7th Hellenic Pasteur Institute Young Scientists Conference 2024

17-18 October 2024

NEUROBIOLOGY

IMMUNOLOGY

BIOINFORMATICS

MICROBIOLOGY

PUBLIC HEALTH

BIOTECHNOLOGY

Hellenic Pasteur Institute, Central Amphitheater K8
127 Vasilissis Sofias Ave, Athens

FREE ENTRANCE

Keynote Speakers

George Paxinos
Mark Mattson
Evangelos Giamarellos-Bourboulis
Emmanouil Maragkakis

CALL FOR ABSTRACTS

21:00 Garden Latin Party & Dinner (18/10)

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ABSTRACT BOOK

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INTRODUCTION

We are happy to present you the abstract book of the **7th Hellenic Pasteur Institute Young Scientists Conference 2024,** that took place on October 17–18, 2024, at the Hellenic Pasteur Institute in Athens, Greece**.** This year we decided to open the Conference up to other institutions and laboratories across Greece and also extended its duration to a two-day event. The idea was to bring together emerging scientists from diverse fields, providing a forum for the exchange of ideas, collaborations, and innovative research.The presentations, discussions, and collaborations that took place during the conference highlighted the talent of young researchers who are poised to make significant contributions to their fields. With topics ranging from immunology, microbiology, and public health to cutting-edge developments in bioinformatics and biotechnology, the research presented reflected the energy and ambition of a new wave of scientific thought. During the event, the HPI amphitheater was filled with young scientists. All oral presentations can be found on Diaulos link (https://diavlos.grnet.gr/event/e4551).

We would like to express our gratitude to the Direction and Administration of the Hellenic Pasteur Institute, and especially to the General Director, Dr. Paraskevi Zisimopoulou, for her invaluable support throughout this period. Additionally, we extend our thanks to Dr. Christina Oikonomopoulou, who was always by our side, ready to assist with anything we requested. Our thanks also go to Ms. Angeliki Lymberopoulou for her impeccable attention to the venue's details and care, as well as to Panos Kalyvas and the entire Technical Support team who were always present. We would also like to the thank the President of the Executive Board of HPI, Prof. Dr. George P. Chrousos. We would also like to warmly thank Ms. Roubini Athanasopoulou for her contributions to the catering and beyond, and Ms. Angeliki Nakou and Ms. Spyridoula Athanasopoulou for their secreteriat support. Finally, special thanks to our friend Stavros Naoum for organizing the photography contest! We owe special gratitude to the external scientific committee, Prof. Dr. Eleni Ntouni, Prof. Dr. Spyros Efthymiopoulos, Dr. Kostas Vekrellis, and Prof. Dr. Georgina Tzanakaki, for their time and dedication in evaluating the 70 research papers, as well as for being present to listen to all presentations and review all posters before selecting the awards. A special thanks to the participants, and the sponsors for their support. Their efforts ensured that the 7th HPI Young Scientist Conference was a landmark event, and we hope to repeat it next year.

We hope that the insights captured in this abstract book will continue to spark curiosity and innovation in the years ahead.

Organizing Committee 2024

Vasileios Gouzouasis, PhD candidate
Athena Boutou, PhD candidate
Anastasia Dagkonaki, PhD
Ilias Roufagalas, PhD candidate
Laboratory of Molecular Genetics, Department of Immunology, Hellenic Pasteur Institute
Research Director Dr. Lesley Probert

Scientific Committee

Dr. Spiros Efthimiopoulos
Professor, Department of Biology, Division of Animal and Human Physiology Director, "Athens International Master's Programme in Neurosciences" National and Kapodistrian University of Athens

Dr. Georgina Tzanakaki
Professor of Public Health Microbiology Head, Laboratory Surveillance of Infectious Diseases (LSID) Head, National Meningitis Reference Laboratory Vice Head, Department of Public Health Policy School of Public Health, University of West Attica

Dr. Eleni Douni
Professor of Animal Biotechnology, Director of the Laboratory of Genetics, Deputy Dean of the School of Applied Biology and Biotechnology, Deputy Head of the Department of Biotechnology, Agricultural University of Athens Affiliated Professor, Institute for Bioinnovation, Biomedical Sciences Research Center 'Alexander Fleming'

Dr. Kostas Vekrellis
Research Director, Neurobiology and Neurodegeneration Investigator A' – Center for Basic Research Biomedical Research Foundation of the Academy of Athens (BRFAA)

Invited Keynote Speakers

Dr.George Paxinos

Scientia Professor George Paxinos studied psychology at The University of California at Berkeley, McGill University and Yale University before taking up a lectureship at The University of New South Wales, in Sydney. He is now an NHMRC Senior Principal Research Fellow at Neuroscience Research Australia and Scientia Professor at The University of New South Wales. He identified 94 hitherto unknown regions in the brain of rats and humans and has published 57 books on the brain and spinal cord of humans and experimental animals and a novel that deals with environmental degradation. Most scientists working on the relationship between brain and emotion, motivation and thought, including neurologic or psychiatric diseases, or animal models of these diseases, use Paxinos' atlases and concepts of brain organization. His first book, The Rat Brain in Stereotaxic Coordinates, is the most cited work in neuroscience. His Atlas of the Human Brain received the American Association of Publishers Award for Excellence in Publishing in Medical Science and the British Medical Association Illustrated Book Award. In a book published in 2024, he was included in the 63 Greek medical scientists considered to have made a historic contribution to medical progress in the period 1821-2021. He served as president of the Australian Neuroscience Society and of the IBRO World Congress of Neuroscience. Twenty-one years in the making, A River Divided is his first novel.

Dr. Mark P. Mattson

Dr. Mark P. Mattson is an American neuroscientist renowned for his contributions to understanding neuroplasticity, aging, and intermittent fasting. He is currently an adjunct professor of neuroscience at Johns Hopkins University School of Medicine and formerly led the Laboratory of Neurosciences at the National Institute on Aging (NIA). Dr. Mattson holds a B.S. in Zoology from Iowa State University, an M.S. in Biology from the University of North Texas, and a Ph.D. in Biology from the University of Iowa. His research has significantly advanced knowledge of the neurotransmitter glutamate's role in neurodegenerative diseases, including Alzheimer's, and he is widely recognized as a leading authority on the health benefits of intermittent fasting. His work on the 5:2 diet has influenced popular dietary practices, and he has authored several books, including The Intermittent Fasting Revolution and Sculptor and Destroyer. Dr. Mattson has received numerous honors, including the Alzheimer's Association Zenith Award and the Metropolitan Life Foundation Medical Research Award, and he was elected a Fellow of the American Association for the Advancement of Science. He also founded NeuroMolecular Medicine and Ageing Research Reviews as their inaugural editor-in-chief.

