ILVs) at multivesicular endosomes (MVEs) was analyzed by the in vitro invagination assay. We developed pHLuorin-CD63, which allows us to quantify the fusion of MVEs with plasma membrane.

Results: When α-syn expression was induced in H4 cells, the number of exosomes released in the media was decreased. However, vesicles positive for CD63, a marker of MVEs, were increased in the cells. The increased α-syn levels did not alter ILV formation, but significantly decreased fusion of MVEs with the plasma membrane, suggesting that α-syn overexpression impairs the process of exosomal secretion. Finally, we confirmed the impaired exosome secretion in SNCA triplication DA neurons.

Conclusions: These results indicate that impaired exosomal secretion by increased α-syn expression may lead to further α-syn accumulation. Targeting this pathway may provide novel therapeutic benefits in PD and associated synucleinopathies.

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Tetrahydroisoquinoline Molecule Identified in Traditional Ayurveda Medicine Modulates Inflammation in MPTP Mice

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Objective: Potential ‘Anti-parkinsonian’ activity of a tetrahydroisoquinoline (TIQ) molecule of traditional ‘Ayurveda’ medicine was tested in a preclinical MPTP mice model for Parkinson’s disease (PD).

Background: PD remains incurable and current treatments are laden with adverse side-effects. Amongst multiple factors, inflammation emerges as one of the prominent potentiators of dopaminergic neurodegeneration in PD. Indian ancient Ayurveda could be a treasure box for PD treatment. Substantial preclinical evidence is essential to validate the efficacy of ‘Anti-parkinsonian Ayurveda’ formulations. A novel Ayurveda molecule 'TIQ' might modulate inflammation to protect in MPTP parkinsonism.

Methods: Adult C57/BL6 mice injected with MPTP neurotoxin (18 mg/kg dose, 4 times at 2 h intervals) served as a reliable animal model for dopaminergic neurotoxicity and inflammatory activation in PD. TIQ was gavaged (200 mg/kg body weight, bi-daily) for 2 -7 days post MPTP and mice were sacrificed by cervical dislocation. Control mice were saline injected or fed with TIQ. Striatal dopamine levels were detected by HPLC electrochemistry. Striatal tyrosine hydroxylase (TH), NADPH oxidase-4 (NOX-4), hemeoxygenase-1 (HO-1) and cyclooxygenase-2 (COX-2) expressions were analyzed by western blot chemiluminescence procedure.

Results: TIQ MPTP mice showed significant recovery of striatal dopamine levels and tyrosine hydroxylase expression 7 days post-MPTP intoxication. MPTP-induced elevation in COX-2 and NOX-4 expression on day 2 post MPTP was significantly attenuated with TIQ treatment. TIQ MPTP mice further showed induction of HO-1 compared to MPTP mice.

Conclusions: Ayurvedic TIQ demonstrates its potency as an ‘Anti-parkinsonian’ molecule by protecting against dopaminergic neurotoxicity in mice. MPTP-induced upregulation of COX-2 -a classical

inflammatory mediator, was significantly reduced with TIQ treatment. TIQ also attenuated MPTP-induced upregulation of NOX-4, a major oxidative marker and inflammation regulator. Further, a very potent antioxidant and anti-inflammatory molecule HO-1, was induced after TIQ treatment in the MPTP mice. Thus, TIQ may be a neuroprotective molecule in PD modulating neuroinflammatory oxidative mechanisms.

1693

Multisite Tissue and Biofluid Sampling for Alpha-Synuclein in Parkinson's Disease: The Systemic Synuclein Sampling Study (S4)

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Objective: To characterize the distribution of alpha-synuclein (aSyn) pathology in multiple tissues and body fluids within the same Parkinson's disease (PD) subjects and compared to healthy controls (HC).

Background: A major obstacle to development of PD therapeutics is the lack of objective disease measures. aSyn is a lead PD biomarker and multiple reports cite altered levels or forms of aSyn in different biofluids and tissues in PD and prodromal PD. However, there are conflicting reports regarding sensitivity and specificity of peripheral tissue aSyn as a diagnostic biomarker, and within-subject distribution of aSyn in different biofluids and tissues is not well described.

Methods: S4 is a multi-center, cross-sectional, observational study to evaluate aSyn pathology in multiple tissues and biofluids in PD and HC. Biopsies of skin, sigmoid colon, and submandibular gland were obtained. Biofluids collected include cerebrospinal fluid (CSF), saliva, and blood including DNA and RNA. Acquisition of all specimens occurred within a 120 day study window. All specimens were acquired and processed in a standardized manner. Paraffin-embedded tissue sections were stained on an automated Ventana platform with the 5C12 monoclonal antibody. Digital images of the slides were interpreted by neuropathologists blinded to diagnosis. aSyn pathology density on each slide was assessed semi-quantitatively. aSyn was measured in CSF, saliva, and blood compartments using the Biolegend total aSyn assay. Biofluid aSyn analysis accounted for hemoglobin values. Clinical assessments included MDS-UPDRS, MOCA, UPSIT, and dopamine transporter SPECT.

Results: The final sample consists of 60 PD (early, middle and late stage) and 21 HC. aSyn pathology was highly specific for a PD diagnosis in all tissue types; the submandibular gland demonstrated the highest sensitivity. Colonic tissue aSyn had poor sensitivity. In CSF, there were significantly lower levels of total aSyn in HC versus PD groups. Within subjects, preliminary analyses suggest that the measured aSyn biomarkers indicated progression of pathology in step with higher disease stage. Comprehensive analyses are ongoing and will be presented in full.

Conclusions: S4 will contribute to understanding of the distribution of aSyn in biological fluids and peripheral nervous system tissue, providing an assessment of the feasibility and utility of peripheral biomarkers for PD.

1696

Salivary Alpha-Synuclein a new tool for the diagnosis of Parkinson's disease?

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Objective: The aim of the PARKSYN study was to test the utility of salivary alpha-synuclein (a-syn) concentration in the differential diagnosis between Parkinson's disease (PD) and Drug-Induced Parkinsonism (DIP).

Background: A previous study found reduced total a-syn and increased a-syn oligomer concentrations in the saliva of PD patients. To date, no previous study has measured a-syn concentrations in DIP patients.

Methods: We collected salivary samples from 30 consecutive PD patients, 30 DIP patients and 30 age- and sex-matched controls from three centers. PD patients were older than 45 years, with PD diagnosis made within the last three years. DATscan was used to differentiate PD and DIP patients, using diminished striatal isotope binding to define the PD group. Clinical recordings of motor and non-motor signs were performed using MDS-UPDRS, UDysRS, PDNMS and MOCA tests. DATscan interpretation and a-syn measurements were made by operators blinded to the clinical group.

Results: In total, 39 subjects were analysed: 16 PD, 11 DIP and 12 controls. Asymmetric parkinsonian syndrome was more frequently found in the PD group compared to the DIP group. The salivary total a-syn, oligomeric a-syn and a-syn oligomeric/total ratio did not differ among PD, DIP and control groups (p=0.89; p=0.25; p=0.47, respectively).

Conclusions: Quantification of salivary a-syn is not a reliable marker for differentiating early PD and DIP.

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GBA haploinsufficiency accelerated alpha synuclein pathology with altered lipid metabolism in a premotor model of Parkinson's disease

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Objective: To make Parkinson's disease (PD) mice model and investigate the mechanism how glucocerebrosidase (gba) heterozygous mutations contribute to asyn pathology and development of PD.

Background: The precise pathomechanism of PD remains unclear, and the appropriate animal model is not available yet. Based on genetic findings from PD patients with asyn multiplication and GWAS in PD, we previously generated human asyn Bacterial Artificial Chromosome (BAC) transgenic (snca tg +/+) mice, but they did not show DA neurodegeneration. Recently, heterozygous mutations in gba gene were reported to be a strong risk factor for PD, but its precise in vivo mechanism remains unclear.

Methods: In the present study, we crossed snca tg +/+ mice with gba heterozygous knockout (gba +/-) mice to make PD mice model and investigated the mechanism how gba heterozygous mutations contribute to asyn pathology and development of PD.

Results: These double mutant (dm) mice express human asyn in a physiological manner by its native promoter. Although behavioral test did not detect motor phenotype of PD, the number of tyrosine hydroxylase (TH) positive cells in substantia nigra pars compacta (SNpc) was significantly decreased, and phosphorylated pathological asyn was accumulated in the early or vulnerable regions in PD. Analysis of lipid metabolism associated with GBA revealed that abnormal lipid accumulation only in dm mice. Moreover, the mild overexpression of asyn solely decreased GBA enzymatic activity in snca tg +/+ mice, and dm mice had tendency to have further decreased level of GBA enzymatic activity. Based on these results asyn accumulation and decreased GBA activity, even if gba is in heterozygous status, might make vicious bidirectional loop in vivo and contribute to PD pathology. Also, dm mice showed a defect in assembly of mitochondrial complex 1 protein, presumably contributing to mild DA cell loss in SNpc.

Conclusions: Dm mice of snca tg +/+ mice crossed with gba +/- mice can be mice model for premotor PD with DA neurodegeneration and pathological asyn accumulation. In addition, considering that the decreased GBA activity was reported in idiopathic PD brains and shown to be enhanced by increased asyn expression, to augment the GBA activity or decrease the level of asyn expression is a reasonable strategy for the treatment of PD with or without GBA mutations.

1699

Reduced vesicular storage of catacholamines enhance MPTP-induced death of dopaminergic neurons in locus coeruleus and olfactory bulb

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Objective: In this study, we employed heterozygous knockout (vmat2+/-) mice to investigate the influence of reduced vesicular storage of catacholamines on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-impaired dopaminergic neurons in locus coeruleus and olfactory bulb.

Background: Cytosolic dopamine (DA) is sequestered by VMAT2 into synaptic vesicle for consequent neurotransmission. Previous studies have shown that mice with a 95% genetic reduction in Vmat2 display motor and nonmotor features of PD and exhibit progressive nigrostriatal degeneration. In addition, reduced vesicular storage of catacholamines mice are more susceptible than WT littermates to MPTP- and amphetamine-induced nigrostriatal neurotoxicity.

Methods: Vmat2+/- mice were provided by professor Uhl GR and bred in SPF Laboratory Animal Center. Genotypes were confirmed by PCR of genomic DNA extracted from tail. 6- month-old Vmat2+/- and WT littermates were investigated in the study and injected MPTP (30mg/kg/day) for 7 days. Two weeks after the last injection, locomotor activity was assessed by total distance moved in the open field test and total time consumed in pole test. TH expression in dopaminergic neurons including substantia nigra, striatum, locus coeruleus and olfactory bulb were demonstrated by westernblot, immunohistochemistry staining and immunofluorescent staining. DA and its metabolites were analyzed via liquid chromatography with electrochemical detection.

