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# IMMUPARKNET

IMMUPARKNET ANNUAL CONFERENCE
2024
Royal Inn hotel,
Belgrade, Republic of Serbia
20th – 22nd April, 2024

## ABSTRACT BOOK

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# Meeting programme

## Saturday 20th April

**09.30** Registration

**10.00** Welcome and Action Presentation by Action Chair: **Prof. Cristoforo Comi**

**10.15 - 10.55** Updates on Working Groups - Meeting and Brainstorming WG1

**10.55 - 11.30 Coffee Break**

**11.30 - 13.00** Updates on Working Groups - Meeting and Brainstorming WG2

**13.00 - 14.00 Lunch Break**

**14.00 - 15.15** Updates on Working Groups - Meeting and Brainstorming WG3

**15.15 - 16.30** Updates on Working Groups - Meeting and Brainstorming WG4

**16.30 - 17.30 Coffee Break**

**17.00 - 18.30** MC Meeting

**18.30** Social events

## Sunday 21st April

**08.30** Registration

**09.00 - 10.15** Updates on Working Groups - Meeting and Brainstorming WG5

**10.15 - 10.50 Coffee break** (2 poster presentations by Marija Jeremić & Elena Rita Simula)

**10.50 - 12.15** Oral Presentations. Chairs: **Marina Romero Ramos & Đorđe Miljković**

Laura Muñoz-Delgado. Peripheral immune profile and neutrophil-to-lymphocyte ratio in progressive supranuclear palsy (online)

Julia Greenland. The effect of azathioprine on peripheral and central inflammation in Parkinson's disease: exploratory biomarker data from the AZA-PD clinical trial

Marta Camacho. Gut function, ghrelin and the immune system in early Parkinson's Disease

Shubhra Acharya. Long non-coding RNAs as neuroprotective targets in Parkinson's disease

Tommaso Schirinzi. Phosphoproteomic profiling of peripheral immune cells from patients with Parkinson's disease

**12.15 - 13.15 Lunch break**

**13.15 – 14.40** Oral presentations. Chairs: **Cristoforo Comi & Özgur Öztop Çakmak**

Augustas Pivoriūnas. A new model describing interactions of extracellular vesicles with human microglia

Frida Lind-Holm Mogensen. PARK7/DJ-1 deficiency impairs microglial activation in response to LPS-induced inflammation

Milorad Dragić. Sustained systemic antioxidative effects of intermittent theta burst stimulation beyond neurodegeneration: implications in therapy in 6-hydroxydopamine model of Parkinson's disease

Nicoleta Moisoi. Mitochondrial proteostasis influences microglia activation in neurodegeneration

Frederico C. Pereira. The interplay between catecholamines and myeloid cells is changed in PD patients

**14.40 – 15.15 Coffee break** (2 poster presentations by presentations by Anđela Stekić & Maria Georgoula)

**15.15 – 16.40** Oral presentations. Chairs: **Franca Marino & Milorad Dragić**

Monica Pinoli. Transcription factor mRNA levels in circulating CD4+ T cells predict phenoconversion in Idiopathic REM Sleep Behavior Disorder (online)

Alessia di Flora. Comparative analysis of T cell phenotype shifts in immunosenescence across young, healthy elderly, and frail subjects

Christiana C. Christodoulou. Unraveling the transcriptomic signatures of Parkinson's disease and major depression using single-cell and bulk data

Christa Nöhammer. Microbiome research tools beyond 16S rRNA and shotgun sequencing

Diego Clemente. Myeloid-derived suppressor cells as biomarkers of

disease severity in multiple sclerosis: relevance to Parkinson's disease?

## Monday 22nd April

**09.30** Registration

**10.00 - 10.45** Oral presentations. Chairs: **Ines Figueira & Tamara Saksida**

Kari Espolin Fladmark. Glial DJ-1 neuroprotection in a zebrafish Parkinson´s disease model

Sanja Blagojević. The effects of prolonged exposure to dopaminergic neurotoxins on AMPK and mTORC2 signaling

Lucia Silvera-Carrasco. Understanding the physiological functions of Activity-Dependent Neuroprotective Protein (ADNP) in microglial responses: linking neurodegeneration and neuroinflammation

**10.45 – 11.20 Coffee break** (2 poster presentations by Marija Adžić Bukvić & Rana Abu Manneh)

**11.20 - 11.50** Oral presentations. Chairs: **Ines Figueira & Tamara Saksida**

Augustas Pivoriūnas. Scaling up production of extracellular vesicles – next step towards clinical therapies against Parkinson's disease

Jinte Middeldorp. Neuroinflammation and neurodegeneration in SARS-CoV-2 infected macaques: A link between COVID-19 and Parkinson's disease?

**12.00** Conclusions and Goodbye

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# Foreword

**Parkinson's Disease** (PD) is a widespread chronic disease affecting over a million people in the European Union. It has currently no cure, hence patients rely only on symptomatic treatments. By consequence PD relentlessly results in serious disability, poor quality of life for patients, families and caregivers, causing high individual and societal costs.

**IMMUPARKNET** is an Action supported by the European Cooperation in Science and Technology (COST) funding organization. Focused on Parkinson's Disease, IMMUPARKNET aims to establish an innovative, multi-interdisciplinary research network, fostering the exchange of expertise among outstanding experts from different countries and institutions, involving scientists not only studying immunity in PD but also immunity in other neurodegenerative diseases.

PD etiology is largely unexplained and several pathogenetic hypotheses have been explored. The role of the immune system has been suggested by important studies, showing significant changes in both central and peripheral immunity. Several approaches exist to target the immune system, thus – would the contribution of immunity in PD be clarified – novel therapeutics could be developed.

Currently only few research groups study the role of the immune system in PD; however methodological and technical approaches are highly variable. Moreover, networking and exchange of expertise between groups working on immunity in different pathologies is still underdeveloped, with the consequence that precious advances are not fully exploited or even precluded. The sharing of experiences, also taking advantage of the efforts made in similar neurodegenerative conditions, will provide unprecedented advantages.

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IMMUPARKNET is thus establishing a first nucleus of a multidisciplinary ecosystem to fight the fragmentation of efforts and approaches, both in research and clinical practice, for boosting research towards the development of innovative treatments for PD.

The abstracts presented in this publication on the occasion of IMMUPARKNET second annual conference, held in Belgrade on April 20-22, 2024, reflect the variety and width of current research in PD developed by different scientists (and their teams) who are active members of our Action.

At the same time, they are the best expression of our network, that currently counts more than 150 members coming from 27 EU and non-EU countries.

A number we hopefully will further increase, hand in hand with the research performed by our members, leading to significative advancements in PD cure.

*The Immuparknet Team*

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# ABSTRACTS - ORAL PRESENTATIONS

# Peripheral immune profile and neutrophil-to-lymphocyte ratio in progressive supranuclear palsy

Laura Muñoz-Delgado[1,2], Antonio Luque-Ambrosiani[1], Belén Benítez Zamora[1], Daniel Macías-García[1,2], Silvia Jesús[1,2], Astrid Adarmes-Gómez[1,2], Elena Ojeda-Lepe[1,2], Fátima Carrillo[1,2,3], Pablo Mir[1,2,3]

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**Objective** This study aimed to evaluate the peripheral immune profile in patients with Progressive Supranuclear Palsy (PSP) compared with patients with Parkinson's disease (PD) and healthy controls (HCs).

**Background** Evidence points to the involvement of peripheral inflammation in the pathogenesis of PSP and suggests that it is a common feature with PD. However, the peripheral immune profile in PSP remains unclear, as well as whether the inflammatory pathways differ significantly from those in PD. Studies on the leukocytes and subpopulations in PSP have shown controversial results. Interestingly, the neutrophil-to-lymphocyte ratio (NLR) in peripheral blood has been proven to be a well-established biomarker of systemic inflammation.

**Methods** We conducted a cross-sectional study including 120 patients with PSP, 127 patients with sporadic PD and 266 HCs. All subjects were examined for exclusion criteria that could influence the immune profile. Leukocytes subpopulations and the NLR were measured in peripheral blood. Multivariate lineal regression and post-hoc tests were applied to determine the differences among groups. Electronic databases were searched in November 2023 to perform a meta-analysis to clarify the peripheral immune profile in PSP.