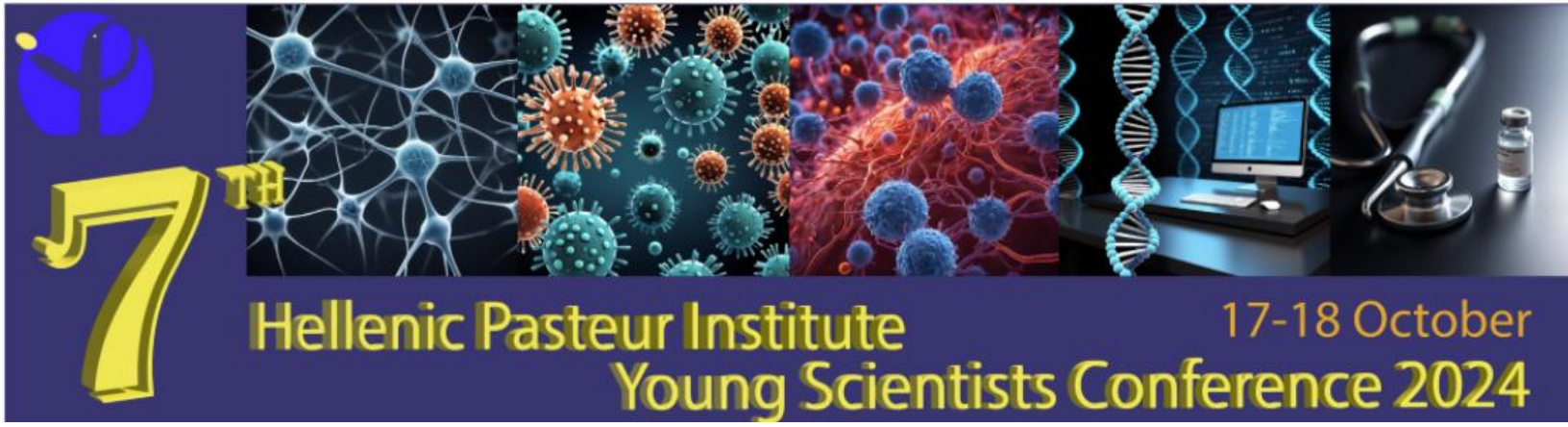
Dr. Emmanouil Maragkakis

Dr. Emmanouil Maragkakis is the head of Computational Genomics Unit in National Institute on Aging, Baltimore, USA. He received his bachelor's degree in Physics from the University of Athens where he focused on computational simulation of neuronal action potentials and signal processing. He received his PhD in a joint program between Biomedical Science Research Center "Alexander Fleming" and Martin Luther University of Halle-Wittenberg. His PhD research involved the analysis and development of microRNA (miRNA) gene target prediction algorithms and the functional analysis of miRNA targeting. He then joined the laboratory of Dr. Zissimos Mourelatos at the University of Pennsylvania where he completed his postdoctoral research work on computational genomics, post-transcriptional gene

regulation, RNA-binding proteins and co-translational mRNA decay. He joined the NIA in 2019 as a Stadtman Tenure-Track Investigator where he leads the Computational Genomics Unit.

Dr. Evangelos Giamarellos Bourboulis

Evangelos Giamarellos Bourboulis is a Professor of Internal Medicine and Infectious Diseases at the Medical School of the National and Kapodistrian University of Athens since 2018. He graduated from the National and Kapodistrian University of Athens Medical School in 1994, where he also completed his PhD in 1998 entitled “The in vitro activity of polyunsaturated fatty acids on Gram-negative bacteria”. He finished his training in Internal Medicine in Laikon Athens Hospital in 2001 and he specialized in the immunology of infectious disease at the Radboud University Nijmegen, The Netherlands in 2006-2007. He is currently Professor of Internal Medicine at Athens Medical School and Supervisor of the Immunology of Infectious Diseases Laboratory and Out-Patient Centre at ATTIKON University Hospital. He is the director of MSc in Infectious Diseases at the National and Kapodistrian School of Medicine in Athens, Greece. His research interests include the pathogenesis of sepsis with emphasis on immunoparalysis, innate immunity and biological therapies in hidradenitis suppurativa, and in vitro activities and pharmacokinetic of antimicrobials and their interactions on multidrug- resistant species. In 2009 he was awarded the Young Investigator Research Award of the European Society of Clinical Microbiology and Infectious Diseases. He is the first who described the complex immune dysregulation of COVID-19. He has published 543 peer-reviewed articles in international scientific journals. He has 36,804 citations with h-index 92. He serves as the current President of the European Sepsis Alliance and he is a board member of the Global Sepsis Alliance. He is also coordinator of the Hellenic Sepsis Study Group.



SCIENTIFIC PROGRAMME

DAY 1 - THURSDAY 17 OCTOBER

09:00-10:00	Registration & Coffee
10:00-10:30	Opening Remarks
10:30-12:00	**Oral Session 1 | Immunology**
	Chair: Dr. Konstantinos Lazaridis, Dr. Vasiliki Kyrargyri
10:30-10:45	Anastasia Dagkonaki, Hellenic Pasteur Institute, *«Maturation of circulating Ly6C^{hi}CCR2^{+} monocytes by mannan-MOG induces antigen-specific tolerance and reverses autoimmune encephalomyelitis»*
10:45-11:00	Eleni Konstantina Loxa, Hellenic Pasteur Institute, *«Application of a competitive ELISA utilizing trimeric spike protein for the detection of neutralizing antibodies in sera of vaccinated individuals against SARS-CoV-2 and patients infected with SARS-CoV-2 variant strain Omicron»*
11:00-11:15	Stavros Naoum, Hellenic Pasteur Institute, *«Tissue factor-expressing neutrophil extracellular traps formation in primary antiphospholipid syndrome is driven by antiphospholipid antibody-mediated platelet activation, subsequent platelet-neutrophil interaction and autophagy induction»*
11:15-11:30	Eleni Ntoukaki, Hellenic Pasteur Institute, *«Investigation of the mechanism of action of an antigen-specific therapy for myasthenia gravis and strategies for pharmacokinetic optimization»*
11:30-11:45	Eleni Papagianni, University of Ioannina, *«The role of neutrophil extracellular traps in the pathogenesis of RA-ILD; the effect of the anti-fibrotic agent Nintedanib in the inflammatory responses of the disease»*

11:45-12:00	Aristotelis Petris, Hellenic Pasteur Institute, *«Neutrophil extracellular trap (NETs) release in Helicobacter pylori infection»*
12:00-12:15	Biotechnological Companies Session: P Zafeiropoulos, «Novel cell-based Applications in Research and Therapeutics»
12:15-14:00	**Poster Session 1 + Lunch Break**
14:00-16:15	**Oral Session 2 | Bioinformatics**
	Chair: Dr. Artemis G Hatzigeorgiou
14:00-14:15	Dimitrios Christos Tremoulis, Hellenic Pasteur Institute, *«A scalable bioinformatics platform for the streamlined analysis of single cell RNA-seq data»*
14:15-14:30	Despoina Kiouri, National Hellenic Research Foundation & National and Kapodistrian University of Athens, *«Deep Neural Network Framework for Predicting Protein-Protein Interactions from Structural Data»*
14:30-14:45	Agoritsa Kalampaliki, BSRC Alexander Fleming, *«A Root Mean Square Deviation Estimation Algorithm (REA) and its use for improved RNA Structure Prediction»*
14:45-15:00	Dimitra Panou, BSRC Alexander Fleming, National and Kapodistrian University of Athens, *«Biomedical Question Answering using a 'Farm' of Open Large Language Models»*
15:00-15:15	Faidon Zacharias Brotzakis, BSRC Alexander Fleming and University of Cambridge, *«Computational design of antibodies against disease relevant molecular targets»*
15:15-16:15	**Keynote 1 |Dr. Emmanouil Maragkakis**
	«The genomics of aging: translating large-scale data to molecular mechanisms»
16:15-16:30	Coffee Break