Results: At 6 months old, total distance moved in the open field test and total time consumed in pole test were similar between WT littermates and Vmat2+/- group. In comparision with MPTP-treated WT mice, MPTP-treated vmat2+/- mice display a decrease in traveled distance and a increase in time spent in pole test. The number of TH positive cells in substantia nigra, striatum, locus coeruleus of vmat2+/- mice did not differ from WT mice. However, MPTP-treated vmat2+/- mice showed more obvious decrease in TH positive cells in substantia nigra, striatum and locus coeruleus. Remarkably, MPTP didn't induce decrease in TH positive cells in olfactory bulb in WT mice while MPTP-treated vmat2+/- mice did show a decrease in TH positive cells in olfactory bulb. In the striatum of vmat2+/- mice, we observed a decrease in DA and increase in DA metabolites in comparision with WT mice. MPTP treatment increased DOPAC:DA in both genotype but more obvious in vmat2+/-, indicating increased DA turn over by MPTP.

Conclusions: Our data demonstrated that Vmat2+/- mice are also more susceptible than WT littermates to MPTP-induced death of dopaminergic neurons in locus coeruleus and olfactory bulb except nigrustriatal system.

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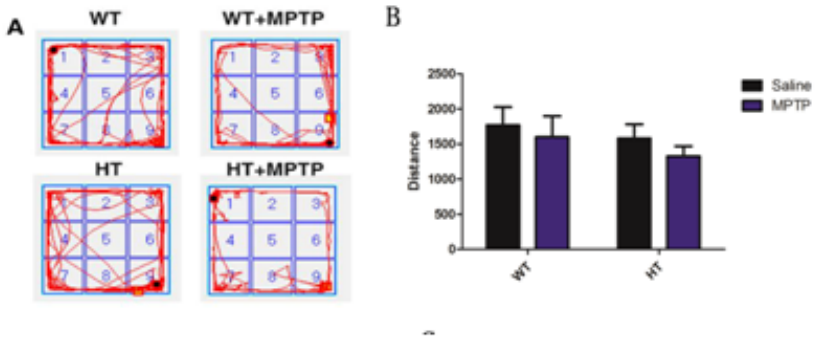

Figure1 .Reduced vesicular storage of catacholamines induce more obvious motor deficits and depressive symptoms. A-B. MPTP-treated vmat2 HT mice display a significant decrease in traveled distance in open-field test in comparision with WT littermates.

FIG. 1 (1699)

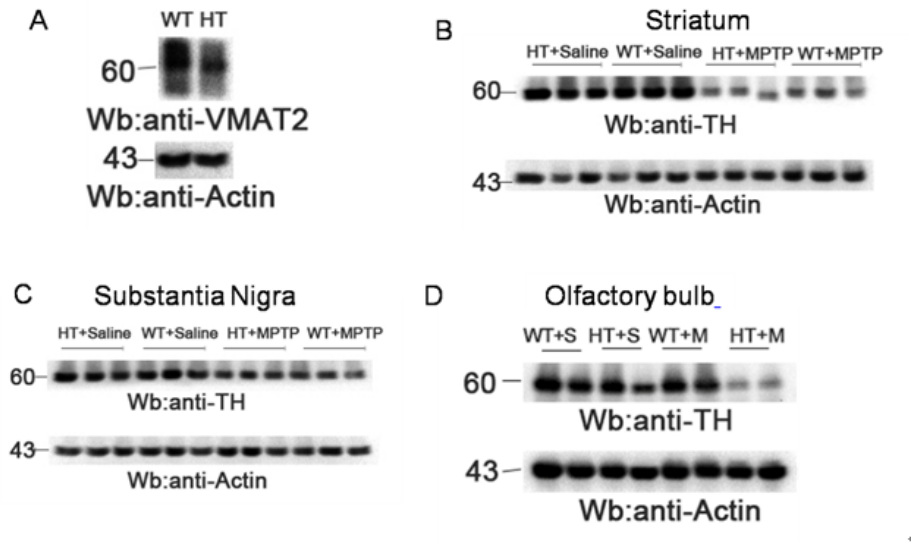

Figure2. Tyrosine hydroxylase expression of substantia nigra, striatum and olfactory bulb. A.Vmat2 expression in vmat2 HT mice and WT mice. B. The west blotting of TH in striatum before and after MPTP treatment. C. The west blotting of TH in s before and after MPTP treatment in substantia nigra. D. The west blotting of TH in s before and after MPTP treatment in olfactory bulb.

FIG. 2 (1699)

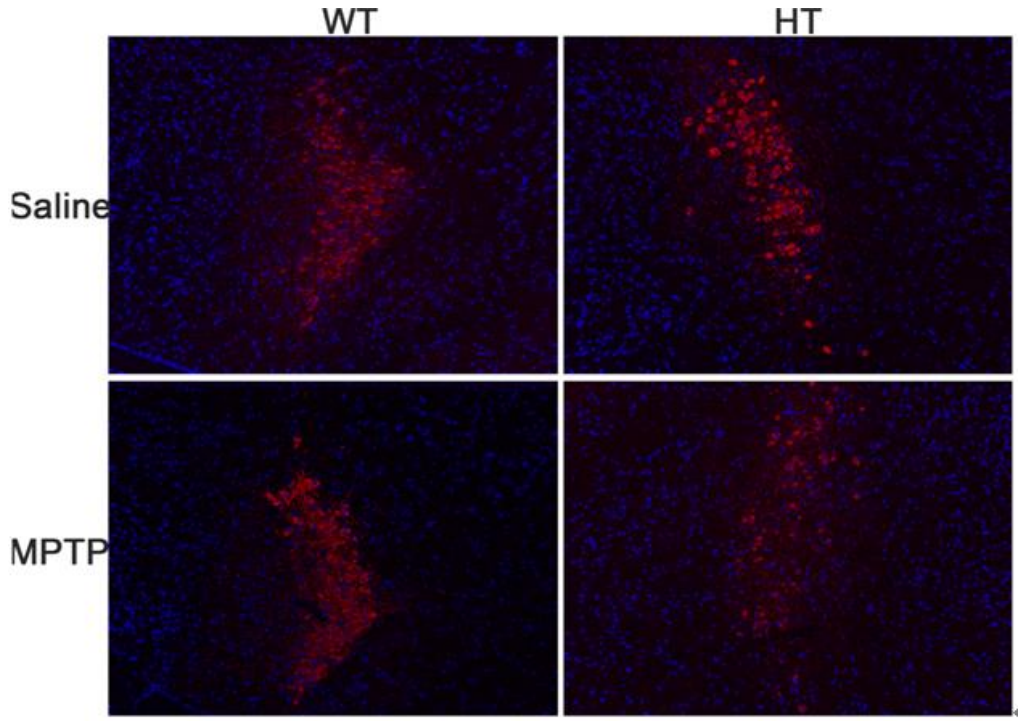

Figure3. Tyrosine hydroxylase immunostaining of locus coeruleus before and after MPTP treatment.

FIG. 3 (1699)

1707

Deletion of GBA2 in neuronopathic Gaucher's disease medaka can't rescue the phenotype

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Objective: This study was performed to determine the pathological role of GBA2 in the CNS in GD and GBA1-related PD.

Background: Parkinson's disease (PD) is one of the most prevalent neurodegenerative disorders characterized by tremor, rigidity, akinesia and postural instability. Recent genetic studies have identified that heterozygous mutations in the GBA1 gene are a strong risk factor for sporadic PD. Homozygous mutations in GBA1 gene are responsible for Gaucher's disease (GD), the common autosomal recessive lysosomal storage disease. We have reported that the GBA1 knock-out (KO) medaka can survive long enough for pathological analysis of disease progression in contrast to the perinatal death of GBA1 KO mice. These GBA1 KO medaka display abnormal swimming movement, non-selective neuronal loss, and α-synuclein accumulation in the brains. These GBA1 KO medaka are useful to investigate the mechanisms of α-synuclein accumulation in GD and GBA1-related PD. The non-lysosomal β-Glucosidase (GBA2), which is localized at the endoplasmic reticulum and Golgi apparatus, cleaves glucosylceramide to glucose and ceramide like GBA1. A recent study has reported that the deletion of GBA2 rescues the visceral manifestations in type1 GD mice model through reduction of sphingosine. To date, it remains unclear whether the deletion of GBA2 can modify the central nervous system (CNS) manifestations of GD.

Methods: We generated GBA2 KO medaka by clustered regularly interspaced short palindromic repeat (CRISPR) / CRISPR-associated nuclease (Cas9) system. Then, we crossed GBA2 KO medaka with GBA1 KO medaka to examine the genetic interaction between GBA1 and GBA2 in GD and GBA1-related PD.

Results: We have successfully generated GBA2 KO medaka by CRISPR / Cas9. GBA2 KO medaka lack both GBA2 enzymatic activity and protein expression. There were no differences in life span or the loss of

dopaminergic cells between GBA1-/-; GBA2+/+ and GBA1-/-; GBA2-/-. Moreover, the deletion of GBA2 in GBA1 KO medaka didn't reduce the amount of sphingosine, the presumptive culprit in the pathophysiology of GD, and increased the amount of α-synuclein in the brains.

Conclusions: The deletion of GBA2 in GBA1 KO medaka didn't reduce the amount of sphingosine or rescue the pathology of CNS. Moreover, the accumulation of α-synuclein was exacerbated by the deletion of GBA2 in GBA1 KO medaka.

1709

Activation of chaperone-mediated autophagy reduces oligomeric alpha-synuclein accumulation in LRRK2(R1441G) knockin mouse model of Parkinson's disease (PD)

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Objective: To determine age-dependent accumulation of oligomeric alpha-synuclein in the brain of aged LRRK2(R1441G) knockin mice; and to explore whether activation of chaperone-mediated autophagy (CMA) can attenuate accumulation of alpha-synuclein oligomers in mutant LRRK2 neurons.

Background: Progressive accumulation and propagation of misfolded alpha-synuclein in the aging brains is a key feature of Parkinson's disease (PD). Impaired alpha-synuclein degradation potentiates aggregation and formation of its toxic pre-fibrillar oligomers. Leucine-rich repeat kinase 2 (LRRK2) mutations form a common cause of familial PD which shares similar features to idiopathic PD. We previously generated a knockin mouse colony carrying the homozygous LRRK2(R1441G) mutation as an in vivo experimental model of PD [1,2]. LRRK2 mutation has shown to perturb lysosomal processes in chaperone-mediated autophagy (CMA) which degrades alpha-synuclein. Here we hypothesize that LRRK2 mutation contributes to alpha-synuclein aggregation into toxic oligomers via impairment of alpha-synuclein degradation.