**Results** In our cohort, the NLR was significantly higher in both patients with PSP and PD compared with HCs ($p<0,001$). No significant differences were found in the NLR between PSP and PD ($p=0.41$). Patients with PSP

had a significant higher neutrophil count compared with HCs (p<0,001). A higher neutrophil count was also found in patients with PD compared to HCs, but it did not reach statistical significance (p=0.06). Interestingly, while a significant lower lymphocyte count was found in patients with PD compared with HCs (p=0.001), the lymphocyte count did not differ between patients with PSP patients and HCs (p=0.97).
The metanalysis supported that a higher NLR and a higher neutrophil count were present in patients with PSP compared with HCs, without differences in the lymphocyte count.
**Conclusions** Patients with PSP and PD show an increased peripheral inflammation and a higher NLR compared with HCs. However, different pathogenic inflammatory mechanisms are probably involved in PSP and PD, since in patients with PSP this altered peripheral immune profile is mainly driven by neutrophils. Understanding the role of neutrophils in PSP may allow for the development of targeted therapies.

# The effect of azathioprine on peripheral and central inflammation in Parkinson's disease: exploratory biomarker data from the AZA-PD clinical trial

Julia Greenland

*Department of Clinical Neuroscience, University of Cambridge, UK.*

Given the substantial evidence implicating the immune system in both the development and progression of Parkinson's disease (PD), we have conducted a clinical trial repurposing azathioprine, an immunosuppressant drug, with the aim of slowing down the progression of the disease and establishing proof of concept for immunosuppression as a disease modifying strategy in PD. AZA-PD is a randomised placebo-controlled, double-blind phase II trial in patients within three years of PD diagnosis and with no immune or inflammatory comorbidities. Participants have taken azathioprine/placebo for one year, with clinical assessments every 6 months and a clinical primary outcome measure of the change in gait/axial subscore of the MDS-UPDRS in the OFF state after 12 months of treatment, a measure which has been shown to be the most sensitive component of the MDS-UPDRS III to disease progression. In addition, we have collected exploratory outcome measures evaluating change in immune activation, using [$^{11}$C]-PK11195 PET neuroimaging and immunophenotyping of immune cells using flow cytometry in the blood and cerebrospinal fluid (CSF).

At the point of submitting this abstract, we are in the final stages of the trial, with all participants having completed their treatment period, and the last trial assessments due to be completed by the end of February 2024. We remain blinded to treatment allocation and are unable to report on clinical outcomes at this point. In the interim, analysis of baseline and 12-month blood, CSF and imaging data has been carried out, and here we present these data demonstrating the impact of azathioprine on the peripheral immune profile and neuroinflammation in PD.

Azathioprine is a peripherally acting immunosuppressant, which does not cross the blood brain barrier. As expected, in the blood there was a depletion in total lymphocyte numbers in ($p<0.0001$) following 12 months of treatment, which was most marked in the CD4+ effector cells ($p= 0.004$) and the NK cells ($p<0.0001$). Monocyte count was unaffected, but there was reduction in classical monocytes ($p=0.002$) and a reduction in monocyte TLR2 expression ($p=0.030$).

Importantly, azathioprine also had an effect on measures of central immune activation. There was a reduction in the number of lymphocytes in the CSF, seen across CD3+ ($p=0.020$) and NK ($p=0.037$) compartments, a reduction in CD28 expression on CD8+ cells ($p=0.010$) and a reduction in classical monocytes ($p=0.023$). Analysis of [$^{11}$C]-PK11195 binding potentials pre and post treatment with azathioprine revealed a significant reduction in neuroinflammatory signal in the pallidum ($p=0.043$) following treatment, and a consistent trend towards a reduction in neuroinflammation compared to the placebo group across multiple brain regions.

As well as providing mechanistic context for the upcoming analysis of the clinical outcomes in the AZA-PD trial, this work has given insight into the interplay between the peripheral immune system and central neuroinflammation. It supports the theory that it is possible to manipulate inflammation in the central nervous system through a peripherally acting immunosuppressive agent, which will inform future trials.

# Gut function, ghrelin and the immune system in early Parkinson's Disease

Marta Camacho

*Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK*

**Background** The mechanisms underlying the association between gastrointestinal dysfunction (GID), immune activation and the clinical progression of Parkinson's disease (PD) are complex and still not clear. Ghrelin, an orexigenic peptide, has been shown to be decreased in PD and studies suggest that it might have an immunomodulatory effect but very few have investigated the relationship between ghrelin, self-reported GID and its association with clinical measures of PD progression.
**Aims** to investigate the association between gastrointestinal symptoms, serum ghrelin levels, and pro-inflammatory cytokines in PD.
**Methods** 69 participants with newly diagnosed PD (<2 years), 30 participants at high risk of developing PD (REM Sleep Behaviour Disorder, RBD) and 48 household controls were assessed at baseline and 12 months later. All participants completed the Gastrointestinal Dysfunction Scale – Parkinson's Disease (GIDS-PD) and the RBD screening questionnaire (RBDSQ). PD and RBD cases were assessed using the MDS-UPDRS. Serum samples were collected for measurement of ghrelin and inflammatory cytokines.
**Results** There were no major differences in gut dysfunction between the groups. However, serum ghrelin levels were significantly lower in RBD when compared to controls at both visit 1 and 2. Ghrelin levels were also lower in PD participants compared to controls at visit 2. There was no significant change in ghrelin levels over time in any group. Lower ghrelin levels correlated with higher IL-6 at visit 1 and with higher RBDSQ scores at both visits.
**Conclusions** Ghrelin was lower in prodromal and in early PD and correlated negatively with IL-6 levels, suggesting it might influence inflammation and warrants further investigation as a gut-related biomarker and potential therapeutic target.

# Long non-coding RNAs as neuroprotective targets in Parkinson's disease

Shubhra Acharya[1,2], Andrew I. Lumley[1], Sandro L. Pereira[3], Sylvie Delcambre[3], Javier Jarazo[4], Katja Badanjak[3], Lu Zhang[5], Semra Smajic[3], Jochen Ohnmacht[3,6,7], Victoria Stopa[1,2], Irene Nada[1,8], Adam Mehdi[1], Lasse Sinkkonen[5], Aleksandar Raković[9], Anne Grünewald[3,9], Yvan Devaux[1], and NCER-PD consortium

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**Background** Long non-coding RNAs (lncRNAs) are implicated in several diseases including Parkinson's disease (PD). Despite advances, novel biomarkers and therapeutic targets for PD are in dire need. LncRNAs can regulate gene expression at transcriptional and post-transcriptional levels. They are tissue-specific and can be measured in various bodily fluids, which highlights their suitability as therapeutic targets and potential biomarkers.

**Aim** Our study aims to discover the role of novel lncRNAs in PD and to study their biomarker and therapeutic potential.

**Methodology** Publicly available RNA sequencing data from PD patients and non-PD controls induced pluripotent stem cell (iPSC)-derived neurons and single cell RNA sequencing data from post-mortem midbrain tissues were re-analysed to identify novel lncRNAs. Differentially expressed lncRNAs were studied via quantitative PCR (qPCR) in the neuroblastoma cell line SH-SY5Y treated with 1-methyl-4-phenylpyridinium (MPP) to mimic mitochondrial dysfunction typically seen in PD. One of the most

promising candidates was selected and further studied to uncover its role in PD. To this end, RNA pull-down, cell type-specific expression and over-expression experiments were performed. Moreover, the biomarker potential of this lncRNA was assessed via qPCR in 319 non-PD and 319 idiopathic PD (iPD) whole blood samples from the NCER-PD cohort.
**Results** Analysis of publicly available RNA sequencing data revealed that 28 lncRNAs were differentially expressed in iPSC-derived neurons obtained from PD patients compared to those from non-PD controls (FDR <0.05). Of the top 10 differentially expressed (DE) lncRNAs, the expression of 1 lncRNA was downregulated; 4 were upregulated and the remaining five were not regulated in MPP treated SH-SY5Y cells. RNA pull-down of one of the top 10 DE lncRNA candidates, named lncRNA1, was found to interact with messenger RNAs coding for genes involved in PD development: tyrosine hydroxylase (TH) and alpha synuclein. Single-nuclei RNA sequencing of postmortem brain tissue and expression quantification in TH-positive iPSC-derived neurons showed lncRNA1 expression was specific to neurons in the midbrain and was found to be exclusively expressed in TH-positive dopaminergic neurons. Overexpression of lncRNA1 significantly increased TH and dopa-decarboxylase protein expression in SH-SY5Y cells treated with MPP as measured by Western blotting. Furthermore, ELISA results indicated a significant increase of dopamine in MPP-treated SH-SY5Y cells following lncRNA1 overexpression. Lastly, lncRNA1 expression was found to be significantly downregulated in whole blood samples from iPD patients compared to non-PD controls (both groups n=319, p=0.002).
**Conclusion** Our study sheds light on the role of lncRNAs in PD. The expression of lncRNA1 was decreased in SH-SY5Y cells treated with MPP and in human iPD whole blood patient samples. LncRNA1 could significantly increase the expression of enzymes involved in the dopamine synthesis pathway, suggesting a neuroprotective role in PD. Further work is needed to validate the therapeutic and biomarker potential of lncRNA1.