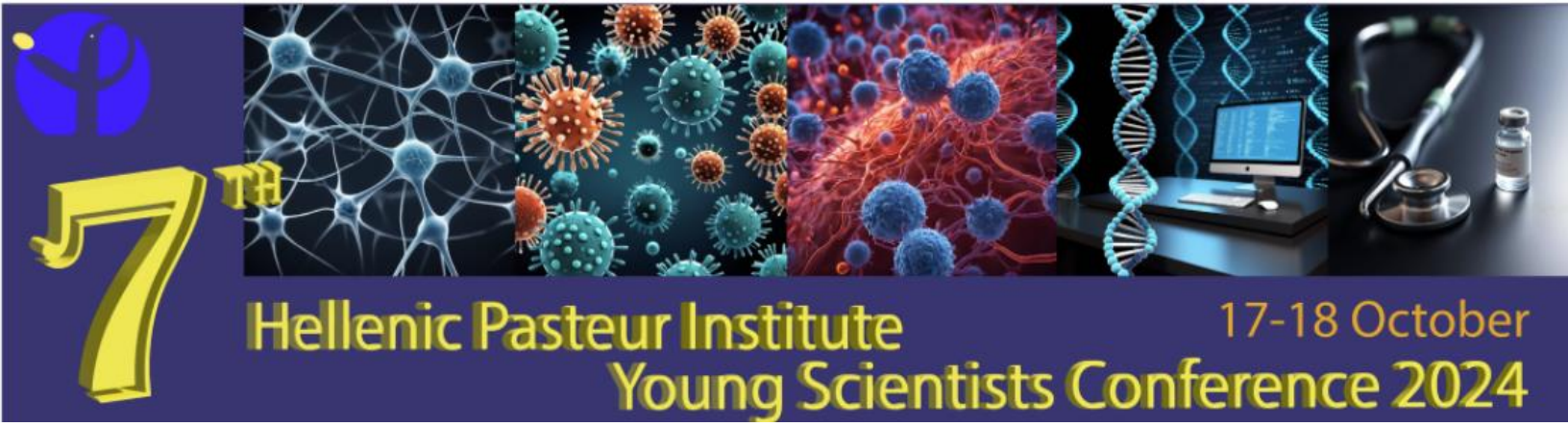
16:30-18:00	**Oral Session 3 | Microbiology**
	Chair: Dr. Haralabia Boleti, Dr. Niki Vassilaki
16:30-16:45	Marianna Arvaniti, Agricultural University of Athens, *«Dormancy induction and outgrowth heterogeneity of Listeria monocytogenes is affected by stress history and type of growth»*
16:45-17:00	Antonia Efstathiou, Hellenic Pasteur Institute, *«Exosomes as a vaccine candidate against visceral leishmaniasis»*
17:00-17:15	Nikolaos Moustakas, Hellenic Pasteur Institute, *«Qualitative and quantitative detection of Non Helicobacter pylori Helicobacter (NHPH) species in human gastric biopsies»*
17:15-17:30	Eleni Paximadi, *«Human Endogenous Retro Viruses (HERVs) and Multiple Sclerosis»*
17:30-17:45	Dimitra Toubanaki, Hellenic Pasteur Institute, *«Utilizing powerful molecular tools to understand environment-threatening viruses: the case of viral encephalopathy»*
17:45-18:00	Vasileios Gouzouasis, Hellenic Pasteur Institute, *«COVID-19 Infection Reactivates Epstein-Barr Virus and Precipitates the First Clinical Episode in a Greek Cohort of Multiple Sclerosis Patients»*
18:00-19:00	**Keynote 2| Dr. Evangelos Giamarellos** ***«Personalized infection treatment»***
19:00-21:00	Official presentation of the 100th Anniversary Edition Pasteur Album
21:00	**Cheese & Wine**

DAY 2 - FRIDAY 18 OCTOBER

09:30-10:45	**Oral Session 4 | Bioinformatics**
	Chair: Dr. Timokratis Karamitros, Dr. Magdalini Bletsa
09:30-09:45	Marina Petsana, University of Thesally and Hellenic Pasteur Institute, *«In Silico Identification and Analysis of Proteins Containing the Phox Homology Phosphoinositide-Binding Domain in Kinetoplastea Protists»*
09:45-10:00	Anargyros Skoulakis, University of Thesally and Hellenic Pasteur Institute, *«Machine Learning-Based Prediction of Antimicrobial Resistance in ESCAPEE Pathogens Using Genomic Data»*
10:00-10:15	Maria Bousali, Hellenic Pasteur Institute, *«HBV Integrations: How Does Viral Load Affect Their Frequency and Genomic Distribution?»*
10:15-10:30	Alexandros Galaras, BSRC Alexander Fleming and University of Thesally, *«LncRNA EPB41L4A-AS1 encodes a novel mitochondrial microprotein with tumor suppressive effects»*
10:30-10:45	Chrysoula Kaligerou, Hellenic Pasteur Institute, *«Development of an analytical NGS workflow for the clinical interpretation of whole exome sequencing data from patients with Multiple Myeloma»*
10:45-11:00	Coffee Break

11:00-12:15	**Oral Session 5 |Structural Biology – Biotechnology & Public Health**
	Chair: Dr. Emmanouil Angelakis, Dr. Marios Zouridakis
11:00-11:15	Oleksii Kupreienko, Hellenic Pasteur Institute and Agricultural University of Athens, *«Towards the development of novel allosteric activators for human angiotensin-converting enzyme 2»*
11:15-11:30	Maria-Evgenia Politi, Hellenic Pasteur Institute and Agricultural University of Athens, *«Expression, purification and characterization of human tyrosinase and crystallographic studies of a bacterial structural surrogate»*
11:30-11:45	Maria Voumvouraki, National and Kapodistrian University of Athens, *«Comparing different lysis conditions for proteomics analysis of amyloid deposits in biopsies for diagnostics»*
11:45-12:00	Constatinos Karamalis, University of West Attica, *«Development of a molecular assay for the identification of Streptococcus spp. directly in clinical samples and the impact on an accurate diagnosis in public health measures»*
12:00-12:15	Eirini Kosta, Hellenic Pasteur Institute & University of West Attica, *«Subtyping of L. infantum strains from Greece by using genomic DNA isolated from cultivated parasites and different PCR-based techniques»*
12:15-12:30	Biotechnological Companies Session: Antisel, “Witness the Next-Generation of Multiomics”
12:30-14:00	**Poster Session 2 + Lunch Break**
14:00-15:00	Scientific Image Session & Neuroholics
	”Science communication: Neuroholic’s point of view”
15:00-15:30	Coffee Break
15:30-16:30	**Keynote 3 | Dr. Mark Mattson**
	«Understanding and Counteracting Brain Aging