Methods: Levels of oligomeric alpha-synuclein in mouse brain lysates at different ages were quantified using oligomer-specific ELISA and dot-blotting. Matured primary cortical neurons from mutant mice were treated with CMA activator, AR7, over 21 days and the amount of oligomers were quantified in both cell lysates and conditioned medium. A novel cell-based flow cytometry assay was developed to measure alpha-synuclein degradation.

Results: There is a greater age-dependent accumulation of oligomeric alpha-synuclein in striatum and cortex of aged LRRK2(R1441G) knockin mice as compared to age-matched wildtype mice. In mutant neurons, AR7 treatment significantly reduced intra- and extracellular alpha-synuclein oligomer levels in a dose-dependent manner. The amount of intracellular oligomers in mutant neurons treated with AR7 was markedly reduced by 44% compared to those without treatment.

Conclusions: Pathogenic LRRK2(R1441G) mutation together with aging resulted in age-dependent accumulation of alpha-synuclein oligomers in the brain. CMA activation to reduce accumulation of alpha-synuclein oligomers in neurons with age may be a viable therapeutic strategy to address LRRK2-associated synucleinopathies in PD.

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1715

Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism

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Objective: (1) To evaluate the differences in gut microbiota among Parkinson's disease (PD), atypical parkinsonism (i.e. multiple system atrophy [MSA] and progressive supranuclear palsy [PSP]) and healthy control subjects (HC). (2) To investigate whether specific microbiota taxa may act as modulators of disease progression and clinical phenotype.

Background: Recent evidences support the hypothesis that PD pathology originates into the gut and propagates to the brain by different pathophysiologic pathways. However, findings are heterogeneous probably due to the presence of several confounders.

Methods: We recruited patients with idiopathic PD (n=193, of whom 39 were de novo), PSP (n=22), MSA (n=22), and HC (n=113). Several confounders have been taken into account, including pharmacological therapy and dietary habits. Information on the type of lactation were also recorded. Early-onset PD (≤50 ys) were screened for mutations on parkin, DJ-1, PINK-1 genes.

Results: Despite simple non-parametric comparison of PD patients and HC showed several differences in relative taxa abundances, the number of significant comparisons was greatly reduced after adjusting for multiple confounders. We observed a constant effect of age on almost all abundances. The use of COMT inhibitors appeared to influence the level of several taxa.
Overall, PD patients had increased Verrucomicrobia, Christensenellaceae, Lactobacillaceae, and decreased Lachnospiraceae and Ruminococcaceae than HC. Reduced level of Lachnospiraceae was significant in all PD duration strata, while many of these differences were associated with disease progression. De novo PD differed from HC only by lower abundance in Lachnospiraceae. Compared to PD, Lachnospiraceae and Ruminococcaceae were not significantly lower in MSA, while in PSP cases other genera of Ruminococcaceae and Lactobacillaceae were higher and comparable, respectively. Increased Lactobacillaceae, Christensenellaceae, Verrucomicrobia and decreased Lachnospiraceae were associated with worse disease severity, including intellectual impairment and other non-motor symptoms, and axial features (gait disturbances and postural instability).

Conclusions: Gut microbiota may play a role in the pathogenesis of PD and act as modulators of individual differences in disease severity, especially non-dopaminergic features (cognitive functions and axial symptoms).

1716

Spatiotemporal patterns of direct and indirect pathway striatal projection neurons in mouse model of Parkinson's disease and dyskinesia

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Objective: To identify patterns of striatal projection neurons (SPN) activities that code for akinetic and dyskinetic movement disorders in a mouse model of hemiparkinsonism and dyskinesia.

Background: L-DOPA is the reference treatment of Parkinson's disease (PD). However, its chronic administration induces abnormal involuntary movements a condition termed L-DOPA-induced dyskinesia (LID). Among all the basal ganglia nuclei, the striatum is attributed a pivotal role in generating PD and LID. PD has been related to overactivity of the striatal projection neurons of the "indirect pathway" (iSPN) over the "direct pathway" striatal projections neurons (dSPN). Conversely, the phenomenology of LID has been broadly associated to a predominant role of dSPN over iSPN. However, the population dynamics of direct and indirect SPNs to the generation of these movement disorders is presently unknown.

Methods: Using in vivo calcium imaging we aimed to identify patterns of striatal SPN activities that code for akinetic and dyskinetic movement disorders in a mouse model of hemiparkinsonism and dyskinesia. We used D1-Cre and A2a-Cre transgenic mice to express the calcium indicator GCamP6f in dSPN and iSPN,

respectively. Mice were implanted with accelerometer devices to record motor activity while imaging striatal activity in vivo in an open field. We measured motor and neural activity in intact and hemiparkinsonian mice at baseline and after administration of dopamine D1-agonist.

Results: Preliminary data show that calcium activity of direct and indirect SPNs increases with movement and during movement initiation in intact and hemiparkinsonian mice. We found a significant correlation between body acceleration and calcium signal activity. Interestingly, this correlation was lost after administration of D1-agonist. Differences in the number and the amplitude of calcium events were observed between intact and hemiparkinsonian mice in both striatal populations, before and after D1-agonist.

Conclusions: These preliminary results show that both striatal populations are modulated by movement in healthy and parkinsonian conditions, and that administration of D1-agonist alters the association between striatal neural activity and movement. Furthermore, dopamine depletion and D1-agonist treatment seem to change the nature of SPN calcium signals.

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Characterization of the preclinical model parkinQ311X

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Objective: To characterize the preclinical model parkinQ311X. Because the PARK2-associated disease in humans is characterized by juvenile onset and slow progression, in order to clarify whether the parkinQ311X model mirrors the human disease, it is important to define whether this model shows early dysfunction or neurodegeneration of SNpc dopaminergic neurons.

Background: Mutations in the PARK2 gene encoding the protein parkin cause autosomal recessive juvenile parkinsonism (ARJP) characterized by early loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The development and characterization of preclinical models is essential to clarify the neurodegeneration mechanisms and lay the groundwork for pharmacological treatments. ParkinQ311X is a transgenic mouse model expressing a mutant parkin variant, found in ARJP patients, selectively in dopaminergic neurons. Previous studies have shown that parkinQ311X mice exhibit dopamine neuron loss at 16 months of age.

Methods: We analysed dopaminergic neuron number in the SNpc of parkinQ311X mice and littermate controls at 1 and 6 months of age by stereological cell count. We also analysed by biochemical assays the levels of previously identified parkin substrates in SNpc and striatum.

Results: We found that parkinQ311X mice display an early dopaminergic neuron loss starting from 6 months of age. We also found that the expression of parkinQ311X leads to dysregulation of some parkin substrates.

Conclusions: These data suggest that this mouse line recapitulates some features of ARJP and provides an appropriate model for the study of the neurodegenerative mechanisms and the screening of neuroprotective drugs.

1720

Neuroprotection effects of probiotics strains on a chronic MPTP-induced mouse model of Parkinson's disease

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Objective: To explore the effects of probiotics strains on the neuroinflammation and degeneration of dopaminergic neurons in chronic Parkinson's disease (PD) mice model.

Background: Microglia-mediated neuroinflammation has been implicated in the pathogenesis of PD. A growing body of evidences from both the clinical and animals' experiments suggested that gut microbiota dysbiosis play a key role in influencing the progress of PD. However, the potential role of the therapeutic

options probiotics, which pointed at modification the gut microbiota composition in the progress of PD is unknown.

Methods: Four-week-old C57BL/6N male mice were oral-administered the probiotics strains Lactobacillus acidophilus NCFM and Bifidobacterium lactis HN019 (109 CFU/day) or saline for 4 weeks prior to testing. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (25 mg/kg) or saline were injected twice a week for 5 weeks. Mice were also oral-administered the probiotics or saline each day during the 5 weeks of MPTP injection. At the end of MPTP injection, gastrointestinal (fecal pellet output) and motor symptoms (open-field and pole tests) was assessed. Pathology of α-synuclein, tyrosine hydroxylase (TH) neuron loss, inflammation and hallmarks of microglial phenotype from the middle brain were also analyzed. Fecal samples were collected and the DNA were extracted and performed 16S rRNA sequencing targeting V3-V4 region.

Results: The supplementary of probiotics could adjusted the changed-community of gut microbiota induced by MPTP, especially with decrease of genus prevotella, which was linked with chronic inflammatory conditions. Probiotics also significantly attenuated gastrointestinal and motor symptoms, along with the decrease of pro-inflammatory cytokines mRNA expression, TH-positive neuronal loss, TLR-2 expression and typical hallmarks of the pro-inflammatory (M1) activation of microglia. Additionally, probiotics upregulated the anti-inflammatory cytokines mRNA expression as well as increasing the expression of typical hallmarks of anti-inflammatory (M2) phenotype.

Conclusions: Overall, the supplementary of probiotics strains was essential for M2 microglia polarization by modifying gut microbiota, and therefore it has a potential role in the switch of microglia phenotypes to show neuroprotective effects in the pathogenesis of PD.

1723

Protective effects of Mucuna pruriens seed on rat brain: A therapeutic potential drug for Parkinson's disease

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Objective: Objective of present study was evaluate neuroprotective and antiparkinson effects of Mp seed extract on Parkinsons brain of rats.

Background: Parkinson's disease (PD) is a multifactorial disorder, which is neuropathologically identified by age-dependent neurodegeneration of dopaminergic neurons in the substantia nigra. PD is a neurodegenerative disorder for which no neurorestorative therapeutic treatment is currently available. Recently studies, Mucuna pruriens (Mp) seed has been shown to possess antiparkinson and neuroprotective effects in animal models of Parkinson's disease. Mp seed extract retard brain aging and help in regeneration of neural tissues besides producing anti-inflammatory and anti-oxidant and memory enhancing effect.

Methods: In present study 6-hydroxydopamine (6-OHDA) model of PD rat (n=8) were used. The symptoms of PD such as tremors, akinesia, rigidity, catalepsy, and vacuous chewing movements (VCMs) were evaluated. The methanolic extract of Mp seed was administered at doses of 200 mg and 500 mg/kg body weight followed by stress. The combination of L-dopa and carbidopa was used as a standard drug. Behavioral studies such as locomotor activity and grip strength were determined, and oxidative stress was evaluated in rat brain with biochemical and histopathological study. ANOVA was used followed by post hoc Turkey test.

Results: Animal exposed to stress showed significant decrease in superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and total protein. This was accompanied by simultaneous increase in thiobarbituric acid reactive substances - TBARS level. Treatment with Mp seed had no significant but moderate effect on antioxidant enzyme (SOD and CAT). Pretreatment with Mp seed dose (200 and 500 mg/kg) significantly reduced the intensity of muscular rigidity, duration of catalepsy, akinesia, the number of tremors, and increase fighting behavior. The locomotor activity and grip strength were significantly increased by Mp seed extract treatment. Treatment with Mp seed extract significantly reduced LPO level and restored the defensive antioxidant enzymes SOD and CAT in rat brain.