**References**

Acharya S, Salgado-Somoza A, et al. Non-Coding RNAs in the Brain-Heart Axis: The Case of Parkinson's Disease. Int J Mol Sci. 2020 Sep 6;21(18):6513. doi: 10.3390/ijms21186513.
Hipp, G., Vaillant, M., Diederich, N. J., Roomp, K., Satagopam, V. P., Banda, P., Sandt, E., Mommaerts, K., Schmitz, S. K., Longhino, L., Schweicher, A., Hanff, A. M., Nicolai, B., Kolber, P., Reiter, D., Pavelka, L., Binck, S., Pauly, C., Geffers, L., Betsou, F., ... Krüger, R. (2018). The Luxembourg Parkinson's Study: A Comprehensive Approach for Stratification and Early Diagnosis. Frontiers in aging neuroscience, 10, p 326. https://doi.org/10.3389/fnagi.2018.00326.

# Phosphoproteomic profiling of peripheral immune cells from patients with Parkinson's disease

Tommaso Schirinzi[1], Giorgia Massacci[2], Jacopo Bissacco[1], Rocco Cerroni[1], Roberta Bovenzi[1], Maximilian Zwiebel[3], Maria Wahle[3], Veronica Venafra[2], Matthias Mann[3], Francesca Sacco[2,4]

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**Introduction** Evidence on the involvement of the peripheral immune response in mechanisms underlying Parkinson's disease (PD) is dramatically increasing. Peripheral blood immune cells may directly participate in pathogenic processes or rather reflect neuropathological events. Regardless, substantial functional changes occur in PD patients' peripheral blood mononucleate cells (PBMCs), which may be theoretically targeted for therapeutic or biomarker purposes. State-of-the-art mass spectrometry (MS)-based (phospho)proteomics allows for profiling the global changes in the phosphorylation and concentration level of thousands of proteins in cells, uncovering activated signaling networks and novel molecular targets.

**Objective** To perform a (MS)-based (phospho)proteomics analysis of PBMCs from PD patients at various disease stages.

**Methods** The study included ten healthy controls and 20 PD patients assessed through conventional scores for disease severity (e.g., MDS-UPDRS part 3, H&Y). PBMCs were isolated from each participant and processed for high sensitive (MS)-based total proteome and phosphoproteome quantification. Principal component analysis (PCA) was run to cluster patients.

**Results** The case-control analysis of the (phospho)proteomic PBMCs profile revealed a global rewiring of pathways involved in oxidative phosphorylation and inflammation.

In addition, expression of a-synuclein, septin-5, and DJ-1 was upregulated in the PD group. The PCA of about 8000 proteins and 12000 phosphosites identified three distinct subgroups at different clinical stages. In particular, crucial kinases involved in the antigen presentation/processing, MAPK, and calcium signaling pathways were differently modulated according to PD severity.

**Conclusions** This unbiased analysis enabled shaping the global (phospho) proteomic profile of PD patients PBMCs, recognizing differential (phospho)proteomic signatures associated with different disease stages, and identifying some biological pathways majorly involved in PD immune cells. A large-scale MS-based characterization of PBMCs might allow for a better understanding of the role of peripheral immunity in PD and discover molecular targets consistent with the disease status.

# A new model describing interactions of extracellular vesicles with human microglia

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Dysregulation of microglial function has been associated with pathogenesis of many neurological disorders, therefore targeting disease-associated microglia represents a promising therapeutic approach. Extracellular vesicles (EVs) attracted a significant interest as a potential therapy against various neurological conditions.

Our previous findings demonstrate that EVs may act as a potent immunomodulators of human microglia by promoting (I) phagocytosis, (ii) motility, (iii) autophagy, and (iv) inducing metabolic reprogramming. Investigation of molecular mechanisms underlying EV – induced effects revealed critical importance of direct interaction with Toll-like receptor 4 (TLR4) and αVβ3/αVβ5 integrin receptors leading to the rapid induction of lipid raft formation in microglia.

We propose that EVs carrying milk fat globule-epidermal growth factor-VIII (MFG-E8) proteins and endogenous TLR4 ligands (HSP70) are recognized by the αVβ3/αVβ5 integrin receptors and TLR4s, respectively, trigger lipid raft formation and enlargement leading to the initiation of motility, phagocytosis and autophagy in human microglial cells.

Our results provide new insights about the molecular mechanisms regulating EV/microglia interactions that could be helpful for the development of new therapeutic strategies against neurological disorders.

# PARK7/DJ-1 deficiency impairs microglial activation in response to LPS-induced inflammation

Frida Lind-Holm Mogensen[1,2], Carole Sousa[1,3], Corrado Ameli[4], Katja Badanjak[5], Arnaud Muller[6,7,8], Tony Kaoma[6], Djalil Coowar[9], Andrea Scafidi[1,2], Suresh K Poovathingal[4,10], Nathalie Nicot[7,8], Aurélien Ginolhac[11], Daniela Maria Vogt Weisenhorn[12,13], Wolfgang Wurst[12,13,14,15], Aurélie Poli[1], Petr Nazarov[6,7], Alexander Skupin[4,16], Anne Grünewald[5,17], Alessandro Michelucci[1]

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The pathogenesis of Parkinson's disease (PD) involves crucial components like oxidative stress and neuroinflammation, which are associated with the activation of microglial cells, the immune effectors of the central nervous system. Approximately 10% of PD cases has a genetic origin, including mutations in *PARK7* that cause DJ-1 deficiency,

leading to autosomal recessive PD. DJ-1 plays diverse roles with a notable function in protecting against reactive oxygen species. However, the precise mechanisms through which DJ-1 deficiency leads to early-onset PD needs further investigations. In this study, we aimed at investigating the role of DJ-1 deficiency in microglia, both at baseline and under acute LPS-induced neuroinflammation. We compared microglial cell phenotypic characteristics in *PARK7*/DJ-1 knock-out (KO) with wildtype (WT) littermate mice following 6- or 24-hour intraperitoneal injection with lipopolysaccharide (LPS) using single-cell RNA-sequencing, deep RNA-sequencing, multicolour flow cytometry and immunofluorescent staining analyses. We conducted corresponding analyses in human *PARK7*/DJ-1 mutant induced pluripotent stem cell (iPSC)-derived microglia and murine bone marrow-derived macrophages (BMDMs). We found that microglia from DJ-1 KO mice peripherally treated with LPS display a diminished transcriptional responsiveness characterized by a downregulation of inflammatory response genes, especially genes involved in type I and type II interferon pathways, compared to microglia from WT littermates. We detected a similar dampened response to LPS in bone marrow-derived macrophages from DJ-1 KO compared to WT mice and in human DJ-1 mutant iPSC-derived microglia. Thus, our findings demonstrate that the observed phenotypic changes in murine microglia are replicated in peripheral macrophages and human microglia. Furthermore, this reduced reactivity was noticeable at the morphological level, as microglia from LPS-treated DJ-1 KO mice exhibited a less amoeboid cellular shape and displayed more processes with increased complexity compared to their WT counterparts. Our findings collectively suggest that in the presence of inflammation, DJ- deficient microglia and BMDMs react differently to a pro-inflammatory stimulus compared to WT microglia and BMDMs. This indicates that the oxidative stress associated with the absence of DJ-1 influences the neuroinflammatory responses of microglia, potentially contributing to the initiation and progression of PD. Further studies are needed to explore how the identified molecular and morphological cues related to *PARK7*/DJ-1 deficiency impact crucial microglial functions.