16:30-18:00	**Oral Session 6 | Neurobiology**
	Chair: Dr. Dimitra Thomaidou, Dr. Florentia Papastefanaki
16:30-16:45	Elissavet Akrioti, Hellenic Pasteur Institute, *«Deciphering and targeting early synaptic dysfunction in pre-clinical models of familial Parkinson's disease»*
16:45-17:00	Spyridon Antoniadis, Aristotle University of Thessaloniki and National and Kapodistrian University of Athens, *«Connecting Connectomes to Physiology via optogenetics and complex network theory: an ex vivo Investigation of Spontaneous Oscillations in the Mouse Motor Cortex»*
17:00-17:15	Olympia Apokotou, Hellenic Pasteur Institute, *«Control iPSC-derived astrocytes rescue P.A53T-αSyn patient-iPSC-derived neurons from Parkinson's disease-related pathology»*
17:15-17:30	Maria Margariti, Hellenic Pasteur Institute, *«In vivo study of the effect of microglial BIN1 deletion on mouse brain under homeostatic and neuroinflammatory conditions»*
17:30-17:45	Myrto Patagia Bakaraki, University of West Attica, *«The Impact of Multisensory Strategies on Functionality in Adults with Neurological Disorders»*
17:45-18:00	Athena Boutou, Hellenic Pasteur Institute, *«Microglia regulate cortical remyelination via TNFR1-dependent phenotypic polarization»*
18:00-19:00	**Keynote 4 | Dr. George Paxinos** ***«Is the brain in the Goldilocks Zone?»***
19:00-20:00	**Award Ceremony | Closing Remarks**
20:00	**Latin Garden Party & Dinner**



POSTER SESSION DAY 1 - THURSDAY 17 OCTOBER

BIOINFORMATICS

Filippos Kardaras, University of Thessaly & Hellenic Pasteur Institute, **«***Impact of Chios Mastic on Oral Health: Reducing Inflammation and Promoting Beneficial Bacteria»*

Vasiliki Kotsira, University of Thessaly & Hellenic Pasteur Institute, **«***Agnodice: indexing experimentally supported bacterial sRNA-RNA interactions»*

Marios Miliotis, University of Thessaly & Hellenic Pasteur Institute, «*TarBase v9.0: Expanding Experimentally Validated miRNA–Gene Interactions to Virally Encoded miRNAs and Cell-Type Specific Contexts»*

IMMUNOLOGY

Errikos Fourlas, Hellenic Pasteur Institute, **«***Brain region specific microglial dynamics in Experimental Autoimmune Encephalomyelitis»*

Maria Kourouvani, Hellenic Pasteur Institute, **«***Spontaneous human CD8 T cell and autoimmune encephalomyelitis-induced CD4/CD8 T cell lesions in the brain and spinal cord of HLA-DRB1*15-positive multiple sclerosis humanized immune system mice»*

Charilaos Spyropoulos, Hellenic Pasteur Institute, *«Investigation of Neutrophil subpopulations and NETs in the peripheral blood of MS patients»*

MICROBIOLOGY

Daniel Carrasco, Hellenic Pasteur Institute & University of Alcalá, **«***Leishmania Dual-specificity Tyrosine-Regulated Kinases: Investigating the role of parasite-specific members and discovering small molecule inhibitors against LinDYRK1»*

Myrto Koutantou, Hellenic Pasteur Institute, *«Interaction of human neutrophils with Bartonella henselae in Cat Scratch Disease»*

Eirini Kyriakopoulou, Hellenic Pasteur Institute, **«***Implication of L-dopa decarboxylase in dengue virus-mediated inhibition of autophagy and induction of cell death»*

NEUROBIOLOGY

Kalliopi Atsoniou, BSRC Alexander Fleming & National and Kapodistrian University of Athens, *«Differential sleep-like deficits of Neurofibromatosis 1 mutations in Drosophila melanogaster»*

Alexandros G. Charonitakis, BSRC Alexander Fleming, ***«****A neuronal circuit approach to Habituation and Dishabituation Mechanisms in Drosophila melanogaster»*

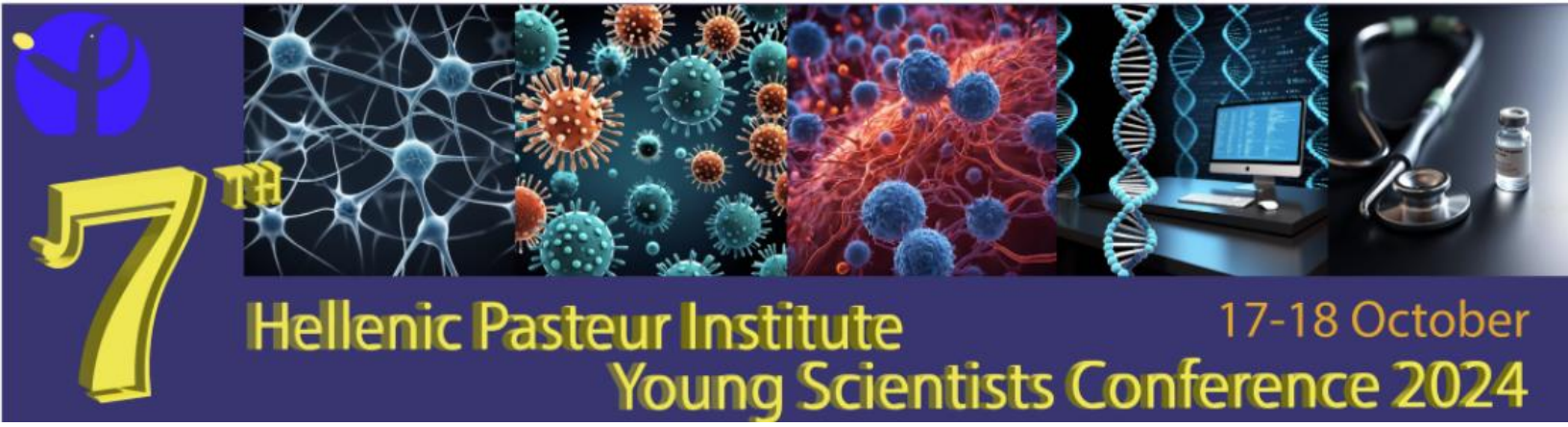
Theodoros Dame, Hellenic Pasteur Institute, ***«****RNA editing contributes to RNA metabolism in Parkinson's disease»*

Sofia Dede, Hellenic Pasteur Institute & Institut Pasteur Paris, ***«****Impaired uptake and clearance capacity of astrocytes in a IPSC-derived Parkinson's disease model from p.A53T a-synuclein patients»*

Evangelia Papoutsi, Nikos Kokkorakis, Hellenic Pasteur Institute, ***«****Mirk/Dyrk1B kinase as a potential drug target in ALS»*