Conclusions: Present study evidences that oral administration of alcoholic extract of Mp extract have shown antiparkinson , neuroprotective and anti-aging effects in animal models of Parkinson's disease. Mp seed extract appreciably improve the neuroinflammatory processes and also restore biochemical and behavioral parameters.

1726

PrP as a receptor of alpha-synuclein in the pancreas of patients with synucleinopathies

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Objective: We have investigated whether the cellular prion protein interacts with alpha-synuclein in pancreatic cells of patients with synucleinopathies.

Background: Neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease are characterized by the progressive dysfunction and death of some nerve cells. Specific protein aggregates constitute the defining neuropathological characteristics of these diesases. One of the key events in the pathogenesis of neurodegenerative diseases is the ability of the amyloidogenic proteins to enter healthy cells and enhance the conversion of the endogenous protein into the aggregation-prone form. In Parkinson's disease, α-synuclein fibrils deposit inside neurons and can be released and taken up by other cells. This fact could cause the spreading of the pathology and the neurodegeneration.

Methods: We studied pancreatic tissue from 39 subjects diagnosed with Parkinson's disease, Lewy body Dementia or incidental Lewy bodies disease, as well as that from 86 neurologically asymptomatic subjects. We studied the pancreatic tissue to assess the accumulation of PrP. Moreover, we performed proximity ligation assays to assess whether this protein interact in the pancreas of these subjects with the alpha-synuclein. We designed two specific PLA assays to detect the interaction between PrP and both the C and N terminal regions of the α-synuclein. Furthermore, we designed a specific PLA assay to detect the interaction between PrP and phosphorylated α-synuclein. Recently it has been shown that PrPC on the cell surface promotes the uptake of different fibrillar forms of α-synuclein through a direct binding by its N-terminal domain.

Results: We found a significant increase in cytoplasmic PRP expression in pancreatic β cells of subjects with synucleinopathies compared with neurologically asymptomatic subjects. Furthermore, we found a PrP/α-synuclein interaction in patients with phosphorylated α-synuclein pancreatic inclusions. This interaction also occurred between the PrP and the phosphorylated α-synuclein.

Conclusions: Our study shows for the first time, histological evidence of the interaction between PrPc and α-synuclein in pancreatic beta-cells of subjects with synucleinopathies. Although further research is needed, our results are in line with previous reports highlighting that the interaction between PrPc and α-synuclein, could lead to α-synuclein fibrils internalization. This process may have therapeutical applications, that will be further discussed.

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1728

Effects of Glial Heme Oxygenase-1 on Neuronal Alpha-Synuclein in the GFAP.HMOX1 Mouse Model of Parkinson's Disease

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Objective: To determine whether astroglial HO-1 transduces environmental and endogenous stressors into patterns of neural damage which promote the toxicity of neuronal alpha-synuclein.

Background: The product of the stressor-inducible HMOX1 gene, heme oxygenase-1 (HO-1) is highly overexpressed in astrocytes of the substantia nigra in patients with idiopathic Parkinson's disease (PD) [1]. There is considerable evidence implicating HO-1 in the pathogenesis of PD, and overexpression of astroglial HMOX1 in vitro to levels seen in post-mortem PD brain promotes pathological iron deposition, oxidative

stress, mitochondrial damage and macroautophagy characteristic of the human disorder. We recently engineered conditional GFAP.HMOX1 transgenic mice that selectively overexpress human HO-1 in astrocytes [2]. Transgene expression in these mice between 8.5 and 19 months of age results in a parkinsonian phenotype characterized by oxidative stress; basal ganglia siderosis; mitochondrial damage; nigrostriatal hypodopaminergia associated with locomotor incoordination and stereotypy; and overproduction of alpha-synuclein mRNA and protein [2,3]. Alpha-synuclein is a key player in PD pathogenesis and a major constituent of hallmark Lewy pathology. While precise mechanisms of abnormal alpha-synuclein aggregation remain disputed, there is fair consensus implicating oxidative reactions in this process [4,5].

Methods: Primary astrocytes and neurons were isolated from homozygous GFAP.HMOX1 or WT matings on postnatal day 1 [2]. RT-qPCR was used to measure gene expression levels and Western blots were used to measure protein levels [2,3].

Results: Primary WT neurons co-cultured with GFAP.HMOX1 astrocytes exhibit enhanced protein oxidation, mitophagy, and apoptosis, aberrant expression of genes regulating the dopaminergic phenotype and imbalance in genes regulating mitochondrial biogenesis. The latter abnormalities were abrogated by siRNA knock-down of alpha-synuclein, implicating alpha-synuclein as a key mediator of HO-1's neurodystrophic effects. We also identified two microRNAs (miRNA) that negatively regulate alpha-synuclein in GFAP.HMOX1 mice, namely miR-153 and miR-223.

Conclusions: Taken together, HO-1 downregulates miR-153 and miR-223 which in turn upregulates alpha-synuclein in the GFAP.HMOX1 mouse brain (Fig.1). These results highlight the importance of alpha-synuclein as a therapeutic target, potentially via miR-153 or miR-223, in the treatment of PD.

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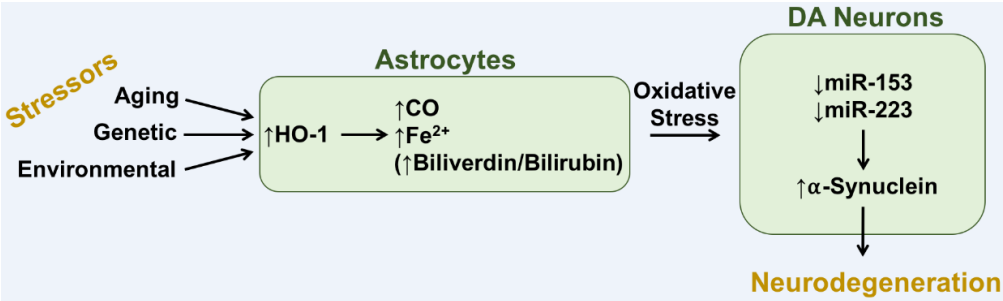

Figure 1. Working model of interaction between HO-1 and α-synuclein in PD.

FIG. 1 (1728)

1731

Enhancing protein palmitoylation is protective in alpha-synuclein dependent cytotoxicity

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Objective: To determine if enhancement of protein palmitoylation benefits alpha-synuclein (aS) dependent vesicular trafficking defects and cytotoxicity.

Background: Recent studies suggest that vesicular trafficking defects caused by aS accumulation play a key role in PD. The mechanism is unknown. Interestingly, palmitoylation, post-translational addition of the

fatty acid palmitate to cysteines, is involved in trafficking by targeting several SNARE proteins to membranes where they mediate vesicle fusion. aS itself is not palmitoylated but binds to membrane vesicles via an amphipathic helix. Amphipathic helices may regulate palmitoylation, which occurs enzymatically at membranes. Here, we hypothesize that abnormal aS accumulation and membrane binding disrupt palmitoylation of SNAREs and subsequent trafficking. We asked whether enhancing palmitoylation might rescue these phenotypes. To that end, we increased cellular palmitoylation using inhibitors of acyl protein thioesterase-1 (APT1), a de-palmitoylating enzyme, followed by assessment of aS-associated cellular pathologies.

Methods: Our lab previously found that "amplification" of the E46K familial PD aS mutant with 2 additional E-to-K mutations in the adjacent KTK(E)GV repeat motifs (E35K, E61K) causes cytotoxicity and aS-rich cytoplasmic inclusions. YFP-tagged versions of these aS "3K" mutants were expressed in M17D human neuroblastoma cells and inclusions measured by automated image analysis (Incucyte). Cytotoxicity was assessed by adenylate kinase release. Phosphorylated aS was examined by Western blotting with a Ser129 phospho-specific antibody. Acyl resin-assisted capture (RAC) was used to measure palmitoylation.

Results: Inclusions in aS 3K M17D cells are comprised of clusters of aS plus many membrane vesicles indicative of disrupted trafficking. Enhancing palmitoylation with the structurally distinct APT1 inhibitors palmostatin B (PSB) and ML348 reduced inclusions. PSB also reduced cell death and levels of phosphorylated aS, a marker of cytotoxicity. In transgenic mice expressing 3K aS, which exhibit a robust parkinsonian phenotype, we found decreased palmitoylation of the vesicular proteins SNAP-25, syntaxin-1, and synaptotagmin-1.

Conclusions: Our findings support the novel hypothesis that impaired protein palmitoylation can contribute to aS-dependent vesicular trafficking defects. Enhancing palmitoylation may be cytoprotective by partially correcting these deficits.

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A post-mortem and in vivo study of neuroinflammation and Toll like-receptors in Parkinson's disease

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Objective: To investigate inflammatory change and Toll-like receptor (TLR) expression in human post-mortem Parkinson's disease (PD) brains and to test the efficacy of TLR-blocking agents in a novel rodent PD model.

Background: Approximately 80% of PD patients have a poor outcome (postural instability, dementia, death) after 10 years from diagnosis. What determines the rate of disease progression is unknown, but neuroinflammation is a possible factor and a promising candidate for disease-modifying interventions. TLRs are a key component of this inflammatory response but their role in PD progression has not been fully explored.

Methods: Post-mortem brain tissue from 18 PD cases and 9 age-matched controls was used. Immunohistochemistry was performed in 8 regions for neuroinflammatory markers (Iba1, HLA-DR, TLR2/TLR4). TLR2/TLR4 expression was evaluated by Western blot and qPCR in 4 brain regions. In a novel rat PD model based on transvascular delivery of alpha-synuclein fibrils, changes in TLR2/TLR4 levels were determined by Western blot at 2,4 and 6 months. Subsequently, we compared the effects of 2 potential TLR-blockers (Candesartan cilexetil and TAK242). Rats were treated for 2 months with Candesartan, TAK242 or vehicle. TLR2/TLR4 expression was evaluated in monocytes using flow cytometry at baseline, 1 and 2 months. After 2 months, TLR-levels were measured in brain by Western blot.

Results: Activated microglia were increased in the hippocampus, amygdala and prefrontal cortex of human PD brains compared to controls ($p=0.054$, $p=0.048$, $p=0.034$, respectively). TLR2 but not TLR4 levels were increased in the hippocampus of PD compared to controls ($p=0.003$, $p=0.192$, respectively). In the rat model an increase in Iba1 and TLR4 levels in the brainstem was observed starting at 2 months, reaching significance at 6 months post-injection. After a 2-month treatment, TAK242 led to a decrease in both TLR2+ and TLR4+ monocytes while Candesartan did not. TLR levels in the brainstem were also

decreased after TAK242 treatment, but not Candesartan. This suggests that TAK242 is a more effective TLR-blocking agent.