# Sustained systemic antioxidative effects of intermittent theta burst stimulation beyond neurodegeneration: implications in therapy in 6-hydroxydopamine model of Parkinson's disease

Milorad Dragić[1,2]

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Parkinson's disease (PD) is manifested by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (*SNpc*) and caudoputamen (*CPu*), leading to the development of various motor and non-motor symptoms. The contribution of oxidative stress to the development and progression of PD is increasingly recognized. Experimental models show that strengthening antioxidant defenses and reducing pro-oxidant status may have beneficial effects on disease progression. Current study examines the neuroprotective potential of intermittent theta burst stimulation (iTBS) in a 6-hydroxydopamine (6-OHDA)-induced PD model in rats seven days after intoxication which corresponds to the occurrence of first motor symptoms. Two-month-old male Wistar rats were unilaterally injected with 6-OHDA to mimic PD pathology and were subsequently divided into two groups to receive either iTBS or sham stimulation for 21 days. The main oxidative parameters were analyzed in the caudoputamen, substantia nigra pars compacta, and serum. iTBS treatment notably mitigated oxidative stress indicators, simultaneously increasing antioxidative parameters in the both structures well after 6-OHDA-induced neurodegeneration process was over. Serum analysis revealed the systemic effect of iTBS with a decrease in oxidative markers and an increase in antioxidant parameter. Prolonged iTBS exerts a modulatory effect on oxidative/antioxidant parameters in the 6-OHDA-induced PD model, suggesting a potential neuroprotective benefit, even though at this specific time point 6-OHDA-induced oxidative status was unaltered. These results emphasize the need to further explore the mechanisms of iTBS, especially its systemic effects and argue in favor of considering it as a therapeutic intervention in PD and related neurodegenerative diseases.

# Mitochondrial proteostasis influences microglia activation in neurodegeneration

Rubab Talpur, Nicoleta Moisoi

*Leicester Institute for Pharmaceutical Innovation, Leicester School of Pharmacy, De Montfort University, Leicester, United Kingdom*

Neuroinflammation is defined as a complex integration of responses from all brain immune cells. It is a 'double-edged sword', with both beneficial and detrimental effects. Increasing evidence suggests that microglia-mediated neuroinflammation and mitochondrial stress signalling and quality control, are key mechanisms in the progression of the neurodegenerative diseases. They have been largely explored independently but their potential interrelationship has begun to come to light only recently. We hypothesized that the regulation of mitochondrial proteostasis has an influence on microglia activation and modulates neurodegenerative processes in Parkinson's and Alzheimer's. To address this hypothesis, we have combined the pharmacological enhancement of proteolysis in the mitochondrial matrix with immune activation triggered by alpha-synuclein preformed fibrils as well as lipopolysaccharide (LPS) as positive control. The study was developed in two human microglia cell models, namely microglia derived from induced pluripotent stem cells and the HMC3 microglia cell line. The endpoints examined included: mitochondrial function (ATP levels), unfolded protein response via qRT-PCR, ROS accumulation, immune response activation (qRT-PCR) and RNA sequencing and metabolomics. The results suggest that our proposed activation of proteostasis improves the viability of microglia undergoing oxidative stress treatments. In addition, enhanced proteostasis modulates the expression of anti-inflammatory cytokines and reduces the toxicity of alpha-synuclein. In microglia-neuron co-cultures, activation of proteostasis, appears to modulate the effects of the alpha-synuclein. These findings indicate that pharmacological activation of mitochondrial proteostasis can modulate immune responses in microglia cells and it has the potential to be used as a neuroprotective strategy.

# The interplay between catecholamines and myeloid cells is changed in PD patients

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**Introduction** Dendritic cells (DCs) and monocytes express dopamine receptors and β2- adrenoceptor and express the enzyme indoleamine 2,3-dioxygenase 1 (IDO1; a key mechanis in immunosuppression). This work aims to characterize DCs and monocytes, including their ability to induce IDO1, in patients with Parkinson's Disease (PD) and to understand the effect of DA and salbutamol in IDO1-expressing DCs and monocytes in PD.

**Material and Methods** Peripheral blood samples were collected from 62 PD patients [66±9 years, 45% females] selected from the Movement Unit of Coimbra Hospital and University Centre with a Unified Parkinson's Disease Rate Scale (UPDRS)III score mean of 19±8 and 40 healthy age-matched controls (HS) [62±8 years, 52% females]. The pharmacological effect of DA (100μM) was evaluated in peripheral blood samples from 14 PD patients [71±11 years, 57% females, UPDRSIII score 24±4] and from 13 HS [65±9 years, 39% females]. The pharmacological effect of salbutamol (1 μM) was analyzed in peripheral blood samples from 6 PD patients [67±10 years, 33% females, UPDRSIII score 11±5] and from 5 HS [57±7 years, 80% females]. In these in vitro studies peripheral

blood was stimulated with lipopolysaccharide (LPS [1 ng/mL], aiming to induce IDO1) in the presence or absence of DA or salbutamol during 6h. Samples were then acquired using FACSDiva software in a FACSCanto II flow cytometer (immunophenotyping, IDO1 induction and cytokine analysis). Data were presented as mean±standard deviation. Unpaired Student's t-tests, Mann-Whitney test, One-way ANOVA with the post hoc analysis -Tukey test – and Friedman test with the post hoc analysis -Dunn test – and two-way ANOVA with the posthoc analysis Tukey's multiple comparisons test – were used to calculate p values. Differences were significant at $p<0.05$. When analysing unstimulated circulating DCs and monocytes, p-values were corrected with the Benjamini-Hochberg (q*) test.

**Results** PD patients showed significantly increased levels of CD16+ double negative classical DCs and increased levels of CD62L+ monocytes, which reached statistically significance in males ($p<.0.05$), when compared to HS. LPS induced a higher decrease of non- classical monocytes (NC-Mo, $p=0.003$) and a higher increase in IDO1+ monocytes populations (Mo;$p=0.0295$; I-Mo; $p=0.0284$ and NC-Mo; $p=0.0255$) in PD patients, when compared to HS. DA seems to further decrease DC and NC-Mo populations in PD patients.

Importantly, salbutamol, but not DA, decreased significantly less IDO1+ monocytes in PD when compared with HS monocytes. DA and salbutamol significantly attenuated the LPS-induced increase in TNF-a and MCP-1 in HS but not in PD patients.

**Conclusions** Our data suggest that DA has anti-inflammatory properties and β2-adrenoceptor contribute to some of the DA effects. Importantly, catecholaminergic modulation of myeloid cells seems to be modified in PD. This may have diagnostic and therapeutic relevance in PD.

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# Transcription factor mRNA levels in circulating CD4+ T cells predict phenoconversion in Idiopathic REM sleep behavior disorder

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**Background** Idiopathic REM sleep behaviour disorder (iRBD) is a major predictor of phenoconversion to chronic neurodegenerative synucleinopathies. Early biomarkers of phenoconversion are crucial to identify individuals at high risk, who could benefit from more aggressive neuroprotective therapy.

**Objective** To assess transcription factor mRNA levels in circulating CD4+ T cells as predictive biomarkers of phenoconversion.

**Methods** Patients with iRBD, originally enrolled in a study investigating transcription factor mRNA levels in CD4+ T cells, were followed prospectively. A receiver operating characteristic ROC curve analysis was used to assess the discrimination between phenoconverted (converters) and not phenoconverted (non-converters) subjects. Phenoconversion was also evaluated by Kaplan-Meier analysis and hazard ratios (HR) were caculated.

**Results** The study includes 31 iRBD subjects, followed on average for 1277 days. During follow-up, 8 subjects phenoconverted. CD4+ T cells from converters had higher *STAT1*, and *GATA3* and *FOXP3*. *STAT1* provided on average good discrimination (mean area under the ROC curve [AUC]: 0.886, 95% CI: 0.765-1.000), *GATA3* excellent discrimination (AUC: 0.918, 95% CI: 0.775-1.000), and *FOXP3* acceptable discrimination (AUC: 0.799, 95% CI: 0.598-0.999). HR was of 58.3 (95% CI: 6.2-547.1) for high *STAT1*, 101.2 (95% CI: 16.8-609.4) for low *GATA3*, and 15.7 (2.7-91.4) for lowe *FOXP3*.