Eleftheria Kotzairaki, Biomedical Research Foundation Academy of Athens, ***«****Integration of diverse platforms to define local mechanisms and long-range signals that mediate brain-heart interactions after injury and during regeneration»*

Elpinickie Ninou, Hellenic Pasteur Institute, *«Microglia-specific phenotypic and functional changes upon neuronal expression of familial Parkinson's disease p.A53T-alpha synuclein»*



POSTER SESSION DAY 2 - FRIDAY 18 OCTOBER

BIOINFORMATICS

Dimitra Panou, BSRC Alexander Fleming & National and Kapodistrian University of Athens, *«Biomedical Question Answering using a 'Farm' of Open Large Language Models»*

Katerina Politopoulou, Hellenic Pasteur Institute, ***«****Longitudinal whole brain RNA sequencing reveals characteristic transcriptomic signatures at hallmark timepoints in cuprizone demyelination and remyelination****»***

Margaritis Tsifintaris, Democritus University of Thrace, ***«****MilkExosOmics: A Comprehensive Relational Database for Analyzing MilkDerived Exosomal Carg****»***

BIOTECHNOLOGY-STRUCTURAL BIOLOGY

Maria-Eirini Lekka, Agricultural University of Athens & Hellenic Pasteur Institute, ***«****Unlocking the Mi-FAR-1 Structure: Expression and Purification of a Nematode Protein****»***

Sampanai Elena, National and Kapodistrian University of Athens, ***«****Exploring Molecular Mechanisms of Resilience to Environmental Stress via Proteomic Analysis of the Mediterranean Plant Cistus creticus****»***

PUBLIC HEALT

Drosos Kourounis, Hellenic Pasteur Institute, ***«****Reduction of Airborne Microbial Bioaerosols in areas of Public Interest, using novel Air filtration-UV Irradiation technology****»***

Nikolaos Moustakas, Olga Papaggeli, Sofia Makka, Hellenic Pasteur Institute, ***«****Development of Photoactivate Surfaces with Antibacterial and Antiviral Activity for a Clean and Safe Environment - APOGEION – ****»***

MICROBIOLOGY

Maria Morakou, Hellenic Pasteur Institute & University of West Attica, ***«****The role of exosomal cargo as viral fingerprint in HCV infection before and after treatment with DAAs****»***

Myrto Panagiotopoulou, Roskilde University, Denmark, ***«****Metabolic activity of vaginal microbiome****»***

Odysseas-Panagiotis Tzortzatos, Hellenic Pasteur Institute, ***«****Effects of polyI:C and LPS on immune status and gene expression of European Sea Bass (Dicentrarchus labrax, L.)****»***

NEUROBIOLOGY

Elsa Papadimitriou, Hellenic Pasteur Institute, ***«****Elucidation of the miR-124/ISX9-mediated transcriptional mechanism instructing the cell fate switch of astrocytes to induced-neurons****»***

Anastasia-Konstantina Papadopoulou, Biomedical Research Foundation of the Academy of Athens, ***«****Cerebral lateralization for writing in left- and right-handers: Attempting to disentangle a Gordian knot****»***

Christina Paschou, Hellenic Pasteur Institute, ***«****Failure of major proteostatic mechanisms in p.A53T-αSyn PD patient iPSC-derived astrocytes, due to lysosomal malfunction****»***

Ilias Roufagalas, Hellenic Pasteur Institute, ***«****Morphological and transcriptomic characterization of cortical astrocytes during early experimental demyelination****»***

Constantinos Sideris, Hellenic Pasteur Institute, ***«****Study of cellular senescence phenotypes in astrocytes derived from Parkinson's disease patient-iPSCs****»***

Irini Thanou, Hellenic Pasteur Institute, ***«****In vivo microglial Bin1 deletion regulates neuroinflammation and Adult Neurogenesis in the mouse hippocampus****»***

Evanthia Tselo, Hellenic Pasteur Institute, ***«****Metabolic impairment in PD-patient iPSC-derived p.A53T-αSyn astrocyte*

ORAL PRESENTATIONS

Oral Session 1: Immunology

OP.01

Maturation of circulating Ly6C^{hi}CCR2^{+} monocytes by mannan-MOG induces antigen-specific tolerance and reverses autoimmune encephalomyelitis

Anastasia Dagkonaki[1], Athina Papalambrou[1], Maria Avloniti[1], Maria-Eleni Androutsou[2], Theodore Tselios[3], Lesley Probert[1]

[1] Laboratory of Molecular Genetics, Department of Immunology, Hellenic Pasteur Institute, Athens, Greece
[2] Research and Development Department, Vianex PLC, Nea Erythrea, Greece
[3] Department of Chemistry, University of Patras, Patras, Greece

Introduction: Autoimmune demyelinating diseases are the main cause of non-trauma disability in young adults. Current medications are effective for managing clinical relapses but induce wide-reaching immunosuppression and are associated with adverse effects. Experimental autoimmune encephalomyelitis (EAE) is a model of autoimmune demyelination useful for the development of specific therapies. Together with antigen-specific T cells, myeloid cells are main effector cells in CNS autoimmune diseases such as multiple sclerosis. We previously show that oxidized mannan-conjugated myelin oligodendrocyte glycoprotein 35-55 (OM-MOG) induces antigen specific tolerance in EAE, by inducing a peripheral type 2 myeloid cell response characterized by increased production of PD-L1- and IL-10 by myeloid cells, and T cell anergy. In this study we further investigated the mechanism of immune tolerance.

Methods: Using the MOG-EAE model we treated C57BL/6 mice with OM-MOG and analyzed the clinical symptoms together with levels of Ly6C^{hi}CCR2^{+} monocytes in peripheral lymphoid tissues by flow cytometry. We analyzed the biodistribution of OM-MOG and the CNS pathology by confocal microscopy. We also tested the role of PD-L1 in OM-MOG-induced tolerance in CD11c^{+} dendritic cell-specific Pdl1 knockout mice and pharmacological blockade of PD-L1.

Results: OM-MOG induces peripheral maturation of Ly6C^{hi}CCR2^{+} monocytes to Ly6C^{hi}MHCII^{+}PD-L1^{+} cells, sufficient to reverse spinal cord inflammation and demyelination in MOG-induced autoimmune encephalomyelitis. Soluble intradermal OM-MOG drains directly to the skin draining lymph node to be sequestered by subcapsular sinus macrophages, activates Ly6C^{hi}CCR2^{+} monocytes to produce MHC class II and PD-L1, thereby preventing immune cell trafficking to spinal cord and reversing established lesions. PD-L1 was essential for OM-MOG tolerance.

Conclusions: These results show the importance of peripheral innate immune responses, in the control of autoimmune demyelination. Maturation of Ly6C^{hi}CCR2^{+} monocytes in the periphery, outside of the target organ, by OM-MOG represents a novel mechanism of immune tolerance that reverses autoimmune encephalomyelitis.