Conclusions: Neuroinflammation in human PD and our novel PD rodent model is associated with an increase in TLRs. Our pilot data indicates that TAK242 is an effective blocker of TLRs and we are now investigating its long-term impact on neurodegeneration in our rodent model, which may have implications for treating human PD.

1737

Partial depletion of peripheral M1 macrophages ameliorates the neuroinflammation and dopaminergic neuronal death in the brain of a MPTP-induced mouse model of PD

A. Yan, Y. Zhang, J. Lin, L. Song, X. Wang, Z. Liu (Shanghai, China)

Objective: Neuroinflammation in the PD brain involves the activation of microglia and the increasing levels of inflammatory cytokines. Several studies have shown that the peripheral immune system is changed in neurodegenerative diseases. However, the function of the peripheral immune system in PD is not fully understood. Therefore, we employed an MPTP induced mouse model of PD to explore the role of macrophages in brain neuroinflammatory events and dopaminergic neuronal death in the SNpc. Our research may give a new clue for the implication of peripheral immune system in PD pathogenesis.

Background: Neuroinflammation plays an important role in the pathogenesis of Parkinson's disease (PD). Inflammatory cytokines in the peripheral immune system can induce neuroinflammation in central nervous system (CNS). Whether the peripheral immune system is involved in PD is unclear. The present study investigated the contribution of the peripheral immune system to the neuronal loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP) model of PD.

Methods: MPTP was intraperitoneally injected into mice to generate a PD model. Mice received clodronate liposomes every 3 days to deplete peripheral macrophages. The percentages of macrophages were measured by flow cytometry at 1,3,7 days after MPTP injection. Neurobehavioral parameters, protein expression, inflammatory cytokines release, and microglia activation were measured by the open field test, western blotting, quantitative polymerase chain reaction (qPCR), and immunofluorescence staining, respectively at 7 days after MPTP injection.

Results: Our research found that the number of macrophages from the spleen was increased in the MPTP model of PD, especially for M1 macrophages. Depletion of M1 macrophages ameliorated the neuroinflammatory reaction and protected the mice against MPTP-induced loss of dopaminergic neurons.

Conclusions: Our results indicated the critical role of M1 macrophages in the pathogenesis of PD and proposed inhibition of M1 macrophages as a promising therapeutic approach for neurodegeneration.

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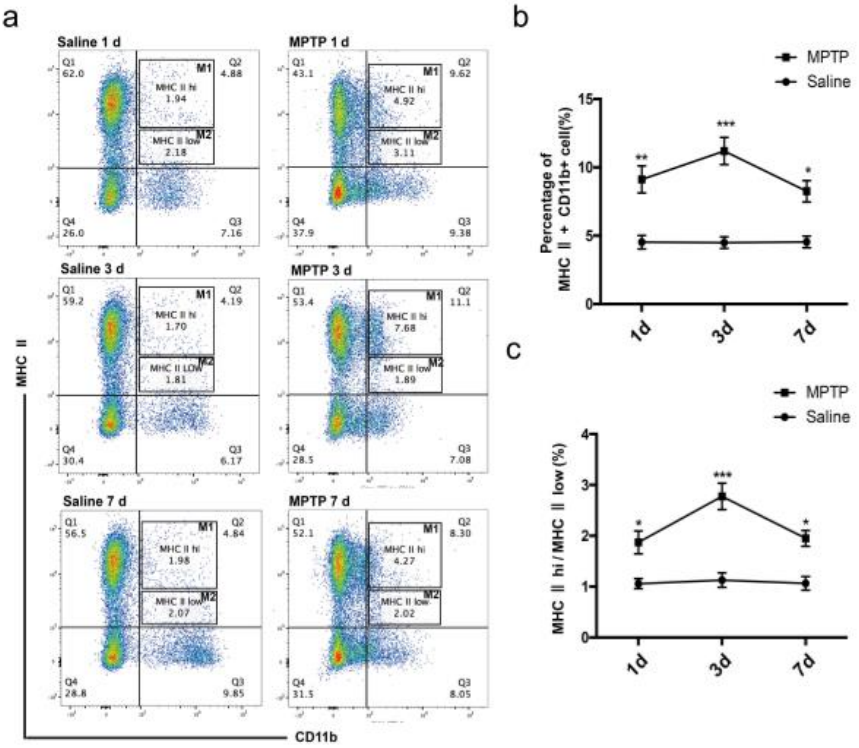

FIG. 1 (1737)

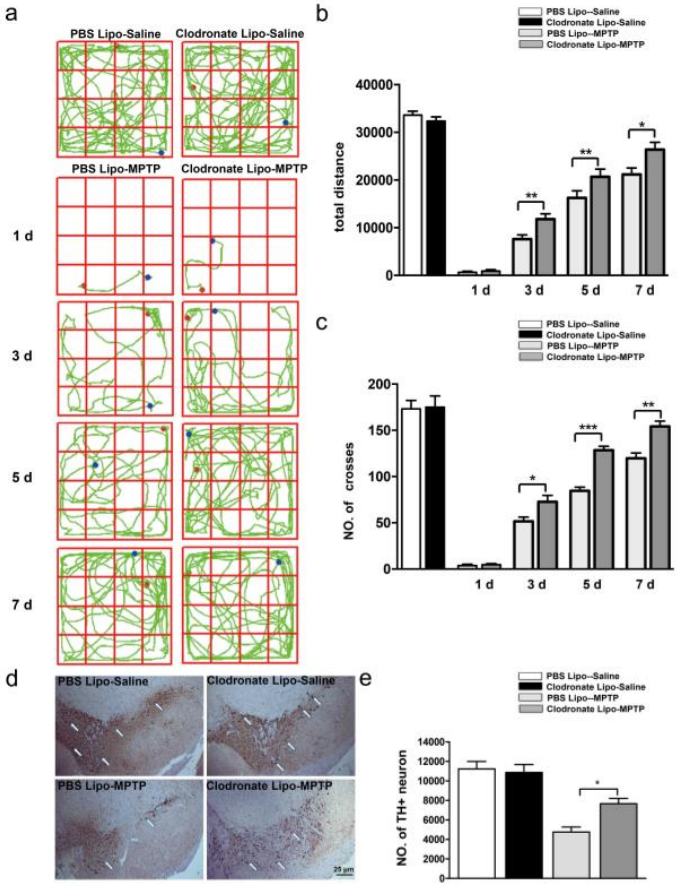

FIG. 2 (1737)

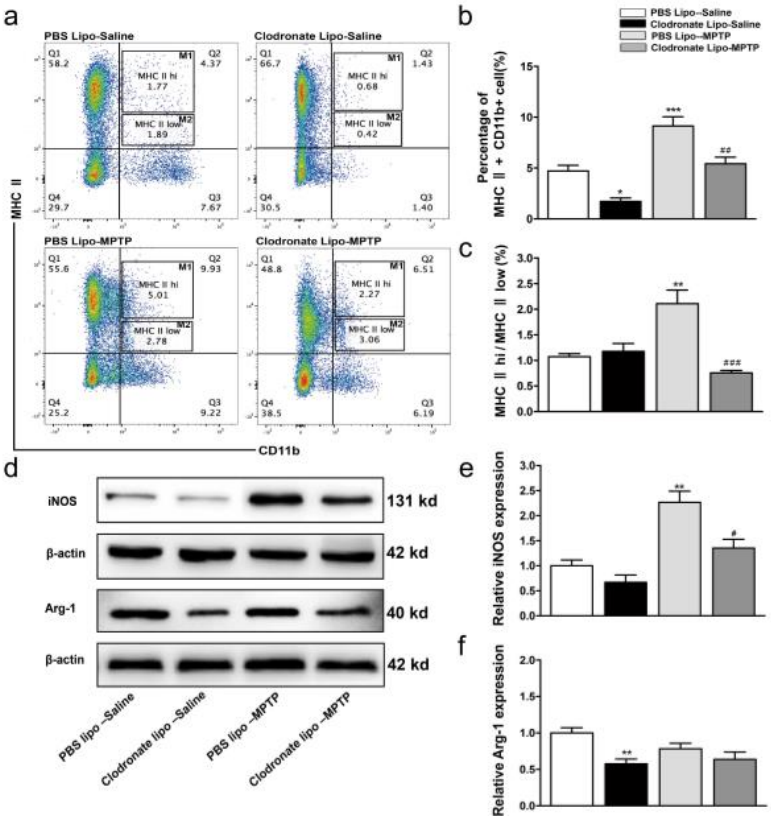

FIG. 3 (1737)

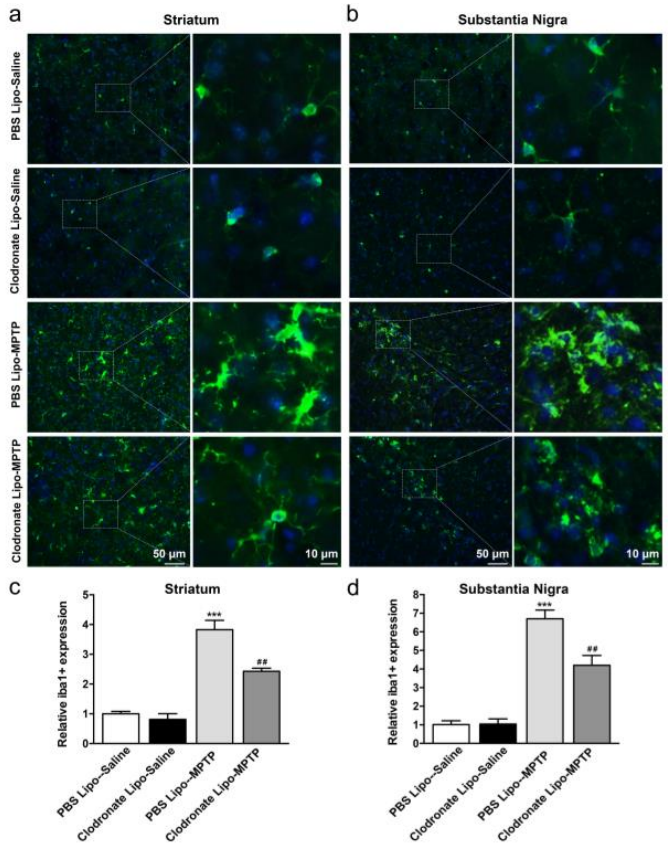

FIG. 4 (1737)

1742

Microglial activation, white matter and hippocampal damage correlate with cognitive impairment in chronic cerebral hypoperfused and MPTP-lesioned mice

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Objective: We hypothesize that cerebral hypoperfusion may influence the cognitive function of PD animal models. To investigate the effects of cerebral hypoperfusion and microangiopathy on Parkinson's disease (PD) cognitive dysfunction in mice and the related mechanisms through the preparation of a PD mouse model of cerebral hypoperfusion.