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**Conclusions** *STAT1*, *GATA3* and *FOXP3* mRNA in CD4+ T cells are promising predictive biomarkers predictive of phenoconversion. Confirmatory studies are needed for biomarker validation. Results also support the early engagement of peripheral immunity as a key contributor leading to neurodegeneration as well as its potential as target for neuroprotective strategies.

# Comparative analysis of T cell phenotype shifts in immunosenescence across young, healthy elderly, and frail subjects.

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Frailty, a multi-organ disorder associated with aging, stems from intricate biological changes involving genomic, biomolecular, and immunological factors[1]. Potential frailty biomarkers, including those related to inflammation, immunosenescence, and cellular aging, have been identified[2,3]. Immunological variations can influence aging, the onset of frailty, and neurodegenerative diseases such as Parkinson's[4].
This study aims to assess functional differences in CD4+ and CD8+ T lymphocyte subsets among healthy young individuals, healthy elderly, and frail elderly subjects.
The study adopts a cross-sectional design with three distinct groups (G): G1: Healthy young subjects (≤55 years); G2: Healthy (non-frail) elderly subjects (>65 years); and G3: Frail elderly subjects (>65 years). Frailty in elderly subjects was determined using the SHARE-FI score based on Fried's criteria[5], which includes assessments such as the walking speed test, time up and go test, short physical performance battery (SPPB), and handgrip and weight evaluations.
The immunophenotypic profile of circulating CD4+ and CD8+ T cells was assessed by flow cytometry, following established procedures[6]. The analysis included subsets such as naïve (NV, CD45RA+CCR7+), central memory (TCM, CD45RA-CCR7+), effector memory (TEM, CD45RA-CCR7-), effector memory CD45RA+ (TEMRA, CD45RA+CCR7-), T helper subsets (Th1, Th2, Th17, and Th1-17), and regulatory T cell subsets (total Treg, naïve Treg, and activated Treg). The expression of CD28 and CD57 was also examined in non-activated/early-activated, activated, activated/early-senescent, and terminally differentiated/senescent T cells.

A total of 111 subjects were enrolled in the study across the three groups. Four subjects from G3 were excluded from the study due to exclusion criteria.

Additionally, some samples were not processed because of technical issues (1 subject from G1 and 4 subjects from G2). Finally, the results reported below were based on the analysis of samples from 102 subjects: 35 from G1 (17F/18M); 31 from G2 (14F/17M) and 36 from G3 (23F/13M).

Notably, a significant reduction in the percentage of CD4+ T Naïve cells (CD45RA+CCR7+) was observed in G2 and G3 compared to G1. This decrease in naïve T cells was associated with an increase in CD4+ TEM (CD45RA-CCR7+) in G2 and G3. Similar trends were observed in CD8+ T naïve cells, where both percentage and absolute counts were significantly lower in G2 and G3 compared to G1. Additionally, CD8+ TEMRA (CD45RA+CCR7-) cells increased in the elderly groups.

Analysis of CD28 and CD57 expression revealed increased CD57+ cells in both CD4+ and CD8+ T cells in elderly subjects (G2 and G3) compared to young subjects (G1), primarily driven by higher percentages of activated CD28+CD57+ and terminally differentiated-senescent CD28-CD57+ T cells. Conversely, non-activated/early-activated CD28+CD57- T cells decreased in elderly subjects. Frail elderly subjects (G3) exhibited higher percentages and numbers of activated/early senescent CD4+CD28-CD57- T cells compared to healthy elderly subjects (G2).

Evaluation of T helper (Th) subsets and regulatory T cells (Treg) showed higher percentages of Th2 cells in elderly healthy subjects (G2) compared to young subjects (G1). Additionally, frail elderly subjects (G3) exhibited lower percentages of Th2 cells than healthy elderly subjects (G2). Circulating total Treg did not differ significantly among groups, except for lower percentages in frail elderly subjects (G3) compared to young subjects (G1). Notably, nTreg cells were lower, while aTreg cells were higher in elderly subjects (G2 and G3) compared to young subjects (G1).

In summary, our findings indicate age-related alterations in T cell subsets and senescence markers.

In conclusion, this study provides comprehensive insights into

immunological profiles across different aging and health conditions, shedding light on potential links between immunophenotyping and frailty indices. Understanding these associations could contribute to a better comprehension of the aging process and the development of age-related disorders, including Parkinson's disease.

**References**

1. Cerreta, F., Ankri, J., Bowen, D., Petrovic, M., Rosa, M., Pillotto, A., ... & Jansen, P. (2016). Baseline frailty evaluation in drug development. J Frailty Aging, 5(3), 139-140.
2. Cardoso, A. L., Fernandes, A., Aguilar-Pimentel, J. A., de Angelis, M. H., Guedes, J. R., Brito, M. A., ... & Trendelenburg, A. U. (2018). Towards frailty biomarkers: Candidates from genes and pathways regulated in aging and age-related diseases. Ageing research reviews, 47, 214-277.
3. Collerton, J., Martin-Ruiz, C., Davies, K., Hilkens, C. M., Isaacs, J., Kolenda, C., ... & Kirkwood, T. B. (2012). Frailty and the role of inflammation, immunosenescence and cellular ageing in the very old: cross-sectional findings from the Newcastle 85+ Study. Mechanisms of ageing and development, 133(6), 456-466.
4. Kouli, A., & Williams-Gray, C. H. (2022). Age-related adaptive immune changes in Parkinson's disease. Journal of Parkinson's Disease, 12(s1), S93-S104.
5. Fried, L. P., Tangen, C. M., & Walston, J. (2001). Newman a B, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci, 56(3), M146-56.
6. Kustrimovic, N., Comi, C., Magistrelli, L., Rasini, E., Legnaro, M., Bombelli, R., ... & Cosentino, M. (2018). Parkinson's disease patients have a complex phenotypic and functional Th1 bias: cross-sectional studies of CD4+ Th1/Th2/T17 and Treg in drug-naive and drug-treated patients. Journal of neuroinflammation, 15(1), 1-17.

# Unraveling the transcriptomic signatures of Parkinson's disease and major depression using single-cell and bulk data

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**Background** Motor symptoms are well-characterized in Parkinson's disease (PD). However, non-motor symptoms such as depression are commonly observed and can appear up to 10 years before motor features, resulting in one third of individuals being misdiagnosed with a neuropsychiatric disorder. Thus, identifying diagnostic biomarkers is crucial for accurate PD diagnosis its prodromal or early stages.
**Methods** We employed an integrative approach, combining single nucleus RNA and bulk mRNA transcriptomics to perform comparative molecular signature analysis between MD and major depressive disorder (MDD). We examined 39,834 nuclei from PD (GSE202210) and 32,707 nuclei from MDD (GSE144136) in the dorsolateral prefrontal cortex (dlPFC) of Brodmann area 9. Additionally, we analyzed bulk mRNA peripheral blood samples form PD compared to controls (GSE49126, GSE72267), as we as MDD compared to controls (GSE39653).
**Results** A higher proportion of astrocytes, and oligodendrocyte cells in the dlPFC of individuals with PD vs MDD. The excitatory to inhibitory neurons (E/I) ratio analysis indicates that MDD has ratio close to normal 80/20, while PD has a ration of 62/38, indicating increased inhibition in the dlPFC. Microglia displayed the most pronounced differences in gene expression profiles between the two conditions. In PD, microglia display a pro-inflammatory phenotype while in MDD, they regulated synaptic transmission through oligodendrocyte-microglia cross talk. Analysis of

bulk mRNA blood samples revealed that the *COL5A*, *MID1*, *ZNF148* and *CD22* genes were highly expressed in MDD. *CD22* is involved in B-cell activation and the negative regulation of B-cell receptor signalling. Additionally, *CD86*, which provides co-stimulatory signals for T-cell activation and survival, was found to be a commonly differentially expressed gene in both conditions. Pathway analysis revealed several immune-related pathways common in both conditions, including the complement and coagulation cascade and B-cell receptor signalling.

**Discussion** The study demonstrates that bulk peripheral immune cells play a role in both conditions, but neuroinflammation in the dlPFC specifically manifests in PD. Integrating these two omics levels offers a better understanding of the shared and distinct molecular pathophysiology of PD and MDD in both the periphery and brain. These findings could lead to potential diagnostic biomarkers, improving accuracy and guiding pharmacological treatments.