OP.02

Application of a competitive ELISA utilizing trimeric spike protein for the detection of neutralizing antibodies in sera of vaccinated individuals against SARS-CoV-2 and patients infected with SARS-CoV-2 variant strain Omicron

Authors
Eleni K. Loxa[1], Annie Mais[2], Alexandros Papazisis[1], Alexios Dimitriadis[3], Ioannis Sarrigeorgiou[1], Marija Backovic[4], Maria Agallou[5], Pelagia Foka[6], Ourania E. Tsitsilonis[7], Marios Zouridakis[8], Evdokia Karagouni[5], Konstantinos Lazaridis[1,3], Avgi Mamalaki[2,3], Peggy Lymberi[1], and Petros Eliadis[1,3]

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Abstract
Since the initiation of the COVID-19 pandemic, there has been a need for the development of diagnostic methods to determine the factors implicated in mounting an immune response against the virus. The most promising indicator has been suggested to be neutralizing antibodies (nAbs), which mainly block the interaction between the Spike protein (S) of SARS-CoV-2 and the host entry receptor ACE2. So far, several studies have used immunoenzymatic methods with RBD as the competitor antigen, while a few have used the trimeric S protein molecule. The aim of the present study was to implement an in-house competitive ELISA, surrogate Virus Neutralizing Assay (sVNA), that pairs S trimer stabilized with 6 molecules of proline (S(6P)) and ACE2 recombinant molecules which could be a biologically more relevant model than the RBD-ACE2 interaction-based assays, to measure neutralizing antibodies from sera of vaccinated individuals and vaccinated individuals who subsequently contracted COVID-19 (hybrid sera). The recombinant ACE2 molecule as well as the S(6P) molecule was produced in our lab after plasmid isolation and transfection to Expi 293 cells. The results of our surrogate Virus Neutralizing Assay (sVNA-S(6P)) were compared against an RBD-ACE2 interaction-based assay (cPass of GenScript, FDA and CE approved). When serially diluted sera from vaccinees were tested, a high correlation of ID50–90 titer values was observed between the two assays. Interestingly, when we tested and compared the neutralizing activity of sera from eleven fully vaccinated individuals who subsequently contracted COVID-19 (hybrid sera), we recorded a moderate correlation between the two assays, while higher sera neutralizing titers were measured with sVNA. Furthermore, when the ratios of cPass-ID50:sVNA-ID50 were compared between the two groups of sera, they were lower for hybrid sera compared to those of the vaccinees' sera. Our data indirectly indicated that the sVNA could identify nAbs more than the RBD-RBM specific ones.

OP.03

Tissue factor-expressing neutrophil extracellular traps formation in primary antiphospholipid syndrome is driven by antiphospholipid antibody-mediated platelet activation, subsequent platelet-neutrophil interaction and autophagy induction.

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Introduction: Antiphospholipid Syndrome (APS) is a rare and complex systemic autoimmune disorder with a poorly understood pathophysiology. APS is characterized by arterial and venous thromboses, microvascular manifestations and pregnancy morbidity in the presence of antiphospholipid (aPL) autoantibodies, namely anticardiolipin (aCL) and anti-β2 glycoprotein I (anti-β2GPI) antibodies and lupus anticoagulant (LA). The involvement of neutrophil extracellular traps (NETs) in the pathogenesis of primary APS (PAPS) is gaining increasing attention. Herein, we examined the underlying molecular mechanisms driving NET release and the thrombogenic effect of NETs in PAPS patients.

Methods: We collected peripheral blood neutrophils and sera from 30 patients with thrombotic PAPS, 5 asymptomatic aPL carriers, and 11 healthy controls (HC). NET release and NET-related proteins were examined by immunofluorescence, immunoblotting, quantitative PCR, and ELISA. We assessed platelet-neutrophil aggregates by flow cytometry (CD61/CD66b staining), and autophagy using LC3B immunofluorescence and immunoblotting. Immunofluorescence was performed in archival formalin-fixed paraffin-embedded kidney, skin ulcer, and endoarterial thrombus tissues from PAPS patients. Statistical significance was at $p<0.05$.

Results: PAPS patient neutrophils exhibited increased NET release compared to asymptomatic aPL carriers and HC. NETs from PAPS patients expressed tissue factor (TF) that induced thrombin generation in platelet-poor plasma from HCs. aPL induced in vitro intracellular TF expression in HC neutrophils. NET release in PAPS neutrophils was not correlated to aPL type, isotype and titers, and aPL could not directly induce NET release in neutrophils. NET release in PAPS neutrophils was driven by aPL-mediated platelet activation, subsequent platelet-neutrophil interaction and autophagy induction. TF-expressing NETs were abundant in kidney, skin ulcer, and endoarterial thrombi tissues from PAPS patients and colocalized with fibrinogen.

Conclusions: In PAPS, neutrophils release TF-decorated NETs, driven by aPL-mediated platelet activation, subsequent platelet-neutrophil interactions, and the induction of autophagy process. Targeting the platelet-neutrophil/autophagy/TF-expressing NETs axis may attenuate thromboinflammation and prevent tissue damage in PAPS patients.

OP.04

Investigation of the mechanism of action of an antigen-specific therapy for myasthenia gravis and strategies for pharmacokinetic optimization

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Background: Myasthenia gravis (MG) is an antibody-mediated autoimmune disorder, which leads to destruction of the neuromuscular junction (NMJ). The autoimmune response is elicited against proteins of the NMJ, with most antibodies targeting the muscle acetylcholine receptor (AChR). Long-term administration of non-specific immunomodulators is the main course of treatment for MG, but these could lead to side effects due to life-long immunosuppression. Therefore, development of antigen-specific therapeutics aiming towards tolerance reestablishment could be a promising strategy. Our team aims towards the development of an antigen-specific approach based on the administration of the extracellular domain of the human AChR alpha-1 subunit (hα1-ECD). We have previously reported the therapeutic efficiency of hα1-ECD administrated intravenously after disease induction in rats with experimental autoimmune MG (EAMG), resulting in significant and long-lasting remission, but the protein exhibited a short serum half-life, potentially limiting its potency.

Methods: We have explored strategies to improve the protein's pharmacokinetics by inclusion in nanoparticles or conjugation with polyethylene glycol (PEG). The modified proteins have been used to assess their *in vivo* therapeutic efficiency in rats following i.v. injection. Additionally, we have initiated studies to elucidate the mechanism of action at play.

Results: We have identified the optimal PEGylation conditions. The pegylated α1-ECD exhibited improved therapeutic efficacy *in vivo* compared to the unmodified α1-ECD at the same dosage. On the other hand, selected nanoparticles showed limited protein release in vitro, and low *in vivo* efficiency, suggesting the need for further optimization. Additionally, lymphocytes isolated from treated rats have exhibited a reduced proliferative response *in vitro*.

Conclusions: Overall, antigen-specific intravenous tolerance is a promising approach for MG treatment. Further studies are underway to elucidate the immunological mechanisms involved. Treatment with hα1-ECD with improved serum half-life could allow improved efficiency and dosage reduction in future studies.