Background: Previous report revealed that abnormal cerebral glucose metabolism and blood flow are related to the motor and cognitive symptoms underlying PD[1].

Methods: PD mice were prepared by intraperitoneal injection of MPTP and probenecid, and the mice were subsequently tested in the Morris water maze. The experimental mice were divided into 7 groups based on the cognitive results and bilateral common carotid artery stenosis (BCCAs) operation. After 28 days of stenosis, the mice in each group were subjected to pole climbing experiments. After behavioral testing, the brain tissue of each group was subjected to tyrosine hydroxylase (TH) staining, Nissl staining, Bielschowsky silver staining, TUNEL and Iba-1immunohistochemistry in each group. The levels of inflammatory cytokines in the plasma of each group were measured by chip-based liquid chromatography. Western blot was used to observe apoptosis and the expression of autophagy-related proteins in each group of mice.

Results: (1) The pole-climbing experiment revealed that BCCAs could significantly prolong the climbing time of mice in the PDMCI group(Figure 1). (2) The immunohistochemistry results suggested that MPTP could reduce the number of TH-positive cells in the substantia nigra of mice, whereas BCCAs could significantly reduce dopamine (DA) neurons in the substantia nigra of PD mice(Figure 2). (3) Compared with the PDCN + BCCAs group, the PDMCI + BCCAs group exhibited white matter damage, significantly increased microglial activation ($P < 0.01$) and significantly increased levels of IL-1β and IFN-γ ($P < 0.05$)(Figure 5,6). (4) Nissl staining, TUNEL immunohistochemistry and Western blot revealed that MPTP injection alone or BCCAs alone could induce neuronal structural changes, reduce neuronal numbers, and increase neuronal apoptosis and that MPTP combined with hypoperfusion could promote the destruction of neuronal structures and neuronal apoptosis. In addition, we also found that the level of intracellular autophagy exhibited a compensatory increase(Figure 3,4,7,8).

Conclusions: Cerebral hypoperfusion can aggravate the impairment of cognitive function in PD mice. This finding may be related to hypoperfusion-mediated deterioration of neuroinflammation, aggravation of white matter damage, promotion of hippocampal neuron apoptosis and activation of autophagy in PD mice.

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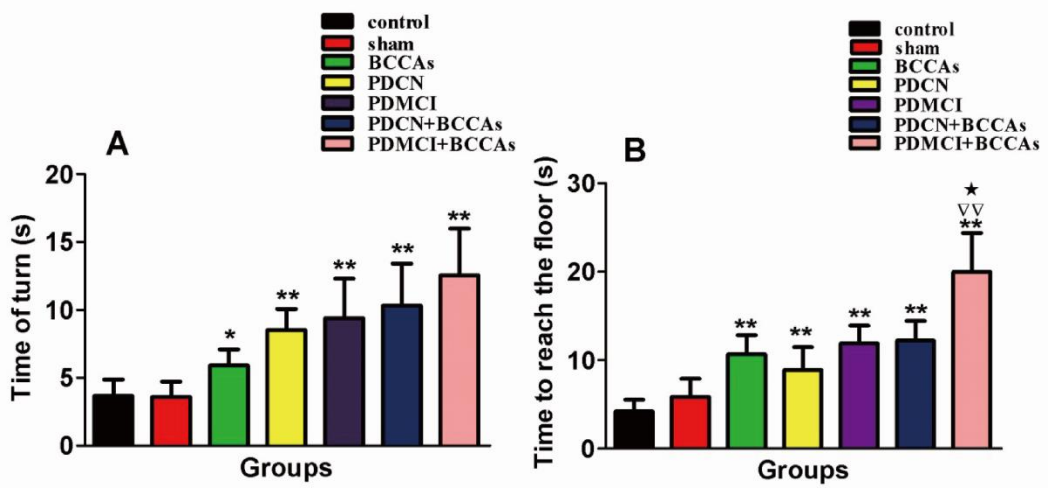


FIG. 1 (1742)

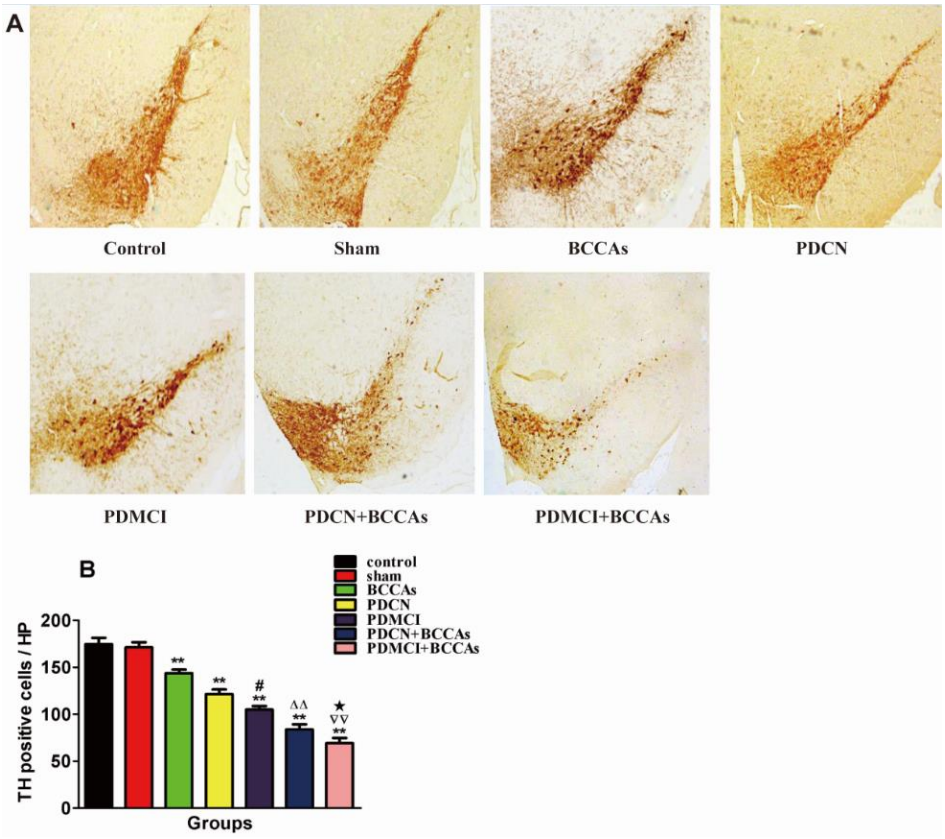


FIG. 2 (1742)

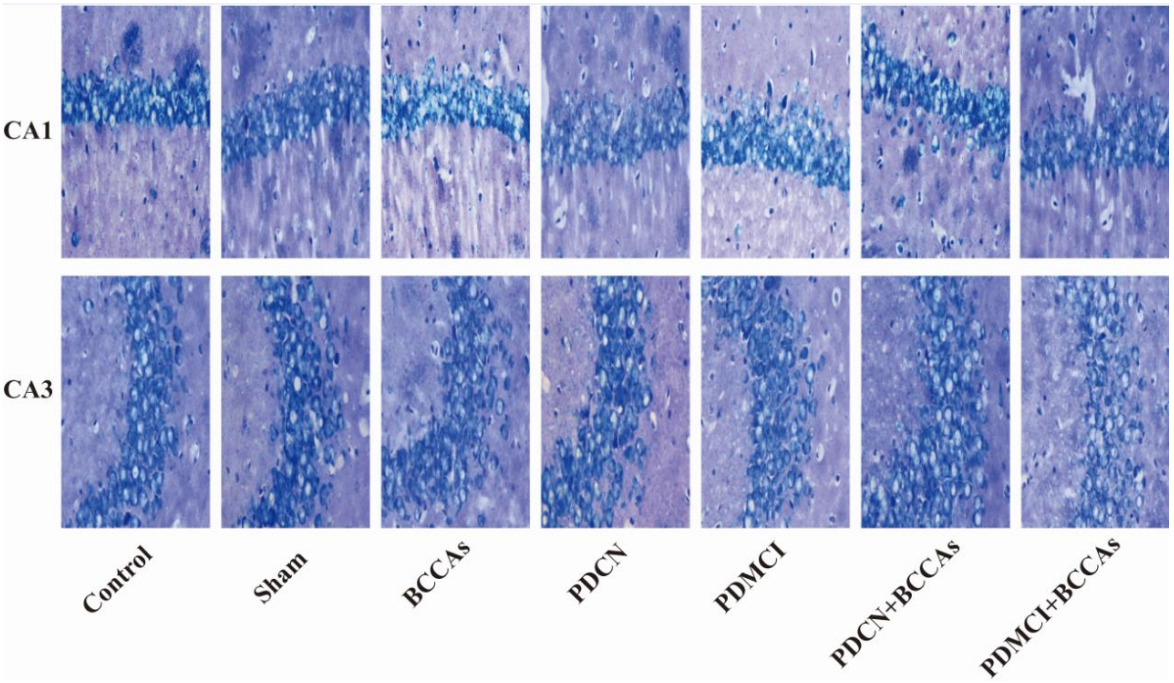


FIG. 3 (1742)

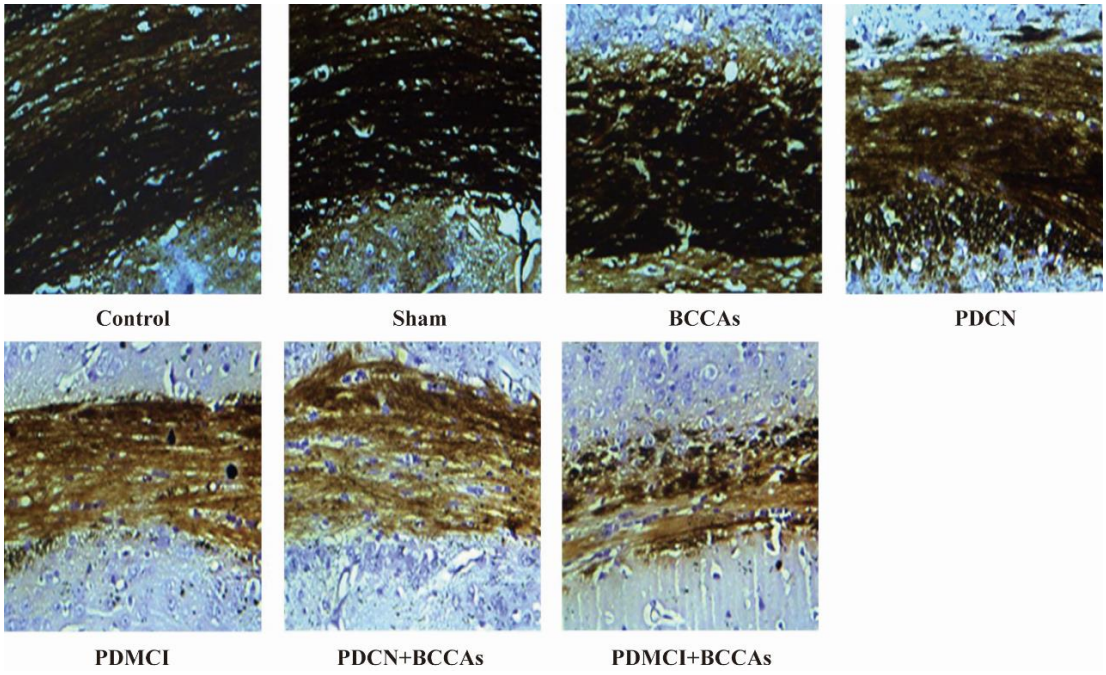


FIG. 4 (1742)