# Microbiome research tools beyond 16S rRNA and shotgun sequencing

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Our research group focuses and aims for the identification of minimally invasive biomarkers for disease diagnostics and therapy response stratification. To achieve in this goal we typically apply a multi-omics approach by investigating mRNA and miRNA expression, DNA methylation, protein- and antibody profiles in parallel taking advantage here of high throughput technologies such as microarrays and next generation sequencing. Multi-omics strategies are at the forefront of personalized medicine and are grounded in the understanding that complex diseases cannot be fully characterized by isolate bio measures but rather by studying the interactions between genes, transcripts, proteins, to elucidate biological pathways underlying the development and trajectory of complex diseases. Not least multi-omics profiling is perfectly suited to study host-microbiome/pathogen interactions. Along these lines we recently added antibody reactivities directed against microbes as an additional layer of biological and potentially disease-related information which we interrogate in plasma via an in-house produced microarray containing crude protein lysates obtained from 253 bacterial and 7 fungal strains. We will showcase a multi-omics study aiming for minimally-invasive biomarkers able to predict the advent of atherosclerotic plaque formation in coronary arteries (stenosis) and report here among others on significant differences detected in antibody reactivities against certain bacteria when comparing stenosis and control patients. Further, we will introduce phage immunoprecipitation sequencing (PhIP-Seq) as a novel approach to study antibody responses against specific, predefined peptides. PhIP-Seq utilises the T7 phage to express antigenic peptides on its surface. After contact with patient plasma, antibody-reactive phage peptides can then be identified by NGS, as the identity of the reactive peptide in the phage genome is reflected by the prior generation of corresponding oligonucleotide pool phage libraries.

# Myeloid-derived suppressor cells as biomarkers of disease severity in multiple sclerosis: relevance to Parkinson's disease?

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Myeloid-derived suppressor cells (MDSCs) have emerged as a novel immune cell population with a crucial role in immune-related disorders, including multiple sclerosis (MS). They comprise a heterogeneous population of immature myeloid cells whose primary function is the promotion of immune tolerance by promoting T cell anergy and/or apoptosis or by inducing Treg. Our laboratory has extensively studied the morphofunctional characterization of monocytic-MDSCs (M-MDCs) throughout the clinical course of the MS animal model, experimental autoimmune encephalomyelitis (EAE). The phenotype and activity of M-MDSCs vary according to the clinical course severity of the animal model. In recent years, we have shown how the severity of the clinical course affects the number and activity of M-MDSCs and that the circulating load of M-MDSCs is useful for predicting the future disease severity in

EAE. Importantly, for the first time, we have characterized the presence and phenotype of M-MDSCs in the central nervous system of patients with MS. Their higher abundance is associated with high inflammatory demyelinating areas as well as milder clinical courses. Our group has pioneered the study of circulating M-MDSCs as biomarkers of disease severity in MS. In this sense, the presence of M-MDSCs in relapsing remitting MS patients is associated with a higher immunoregulatory environment one year later. Abundance of M-MDSCs, but not other regulatory cells such as Treg, appears to be a prognostic factor for complete recovery from relapse. Lastly, M-MDSCs (along with so-called early-MDSCs-eMDSCs) are specifically underrepresented in benign MS blood many years after disease onset. Although the increased level of MDSCs has been also described in the blood of patients with Parkinson's disease, its relationship with the different stages of the disease or its value as a biomarker of this pathology remains fully unexplored. In sum, our data indicate that circulating MDSCs are a very promising biomarker for predicting disease severity in MS, which opens the door to analyze their usefulness as a prognostic biomarker in other neurodegenerative pathologies.

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# Glial DJ-1 neuroprotection in a zebrafish Parkinson´s Disease model

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The *park7* encoded DJ-1 is a multifunctional protein with particular role in oxidative stress regulation. A non-functioning DJ-1 is associated both to familiar Parkinson´s disease and spontaneous neurodegenerative diseases suggesting that DJ-1 may have a general neuroprotective function. In particular, glial DJ-1 seems to be highly important in order to protect neighbouring neurons. To elucidate the driving mechanisms behind glial DJ-1´s neuroprotective role we have established knock-out and transgenic zebrafish models of DJ-1. This presentation will show the response to DJ-1 loss and re-insertion of glial DJ-1 with emphasis on methods and lines available for collaboration.

# The effects of prolonged exposure to dopaminergic neurotoxins on AMPK and mTORC2 signaling

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Standard models used in *in vitro* studies of dopaminergic neurons' (DA) deterioration, found in many neurodegenerative diseases, include application of the 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium ($MPP^+$). Multiple cell signaling pathways have been found to be impaired in these diseases, including the adenosine monophosphate activated protein kinase (AMPK) signaling pathway, major energy-regulating system within the cell, as well as the mammalian target of rapamycin complex 2 (mTORC2), important for cell survival, metabolism and cytoskeleton organization. However, the exact role of the mTORC2 and the interaction between mTORC2 and AMPK in DA neurodegeneration models remain insufficiently elucidated.

We aimed to explore the effects of prolonged 6-OHDA and $MPP^+$ treatments on the activity of AMPK and mTORC2 components, as well as mTORC2 downstream targets.

The experiments were performed on the SH-SY5Y cell line. The neurotoxin concentrations were determined using MTT viability assay after treatment with decreasing concentrations of 6-OHDA (30-0.23μM) or $MPP^+$ (1-0.008mM) for 7 days. After 3, 5, and 7 days of treatment with 6-OHDA (15μM) or $MPP^+$(0.25mM), using the immunoblot method, we analyzed the activity of AMPK and mTORC2 components, as well as their downstream targets (Akt, GSK-3β), and expressed it as the level of phosphorylated compared to total forms of these enzymes, or the protein level compared to β-actin expression.

The prolonged 7-day treatment caused a dose-dependent decrease in cell viability, with the $IC_{50}$ values of 15μM for 6-OHDA and 0.25mM for $MPP^+$. The immunoblot analysis revealed increase in activity of AMPK

and mTORC2 component Sin1 following the 3-day treatment with $MPP^+$, but not 6-OHDA. The 5-day treatments with either neurotoxin caused an increase in phosphorylation of AMPK and mTORC2 components Sin1, Rictor and DEPTOR, as well as mTORC2 downstream targets, Akt and GSK. The increase in phosphorylation continued for the 7-day treatment for AMPK, Sin1, DEPTOR and Akt, with decrease in activity for Rictor and GSK.

Our results indicate that prolonged neurotoxin treatments lead to the activation of the AMPK and some of the mTORC2 components, but the exact relationship and role of these proteins in neurotoxin-induced DA damage warrants further investigation.

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# Understanding the physiological functions of Activity-Dependent Neuroprotective Protein (ADNP) in microglial responses: linking neurodegeneration and neuroinflammation

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The Activity-Dependent Neuroprotective Protein (ADNP) is well stablished as an essential factor for neuronal differentiation and functioning. Some studies have demonstrated lower ADNP expression levels in Parkinson's disease at early stages.
Moreover, ADNP has been uncovered as a participant in microtubule dynamics regulation, which is crucial in tauopathy, a sign of neurodegeneration present, among others, in Parkinson's disease patients. Additionally, the implication of microglial cells in the inflammatory dysregulation that accompanies neurodegeneration has been extensively reported. Although very recently ADNP has been associated to acquired immune responses in T cells, the physiological role of ADNP in innate microglial responses is still unknown. Thus, we have generated ADNP-KO SIM-A9 microglial cells, which have been found to exhibit key alterations in both MyD88-dependent and independent inflammatory pathways. ADNP-KO cells also display overactivation in TANK-binding kinase 1 signaling, and defective autophagy/mitophagy mediators as well as impairment in mitochondrial metabolic activity. This correlates with genomic instability in primary neurons in culture. Our results disclose for the first time a physiological role of ADNP as an inflammatory repressor protein in microglia. Furthermore, they might help to intervene in altered neuroinflammatory responses in neurodegenerative conditions such as Parkinson's Disease.

# Scaling up production of extracellular vesicles – next step towards clinical therapies against Parkinson's disease

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We and others have demonstrated excellent therapeutic efficacy of extracellular vesicles (EVs) from human dental pulp stem cells (DPSCs) in unilateral 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease (PD). These proof of concept studies have been performed with EVs isolated using laboratory scale methods. However, therapeutic applications require large amounts of EVs that can only be obtained by scaling up production and isolation.