OP.05

The role of neutrophil extracellular traps in the pathogenesis of RA-ILD; the effect of the anti-fibrotic agent Nintedanib in the inflammatory responses of the disease"

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Introduction: Interstitial lung disease (ILD) that is associated with rheumatoid arthritis (RA) constitutes a serious extra-articular manifestation of the disease with high morbidity and mortality. The release of neutrophil extracellular traps (NETs) can contribute to the perpetuation of interstitial inflammation and fibrosis, key characteristics of the fibrotic lung. In this study, we tried to investigate the role of NETs in RA-ILD pathophysiology. At the same time, we examined the potential effect of the anti-fibrotic factor nintedanib in advanced stages of the disease.

Methods: Nine RA-ILD patients and nine healthy individuals (control group) were enrolled in the study. The presence of NETs in patients' circulation, the expression of inflammatory markers and the activation of complement (sC5b-9) were examined. To assess the interaction between NETs and human pulmonary fibroblasts (HPFs), *in vitro* studies were deployed. Furthermore, five RA-ILD patients were treated with nintedanib and evaluated sixteen weeks after initiation of the therapy.

Results: RA-ILD patients showed increased levels of NETs, enriched with tissue factor (TF) and interleukin-17A (IL-17A), compared to healthy individuals. Moreover, HPFs were activated and enhanced their migration capacity upon incubation with isolated NET structures. Treatment with DNAse-I or/and monoclonal antibodies against TF and IL-17A attenuated this phenomenon. Moreover, RA-ILD patients treated with nintedanib showed reduced levels of NETs and sC5b-9 in circulation.

Conclusions: The findings indicate NETs as a key mechanism in RA-ILD pathogenesis. They also support that nintedanib can exert a beneficial effect in the process of the disease by decreasing specific inflammatory events. Further studies will contribute to better understanding of these mechanisms, to the investigation of new pathways and to the optimization of therapeutic manipulations in RA-ILD.

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OP.06

Neutrophil extracellular trap (NETs) release in *Helicobacter pylori* infection

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Background: *Helicobacter pylori* (*Hp*) is a pathogen colonizing the human gastric mucosa in early childhood, thus increasing the lifelong risk of gastric cancer development. Although neutrophil infiltration in the lamina propria is the hallmark of *Hp* infection and considering their capacity to exert a multitude of antimicrobial responses, neutrophils fail to clear the infection. Why?

Material and methods: We investigated the interactions of freshly isolated human peripheral blood neutrophils with *Hp* clinical isolates and laboratory-adapted strains *in vitro*, in terms of their ability to phagocytose and release neutrophil extracellular traps (NETs), visualized by confocal microscopy, while ROS generation was detected by flow cytometry.

Results: We documented the capacity of neutrophils to phagocytose all *Hp* strains, irrespective of CagA EPIYA phosphorylation status and produce ROS, albeit at significantly lower levels to those observed when phagocytosing *E. coli*. Interestingly, *Hp*-induced NETs appeared to be degraded and demonstrated only faint presence of NET-related proteins compared to *E. coli* controls or strong sterile stimulus induced by ionomycin. To visualize and further validate these degradation processes, a live imaging protocol was carried out on *Hp*-infected neutrophils, using SYTOX-GREEN and Hoechst 33342 stains, observed by confocal microscopy. Images were captured every 3 minutes and demonstrated progressive reduction of extracellular staining in *Hp*-induced NETs, compared to those released by sterile stimulus, suggesting a degradation process due to *Hp* presence.

Conclusion: These findings indicate that *Hp* infection induces neutrophil activation, yet a progressively degraded NET release points towards a possible immune-evasion strategy to host neutrophilic response.

Oral Session 2: Bioinformatics I

OP.07

A scalable bioinformatics platform for the streamlined analysis of single cell RNA-seq data

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Introduction: Single Cell RNA sequencing (scRNA-seq) has been very effective in unraveling the complexity of biological systems. scRNA-seq data analysis includes a multitude of steps such as quality control, normalization, clustering and cell annotation, each with its own thresholds and hyperparameters. These parameters are largely experiment-dependent and often cannot be determined a priori. Thus, we have developed a scalable bioinformatics platform to accelerate the estimation of those parameters and το make scRNA-seq analysis more accessible.

Methods: Programming languages bash and R were used to integrate state-of-the-art bioinformatics tools namely DecontX (quality control), Monocle and Seurat (normalization, clustering, differential expression analysis, trajectory analysis), SingleR (cell type annotation), LIANA (Ligand-Receptor analysis), inferCNV (CNV detection) and UCell (gene-set scoring). Critically, we created an automated optimization algorithm which takes as an input a range of parameter values in each step of the pipeline and can aid in the estimation of the most appropriate value of the said parameter. Parallel-R package was used to parallelize most of the steps of the pipeline, in order to ensure its scalability and maximize the usage of the available hardware infrastructure.

Results: This pipeline was used to analyze public breast cancer and reductive mammoplasty scRNA-seq datasets (ENA accession numbers: PRJNA702767, PRJNA749859). Its scalability and ease of parameter estimation significantly reduced the computation time and enabled us to focus more on the underlying biological phenomena of the study. The streamlined visualization of various plots allowed us to rapidly detect important biomarkers such as GDF15 which is detected only in a subset of cancer patients and has been associated with unfavorable prognosis in breast cancer.

Discussion: Considering the major challenges in the scRNAseq field, we anticipate that our scRNA-seq analysis platform will be of great use and will reduce the complexity and the hands-on time significantly.

OP.08

Deep Neural Network Framework for Predicting Protein-Protein Interactions from Structural Data

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Introduction: The gut microbiome, a complex ecosystem of microorganisms, plays a pivotal role in human health and disease. The gut microbiome's influence extends beyond the digestive system to various organs, and its imbalance is linked to a wide range of diseases. Despite its significance, the interactions between gut bacteria and human proteins remain understudied, with less than 20,000 experimentally validated protein interactions between the host and any bacteria species. This study addresses this knowledge gap by predicting a protein-protein interaction (PPI) network between gut bacterial and human proteins using a novel structure-based deep neural network.

Methods: The developed deep learning model was designed to predict PPIs within the human-gut microbiome using structure-based protein representations extracted from AlphaFold database. The model was trained with ~1M experimentally validated pan-bacterial-human PPIs, human PPIs, as well as inter- and intra- species PPIs from different organisms including viruses, plants, and animals.

Results: The model achieved an Accuracy of 0.97, and an Area Under the Curve (AUC) score of 0.836, reflecting high precision and recall for predicting positive interactions while maintaining strong discrimination between classes (i.e., interaction and non-interaction). For highly confident results, the decision threshold value was set to 0.7 (i.e., any possible interaction with a predicted interaction probability equal to or greater than 0.7 is a PPI).

Conclusions: This study introduces a predictive model of the interactions between gut microbiome proteins and the host, offering a foundation for experimental validation and novel therapeutic strategies. By pinpointing critical regulatory elements, the model will aid in the design of targeted experiments, enhancing personalized interventions to modulate gut microbiome dynamics for improved health outcomes.