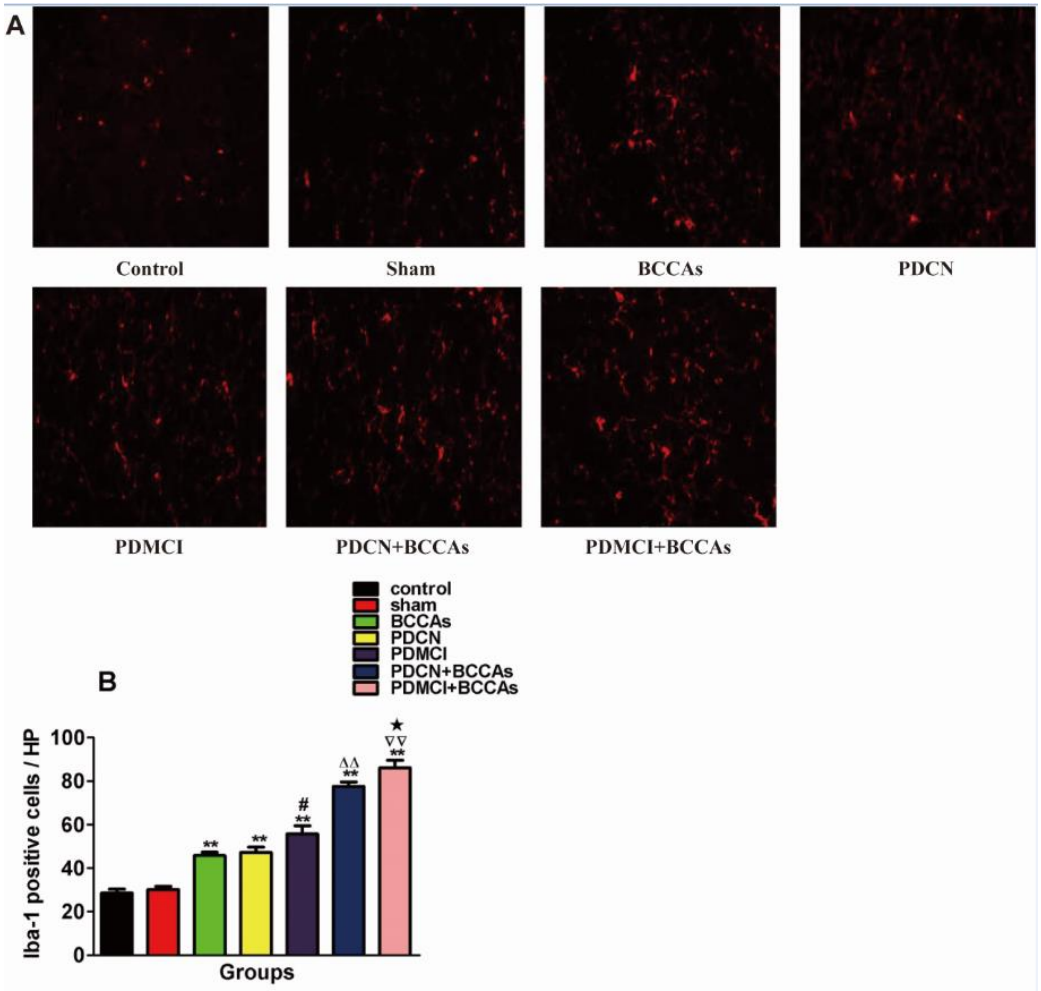
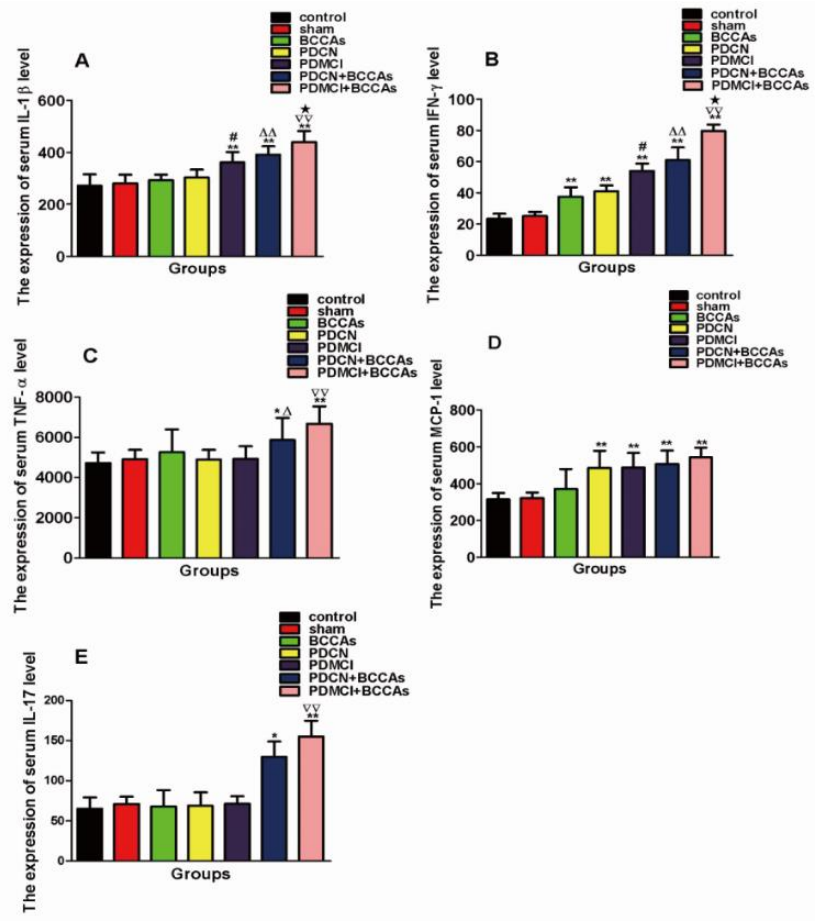


FIG. 5 (1742)



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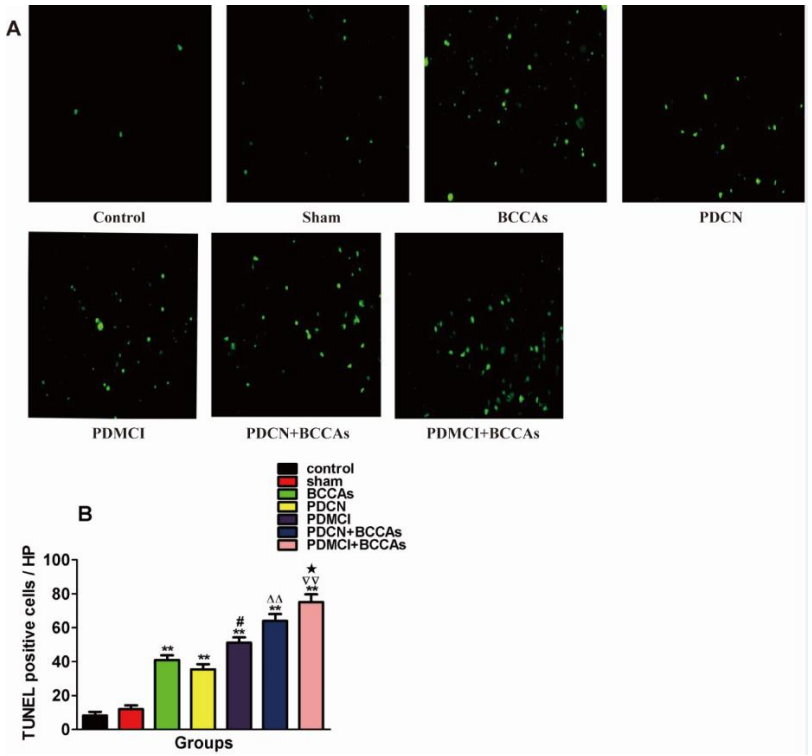

FIG. 7 (1742)

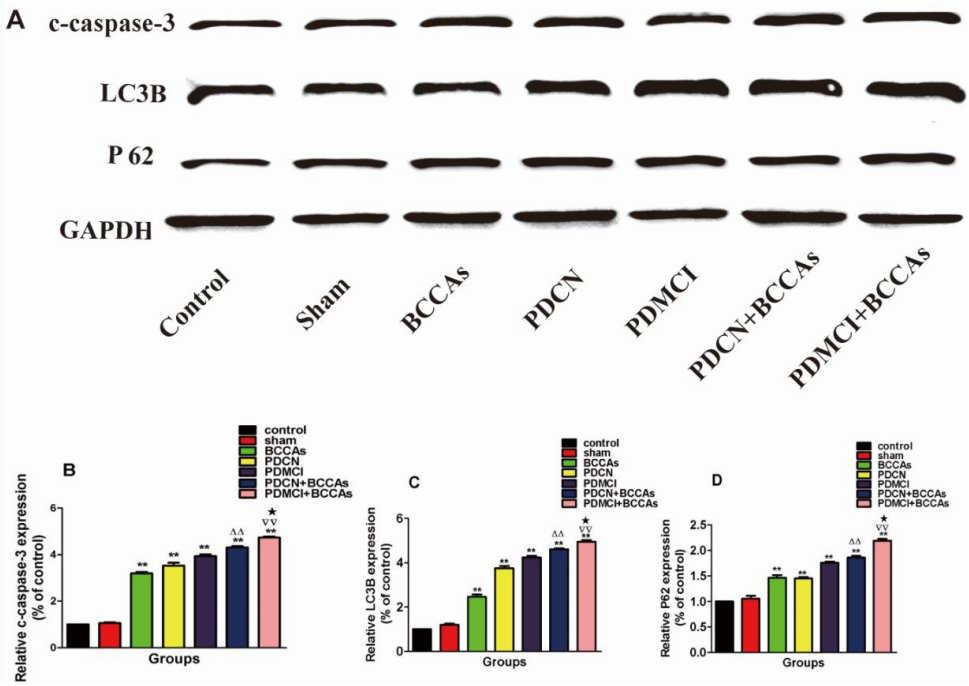

FIG. 8 (1742)

1743

Investigating the role of microRNA biogenesis pathway in neuroprotection in primary neuronal culture model of Parkinson's disease

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Objective: Here we study the effect of microRNAs (miRs) biogenesis on the dopamine (DA) neuronal maintenance and protection from different stressor-induced insults.