In this study we used immortalized human DPSC line to select optimal combination of xeno-free medium and microcarriers for optimization of EV production in the stirred tank bioreactors. Isolation of EVs using tangential flow filtration/size-exclusion chromatography (TFF/SEC) resulted in an impressive 463-fold increase in yield when compared with standard laboratory scale method (conventional 2D cultures, EV isolation performed using differential centrifugation).

Characterization and comparison of the EV cargo between preparations isolated using standard lab scale and scaled up protocols revealed that (i) both cell culture and isolation methods affect proteomic and RNA cargo of the EVs; (ii) isolation using TFF/SEC decreased complexity of proteome and RNA species of EVs.

We next compared therapeutic efficacy of EVs derived by both methods in the 6-OHDA rat model of PD. For intranasal applications we used the same amounts of EVs (as determined by NTA analysis). Healthy and PD-type Wistar male rats (11-12 weeks old) were treated with vehicle or EVs for 17 consecutive days. CatWalk and Morris water maze tests were performed to determine the effects of EVs on motor

and cognitive functions, respectively. Changes in protein levels involved in neuroinflammation (Iba-1, GFAP), dopamine production (tyrosine hydroxylase) and inflammasome activity (IL-1β) were analyzed using immunohistochemistry (IHC). Gait and spatial learning/memory of EV-treated rats were significantly improved compared to 6-OHDA group. Dopamine production was preserved, and inflammatory protein levels were decreased in 6-OHDA group rats treated with EVs.

Functional and IHC studies revealed that both EV preparations showed very similar therapeutic efficacy. We therefore focused on the miRNAs and proteins that were most abundantly expressed and presented in both groups. Bioinformatic analysis of proteomic cargo revealed that both EV preparations were enriched in proteins (i) associated with various aspects of lipoprotein particle transport and metabolism; (ii) innate immune responses; and (iii) proteasome-related processes.

Finally, transciptomic analyses were performed from the RNA extracted from the *striatum* and *substantia nigra* of experimental animals. Bioinformatics revealed that in 6-OHDA treated rats EVs significantly affected expression of genes associated with several biological processes including synaptic transmission, negative regulation of neuronal death and neurogenesis.

Our results underscore the potential of large-scale EV production and emphasize the therapeutic advantages of EV-based interventions against PD.

# Neuroinflammation and neurodegeneration in SARS-CoV-2 infected macaques: A link between COVID-19 and Parkinson's disease?

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Infection with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) results in respiratory symptoms, yet neurological symptoms are common as well, both in acute phase as well as months later in patients with post-COVID syndrome. COVID-19 induced systemic inflammation and neurological damage could contribute to the development of neurodegenerative diseases. With more than 700 million reported coronavirus infections worldwide, the impact of such late-onset outcomes is inconceivable.

Viral infections can trigger α-synucleinopathies, and SARS-CoV-2 infection is considered responsible for the development of parkinsonism, however, the mechanism by which COVID-19 triggers neurodegeneration remains to be determined.

In SARS-CoV-2 infected macaques, a model for mild COVID-19, we showed ongoing neuroinflammation throughout the brain by longitudinal TSPO-PET imaging. In addition, we showed significant alterations in glial cells and infiltrated immune cells by post-mortem immunostainings in various brain regions, including the hippocampus and pons. When focusing on the substantia nigra we not only find signs of neuroinflammation, but also a decline in the number of dopaminergic neurons, accompanied by aberrant α-synuclein. Moreover, (phosphorylated) α-synuclein aggregates are seen in the meninges and choroid plexus of infected animals. This study shows a direct link between SARS-CoV-2 infection and neuropathology associated with Parkinson's disease, in a model that closely resembles humans.

We are currently exploring this further in brain tissue from macaques 2 weeks and 5 months after infection. Moreover, we are analyzing

the proteomic signature of cerebrospinal fluid samples from these animals before and after infection.
Our models are valuable for studying the long-term consequences of SARS-CoV-2 infection on the brain and can help to understand the risk for neurodegenerative disease following the COVID-19 pandemic. In addition to studying the pathophysiological mechanisms, our models can aid in biomarker and target discovery for the development of diagnostic tools and therapies.

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# ABSTRACTS - POSTER PRESENTATIONS

# The effect of adenosine monophosphate-activated protein kinase (AMPK) activation on the mTOR complex 2 signaling pathway in neurotoxin-induced cell death

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**Introduction** 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl pyridinium (MPP+) are commonly used neurotoxins in neurodegeneration studies. The mammalian target of rapamycin (mTOR), a serine/threonine kinase, is present in the cell in two forms, mTORC1 and mTORC2. While the functions of mTORC1 are well understood, the roles of mTORC2 still need to be clarified. However, it is believed that its activation is involved in cell survival. Adenosine monophosphate-activated protein kinase (AMPK) functions as an energy sensor in the cell and is activated in conditions of increased AMP/ATP ratio. It has recently been shown that AMPK can directly phosphorylate mTORC2 components to increase mTORC2 activity, but their interaction in neurotoxin-induced models of neurodegeneration is still insufficiently elucidated.

**Aim** Our study aimed to examine the effect of constitutive activation of AMPK on the mTORC2 signaling pathway in neurotoxicity caused by MPP+ and 6-OHDA.

**Material and methods** Experiments were performed on the SH-SY5Y human neuroblastoma cell line. The cells were divided into the control group (cells transfected with the control plasmid) and the CA-AMPK group (cells transfected with plasmid carrying information on constitutive AMPK activation). We used an MTT assay to determine cell viability after the treatment with MPP+ or 6-OHDA. The levels of phospho- and total forms of mTOR, Sin1, Rictor, and Akt after MPP+ or 6-OHDA treatment were determined using the immunoblot method.

**Results** MPP+ and 6-OHDA decreased the viability of SH-SY5Y cells. Activation of AMPK increased the survival of cells treated with $MPP^+$ but increased the neurotoxicity of 6-OHDA. Both MPP+ and 6-OHDA treatment caused the increase in the expression of p-mTOR, p-Sin1, and p-Akt in the control group. AMPK activation decreased the levels of phosphorylated forms of mTOR and Rictor after MPP+ and 6-OHDA treatment. Also, in the CA-AMPK group, neurotoxin treatments increased the expression of p-Sin1 and p-Akt compared to control cells.

**Conclusion** The role of AMPK exhibits a distinct effect in the neurotoxicity induced by MPP+ compared to the 6-OHDA mediated toxicity. An interrelation exists between AMPK and mTORC2, but the underlying mechanism warrants additional exploration.

# The potential role of microRNAs in modulating HERV-K expression in Parkinson's disease

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Parkinson's disease (PD) represents one of the most debilitating neurodegenerative motor disorders. Recently, the involvement of Human Endogenous Retroviruses (HERVs) in this condition has been documented. PD patients present upregulation of HERV transcripts in prefrontal cortex tissue, prefrontal neurons, and blood samples compared to healthy controls, but the mechanisms triggering their overexpression are unknown. Several studies have highlighted the deregulation of specific miRNAs in PD. Within this context, our study explores the regulatory mechanisms of microRNAs (miRNAs) on HERV expression with a specific focus on HERV-K, the most recently integrated endogenous retrovirus. Based on in silico analyses we foretold the potential binding affinity between specific miRNAs and the HERV-K transcripts hypothesizing the downregulation of miRNAs as a mechanism that prompts an upregulation of HERV-K in PD. To validate this theory, we performed co-transfections of the human embryonic kidney 293 (HEK-293) cell line with a vector encoding a HERV-K consensus sequence together with selected miRNAs and we measured the levels of HERV-K transcripts and proteins by PCR and western blot, respectively. To further elucidate the role of these miRNAs, we performed microRNA-only transfections in the TERA-1 cell line. It is noteworthy that this peculiar cell model naturally exhibits high endogenous HERV-K expression, thus offering a closer approximation of in vivo conditions. Our data suggests a differential regulation of HERV-K expression by miRNA

182, which inhibits translation, and miRNA 221, which promotes mRNA degradation. However, subsequent experiments conducted in Tera-1 cells did not demonstrate a significant effect post- transfection underscoring a potential variability in the physiological impact of miRNAs on various HERV-K loci across distinct cell types. We are currently investigating the expression levels of miRNAs in PD patients versus healthy controls. This data will be cross-referenced with the gene expression levels of HERV-K aiming to identify potential correlations. Our goal is to elucidate the molecular mechanisms regulating HERVs, which eventually point out the use of miRNAs as regulators of their expression.