Background: Despite many years of extensive research, Parkinson's disease (PD) remains one of the commonest neurodegenerative disorders with mostly unknown etiology. Our recent data show that miRs, small non-coding RNAs regulating translation and stability of mRNA targets, may be implicated in PD pathology. We have demonstrated that selective homo- and heterozygous deletions of miRs processing enzyme Dicer in vivo cause development of PD-like symptoms. In addition, pharmacological stimulation of miRs biogenesis with enoxacin results in improved survival of primary DA neurons and attenuates their vulnerability to endoplasmic reticulum stress (1).

Methods: To elucidate the role of miRs biogenesis pathway in survival and stress resistance of DA neurons, we used several approaches to either boost or impair miRs maturation process. To stimulate miRs biogenesis, we overexpress Dicer and its interaction proteins, such as Trbp and Pact, in primary DA cultures using corresponding lentiviral vectors (LV). For impairing miRs maturation, we utilize Cre-recombinase delivered by LV to delete "floxed" Dicer1 gene allele in primary DA cultures from corresponding genetically modified mice. Alternatively, we are also setting up the single vector LV delivery system utilizing CRISPR/Cas9 gene editing to target Dicer and other members of miRs biogenesis machinery. Cells transduced with LV are further treated with different stressors, such as alpha-synuclein preformed fibrils, and their survival is analyzed by immunohistochemistry against tyrosine hydroxylase.

Results: Currently we are assessing the effect of overexpression of Dicer and other proteins involved in miR biogenesis on survival and protection of DA neurons from various PD-associated stressors. Additionally, obtained DA neurons with homo- and heterozygous deletion of Dicer are tested for their ability to withstand various stress conditions.

Conclusions: In summary, our data will assess the role of miR biogenesis in supporting survival and stress-resistance of primary DA neurons and evaluate the possibility of targeting Dicer and its interaction proteins for PD therapy.

References: 1) Chmielarz P, Konovalova J, Najam SS, Alter H, Piepponen TP, Erfle H, et al. Dicer and microRNAs protect adult dopamine neurons. Cell Death Dis. 2017;8(5):e2813.

1751

Peripheral Inflammatory Mediators in Parkinson's Disease - A Potential Biomarker

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Objective: i.To determine if post-translationally modified phosphorylated α-synuclein levels are altered in the peripheral blood of Parkinson's disease (PD) patients compared to control subjects. ii.To determine if inflammatory mediators are dysregulated in the peripheral blood of PD patients compared to control subjects.

Background: The molecular mechanisms of PD etiopathogenesis and its progression remain elusive. Inflammatory transcripts and peripheral immune cell infiltration in post-mortem PD brains indicate the involvement of peripheral blood inflammation. Recent in vitro and animal experiments demonstrate potency of misfolded α-synuclein to trigger Nod-like receptor protein-3 (NLRP-3) inflammasome activation. Therefore, NLRP-3 inflammasome activation in the peripheral blood of PD patients may act as a potential indicator of disease progression in PD.

Methods: PD patients (n=27) and age- and sex-matched healthy volunteers (n=15) were recruited from the hospital outpatient department following inclusion criteria. Peripheral venous blood was collected from the participants. Post-translationally modified phosphorylated α-synuclein levels were estimated by ELISA. NLRP-3 inflammasome activation was determined by estimating NLRP-3 and IL-1β levels by ELISA.

Results: Phosphorylated α-synuclein levels were not statistically different (p=0.07) between PD and controls (unpaired student t test). Compared to controls, PD patients showed significant elevation of serum NLRP3 inflammasome (p = 0.0008) and IL-1β levels (p =0.0019).

Conclusions: Alpha-synuclein in the peripheral blood are yet to be proven as a reliable biomarker in PD. The findings from this study indicate consistent inflammasome signalling with dysregulation of proinflammatory cytokine IL-1β in the peripheral blood of PD patients. Thus, inflammatory processes and mediators in PD might serve as potential peripheral biomarkers of PD.

1753

α-synuclein antibody 5G4 identifies manifest and prodromal Parkinson's disease in colonic mucosa

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Objective: The aim of this study was to evaluate colonic mucosa staining using the 5G4 antibody, specific only for the disease-associated form of α-synuclein, in a deeply phenotyped cohort of clinically manifest PD patients, patients meeting MDS research criteria for prodromal PD (pPD), and healthy controls (HC) not meeting the prodromal PD criteria.

Background: Previous studies point to a potential role of peripheral tissue biopsies as potential biomarkers of PD including its prodromal stages. Recently, a monoclonal α-synuclein antibody (clone 5G4) has been reported to show high reactivity for disease-associated forms, including oligomers, of α-synuclein, with superior results in comparative immunohistochemical studies in the CNS.1

Methods: Patients undergoing diagnostic colonoscopies were screened for all risk and prodromal markers from the MDS research criteria for prodromal PD, except genetic testing and PET/SPECT studies. Immunoreactivity for 5G4 antibody was performed in formalin-fixed in vivo biopsy samples of the colonic

mucosa of patients with manifest PD (n=6), pPD (n=7) and HCs (n=17) and were evaluated by two experienced independent and blinded raters.

Results: Pathological 5G4 positive neuritic structures were present in 5/6 clinically manifest PD patients, 4/7 pPD subjects and 2/17 HCs, yielding sensitivity 83.3%, specificity 88.2%, positive predictive value 71.43% and negative predictive value 93.75% for distinguishing manifest PD from healthy controls; and sensitivity 57.1%, specificity 88.2%, positive predictive value 66.7% and negative predictive value 83.3% for distinguishing probable pPD subjects from HCs. Both HCs with positive 5G4 immunostaining reported previous frequent pesticide exposure, while one of them had also constipation, hyposmia and increased MDS-UPDRS part III score, however, she did not exceed the threshold for probable prodromal PD.

Conclusions: 5G4 immunoreactivity in colonic mucosa is able to distinguish clinically manifest and prodromal PD patients from healthy controls. Although these results need to be confirmed in independent and larger cohorts, our study suggests a predictive value of diagnostic colon biopsies containing mucosal tissue with nerve structures.

References: 1. Kovacs GG, Wagner U, Dumont B, et al. An antibody with high reactivity for disease-associated α-synuclein, reveals extensive brain pathology. Acta Neuropathol 2012; 124: 37-50.

1756

Modulation of CaMKIIa-NR2B interaction in levodopa-induced dyskinesia in 6-OHDA- lesioned Parkinson's rats

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Objective: In the present study, therefore, we examined the relationship between CaMKII and NR2B in levodopa-induced dyskinesia rats.

Background: Long-term treatment with L-dopa leads to involuntary aimless movements called L-dopa-induced dyskinesia (LID) has hindered its use in Parkinson's disease (PD) patients. Emerging evidence suggests a possible role of CaMKIIa and its interacting partners in the development of LID.

Methods: To address this issue, we produced a rat model of PD, and valid PD rats were intrastriatal administrated with different doses of KN-93 (CaMKIIa inhibitor, 5 ug/kg and 10 ug/kg) and MK-801 (NR2B receptor antagonist, 0.1 mg/kg and 0.3 mg/kg), respectively.

Results: We found that CaMKIIa was found to form complexes with NR2B after chronic administration of L-dopa in adult rat striatal neurons. Intrastriatal injection of KN-93 significantly reduced the level of NR2B in CaMKIIa precipitates with a dose dependent response, as well as reduced the Global ALO AIM score without ablation of the therapeutic response to L-dopa. In parallel, intrastriatal injection of MK-801 significantly alleviated the level of CaMKIIa in NR2B precipitates compared to LID group ($p < 0.01$), and this is accompanied by realizing improvement of the Global ALO AIM score also without affect the efficacy of L-dopa.

Conclusions: In summary, the present study indicated that CaMKIIa-NR2B interaction had an important role in the development of LID. Disrupt of this link by intrastriatal infusion of KN-93 or MK-801 ameliorated dyskinesia in 6-OHDA-lesioned PD rats.

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Alpha-synuclein oligomer and rotenone treatments injury the dopaminergic neuron via inhibiting the expression of gene SEMA6D

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Objective: To investigate the expression of Semaphorin6D (SEMA6D) and its interaction with Plexin-A1 in cellular Parkinson's disease(PD) models induced by α-synuclein and rotenone.

Background: In order to uncover the molecular pathological process in Parkinson's disease (PD) ,we employed a microarray analysis upon the alpha-synuclein oligomer induced cellular PD model and investigate the significant differentially expressed genes(DEGs) screened from the microarray analysis.

Methods: SY-SH5Y cells cultured in vitro were divided into three groups:normal control group, α-synuclein oligomer-induced group, rotenone-induced group.The last two groups were treated with α-synuclein oligomer and rotenone respectively to establish the cellular PD models. The mRNA levels of SEMA6D and plexin-A1 were evaluated using real-time polymerase chain reaction analysis(rt-PCR), and the determinations had also been made on related proteins by Western blot analysis. The interaction between SEMA6D and Plexin-A1 was validated by co-immunoprecipitation. Immunofluorescence and co-focusing experiment were used to investigate the co-location of SEMA6D and PlexinA1 inside the cell.

Results: As the result showed, the expression of SEMA6D was significantly decreased in cellular PD model. KEGG Pathway analysis showed that SEMA6D was closely related to the network of MARK pathway. Comparing with the normal control group, the expressions of SEMA6D in cellular PD model groups were down-regulated obviously. Immunoprecipitation analysis and Immunofluorescence co-localization analysis comfirmed that the association of SEMA6D with Plexin-A1 was reversed in PD models.

Conclusions: α-synuclein and rotenone treatments inhibit expression of SEMA6D and its interaction with Plexin-A1. The decline of their interaction could trigger the MAPK signaling pathway, which is associated with the progress of PD.Our results suggest that SEMA6D is a critical factor in the pathogenesis of PD, and therefore could be an important drug target for novel treatments towards PD.