# Olfactory dysfunction is related to impaired plasticity of neuronal networks and dopamine metabolism in the olfactory bulb in a rat model of multiple sclerosis

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Multiple sclerosis is a chronic immune-mediated, neuroinflammatory and neurodegenerative disease of the central nervous system that usually affects young adults between the ages of 20 and 40 and manifests itself in a variety of sensory, motor and cognitive symptoms. Olfactory dysfunction is a common early sensory symptom of various neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease and multiple sclerosis (MS). In most patients, it appears long before the onset of motor and cognitive symptoms. Therefore, an impaired sense of smell could serve as a valuable diagnostic marker for the prodromal phase of the disease, disease progression and even response to therapy. The aim of this study was to investigate the plasticity of neuronal networks and neurochemical changes in the olfactory bulb (OB), focusing on the role of dopamine in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. The animals used in this study were 2-month-old male Dark Agouti rats immunized with spinal cord homogenate in complete Freund's adjuvant supplemented with Mycobacterium tuberculosis. Rats were examined daily and sacrificed at the peak of disease (11-13 dpi) to isolate olfactory bulb tissue for immunohistochemistry, immunoblot, and high-performance liquid chromatography. In EAE, we observed decreased density of dopaminergic periglomerular neurons in the OB, but increased tyrosine hydroxylase (TH) protein expression and elevated dopamine (DA) levels compared to healthy controls. The decreased density of DA neurons likely alters the resting activity of mitral cells, contributing to olfactory dysfunction. In terms of dopamine, the effect of reduced DA neuron number is possibly reversed by an increase in the expression of the dopamine-producing enzyme TH, as confirmed by an increased dopamine concentration in the OB, leading to a deterioration of olfactory ability. Therefore, the characterization of changes in the olfactory network and the neurochemical profiling of OB could play a very important role in the diagnosis of neurodegenerative diseases in the future.

# Sortilin expression in immune cells as a potential biomarker for the Parkinson's disease patients

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Parkinson's disease (PD) is one of the most common neurodegenerative disorders, affecting mainly the elderly human population worldwide. The extreme variability of symptoms is one of the hallmarks of Parkinson's disease. Because of this heterogeneity, finding biomarkers to support diagnosis, pharmacological treatment, or/and monitoring the disease's progression is an essential aspiration. In this study, a novel cohort of patients was recruited that consisted of 11 healthy volunteers (7 males and 4 females) and 10 PD patients (4 males and 6 females) at the outpatient clinic at General Hospital of Larissa, Thessaly, Greece. We investigated whether circulating innate and adaptive immune cells are affected in PD patients, utilizing flow cytometry. We observed a markedly reduced proportion of B cells and monocyte populations in PD patients, whereas T cell numbers were similar between the two groups. We also examined the surface levels of sortilin, a member of the VPS10P (Vacuolar Protein Sorting 10 Protein) family of receptors, that has been correlated with Alzheimer's disease. Sortilin levels appeared to be higher in all immune cells examined in PD patients compared to healthy donors, without achieving statistical significance. At the same time intracellular flow cytometry was performed for the transcription factor p65/NF-kB, which is an important regulator of *SORT1*, the gene that encodes human sortilin. We observed statistically significant high levels

of p65/NF-kB in T cells from PD donors (MFI 2.316 ±0.43) compared to healthy individuals (MFI 1.007±0.063), ($p$<0.01). Flow cytometry on PBMC-derived macrophages from PD patients showed higher sortilin expression levels compared to cells from healthy donors. When PBMC-derived macrophages were treated with ox-LDL they formed foam cells. Moreover, cells from PD patients that expressed high levels of sortilin on the cell surface showed high number of foam-cell formation (62.74±5.6) compared to cells from healthy donors (46.5±5.7). These results indicate that sortilin may be a a potential biomarker for Parkinson's disease and a novel candidate as therapeutic target to prevent the development of atherosclerotic plaque in PD patients.

# Parkinson's disease therapeutic strategies applied in SH-SY5Y neuron-like cell in vitro model – emphasis on neuron protective potential of Magnetic stimulation

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SH-SY5Y is a cell line derived from human neuroblastoma, and it is frequently used in vitro model to study Parkinson's disease (PD). PD is a neurological condition with selective progressive degeneration of dopaminergic neurons, being handled with only shortly effective symptomatic or palliative therapies. Searching through the horizon of new therapeutic approaches and ideas in the battle with PD, our team explored the repetitive transcranial magnetic stimulation (rTMS), which is shown to be beneficial in several animal models of neurodegeneration, including PD. Along a comprehensive in vivo study and ample amount of data gained in the 6-hydroxydopamine (6-OHDA) induced experimental model of PD, we have decided to extend the perspective of magnetic stimulation (MS) impact directly on cellular level. Thus, we have utilized the in vitro modeling strategy for PD: 6-OHDA mediated toxicity in SH-SY5Y differentiated neuron-like cell cultures. It was our interest to analyze the general electrophysiological functionality of these cultures and the MS therapeutic potential in the neuron recovery from the 6-OHDA induced damage. Additionally, the levels of oxidative stress and antioxidative protection parameters will be determined with or without the MS, paralleling our recent research in the in vivo study. Alternatively, we aim to screen for the therapeutic effect of istradefylline (a nondopaminergic drug that exerts its effects via adenosine A2A receptor antagonism, which is a potential therapeutic in PD) and preventive effect of ALA

(α-lipoic acid, which has strong antioxidant characteristics) in these 6-OHDA affected neuron-like cell cultures. In perspective our in vitro study will be broadened with the analysis of purinergic signaling system components, which play an impactful role in neuroinflammation, and the development in neurodegeneration.

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# Genetic Study of Early Onset Parkinson's Disease in Cyprus

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**Background** Parkinson's Disease (PD) is a multifactorial neurodegenerative disease characterized by motor and non-motor symptoms. The etiology of PD remains unclear. However, several studies have demonstrated the interplay of genetic, epigenetic, and environmental factors in PD. Early-onset PD (EOPD) is a subgroup of PD diagnosed between the ages of 21 and 50. Population studies have demonstrated great genetic variability amongst EOPD patients, suggesting that geographic location and ethnic origin influence the detection outcome. Inclusivity is very important in PD research and hence filling the genetic gap in underrepresented populations is very useful for better disease understanding. Hence, this study aimed to obtain a genetic landscape of EOPD in the Cypriot population.

**Methods** Greek-Cypriot EOPD patients (*n* = 48) were screened for variants in the six most common EOPD-associated genes (*PINK1*, *PRKN*, *FBXO7*, *SNCA*, *PLA2G6*, and *DJ-1*). This included DNA sequencing and Multiplex ligation-dependent probe amplification (MLPA) to detect single nucleotide variants (SNVs), insertions or deletions (Indels) and copy number variation (CNV) in the aforementioned genes.

**Results** One previously described frameshift variant in *PINK1* (NM_032409.3:c.889del) was detected in five patients (10.4%)—the largest number to be detected to date. CNVs in the *PRKN* gene were identified in one homozygous and 3 compound heterozygous patients

(8.3%). No pathogenic variants were detected in the other 4 genes (*FBXO7*, *SNCA*, *PLA2G6*, and *DJ-1*) under investigation in this study.
**Discussion** The EOPD-associated genes that were under investigation in this study seem to interact with each other on a structural and functional level. The function of the EOPD-associated genes has been linked to protection against mitochondrial dysfunction, the mediation of mitophagy, and to roles in synaptic transmission and phospholipid remodeling. Hence, variants in either one of the aforementioned genes have the potential to alter cellular processes and potentially increase PD susceptibility. Currently, the diagnosis of PD is clinical and based on the presence of motor features. Early onset patients have a challenging journey towards a PD diagnosis as their initial symptoms may vary and their young age of onset may lead to differential diagnoses. To date, the pathogenic variants identified in this study have explained the PD phenotype for 18.8% of the EOPD cases. Almost 1 in every 5 patients in our cohort has been identified as a carrier of either a *PINK1* or *PRKN* pathogenic variant. Hence, the results of this study may contribute to the genetic screening of EOPD in Cyprus.

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