OP.09

A Root Mean Square Deviation Estimation Algorithm (REA) and its use for improved RNA Structure Prediction

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Intoduction: The 3D structure of RNA is crucial for biotechnological applications and to comprehend its biological function. Recent developments using AlphaFold-inspired deep neural networks improved the prediction of 3D structure from RNA sequence, but evaluation of the accuracy of these predictions is still necessary. We present the RMSD Estimation Algorithm (REA), a feed-forward neural network to predict the root-mean-square deviation (RMSD) of a 3D RNA structure from its native conformation using its Molprobity [1] stereochemical validation features. We obtain higher prediction accuracy than each of 3 prediction algorithms alone by selecting the best predicted structures with REA.

Methods: REA was trained on structures predicted by the DeepFoldRNA [2] and trRosettaRNA [3] transformer-based deep neural networks on a set of 182 models of RNA structures.

Results: We compared REA with ARES [4], a state-of-the-art deep learning algorithm for predicting the RMSD of RNA structures, assessing the prediction accuracy on novel RNAs with and without pseudoknots. REA outperformed ARES on both test sets with smaller absolute difference between the true and the predicted RMSD.

Using a combination of REA and a Support Vector Regression (SVR) trained on the same data as REA, we can select RNA structures predicted with DeepFoldRNA, trRosettaRNA and Rhofold [5] to achieve a significantly higher prediction accuracy than any of the prediction methods used alone. This was shown on a validation set with 261 novel RNA chains from the Nonredundant 3D Structure Dataset [6] and a test set with 55 novel RNA chains from RNA-Puzzles [7].

Conclusion: REA proves to be a valuable tool for evaluating RNA 3D structures. The selection based prediction can easily incorporate additional prediction algorithms. RNA 3D structure prediction and assessment with REA, given a sequence, will soon be available via a web-service.

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OP.10

Biomedical Question Answering using a 'Farm' of Open Large Language Models

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Keywords: Biomedical Question Answering, BioASQ, Large Language Models, Retrieval-augmented generation

Abstract. Biomedical text mining and question-answering are essential and complex tasks driven by the need to access and process the ever-expanding volume of biomedical data. With the exponential growth of published biomedical literature, effective retrieval and accurate question-answering systems are crucial for researchers, clinicians, and medical experts to make well-informed decisions. The emergence of open-source Large Language Models (LLMs) marks a significant trend in the tech landscape, with these models increasingly tailored to address diverse tasks. In this work, we present our participation in the twelfth edition of the BioASQ challenge, which involves biomedical semantic questionanswering for task 12b and biomedical question answering for developing topics for the Synergy task. We deploy a selection of open-source LLMs for embedding and retrieval of documents and snippets, as well as retrieval-augmented generators to answer biomedical questions. Dense retrieval methods, leveraging distances between dense representations of documents and questions obtained from LLM embeddings, and hybrid sparse/dense approaches, outperform traditional sparse retrieval methods in terms of mean average precision. We also implement a 'farm' of open-source LLMs to provide exact answers to biomedical Yes/No type questions. A variety of models process the prompts, and a majority voting system combines their outputs to determine the final answer. Ideal answers, summarizing the most relevant information for each question type, are generated by the MIXTRAL LLM. In the four rounds of the 2024 BioASQ challenge, our system achieved notable results: 1st and 2nd place in two rounds for 'exact answers,' 2nd place in one round for 'documents,' 2nd place in one round for 'ideal answers,' and 1st place in one round for 'snippets'.

OP.11

Computational design of antibodies against disease relevant molecular targets

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Introduction: Owing to their outstanding performances in molecular recognition, antibodies (Abs) are widely used in molecular biology, biotechnology and medicine. From a drug discovery perspective, first (in-vivo) and second (in-vitro) generation of antibody discovery methods might elicit Abs of decreased therapeutic and safety capacity caused by poor biophysical properties and adverse immune responses, thereby increasing R&D costs for effective Ab drugs, spanning 3-6 years and $13 mln to $1 bn. Hence, there is an extensive need to reduce the cost and time for R&D of antibody therapeutics by pushing the boundary of third (in-silico) generation antibody discovery methods.

Methods: We propose a bottom-up approach that integrates state of the art physics-based molecular simulations (Metadynamics) that efficiently uncover the atomistic mechanism of Ab engagement to targets, with AI algorithms (Inverse folding) that efficiently explores the vast Ab sequence space, for proposing de-novo Abs with optimal a) biological activity: bind predetermined molecular targets, with high affinity and slow dissociation rates b) developability properties: high conformational stability, low immunogenicity. We consider the SARS-CoV-2 Spike protein, as a proof of principle molecular target for designing nanobody binders of spike, aimed at inhibiting SARS-CoV-2 Spike mediated cell entry.

Results: The platform enables performing aminoacid substitutions at the CDR3 (2015 sequence diversity) of nanobody fragments which bind to SARS-CoV-2 spike by dissociating up to 20fold slower than phage-display derived nanobodies, while maintaining their conformational stability. A list of hit nanobodies is currently expressed in bacteria and purified for biological evaluation of inhibition of viral infectivity in cell assays, by quantification of the Cytopathic Effect in terms of the TCID50.

Conclusions: We developed a computational platform to de-novo antibody design showcased to target the key antigen of SARS-CoV-2 spike protein with up to 20fold higher predicted affinity and slower dissociation rates, while maintaining conformational stability.

Oral Session 3: Microbiology

OP.12

Dormancy induction and outgrowth heterogeneity of *Listeria monocytogenes* is affected by stress history and type of growth

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Introduction: Adaptive response of *Listeria monocytogenes* to sublethal stresses may induce injury and the Viable-But-Non-Culturable state (VBNC), that are stochastically expressed at single-cell level, with varying capacity in subsequent recovery. The objectives of this study were: (i) to investigate dormancy phenomena induced by oxidative and acid stress at single cell level and (ii) to examine how stress history and type of growth (colonial *vs* planktonic) affects outgrowth heterogeneity.

Materials and Methods: Acidic conditions (acetic and hydrochloric acid adjusted to pH 2.5-3.0, 20°C for 5h) and peracetic acid (PAA) (20-40 ppm, 20°C for 3h) were used to evaluate induction of dormancy in *L. monocytogenes* Scott-A. To quantify injured ($CFDA^+/PI^+$) and VBNC ($CFDA^+/PI^-$) cells flow cytometry coupled with CFDA (metabolically active) and PI (dead) staining was used. Stressed $CFDA^+/PI^-$ cells were sorted on Tryptic Soy Agar (TSAYE) or Broth (TSBYE) supplemented with 0.6% Yeast Extract to evaluate culturability. After treatment colony growth was monitored on TSAYE by phase-contrast time-lapse microscopy (37°C). Fitting of the colonial growth curves to sigmoidal (Baranyi and tri-linear) model was carried out for the estimation of growth kinetics. Data and statistical analyses were performed in R.

Results: The choice of recovery medium and stress history had an impact on the resuscitation capacity of CFDA+-sorted cells. Treatment with 30 ppm PAA and AA pH 2.5 induced phenotypic variance. This survival strategy helps *L. monocytogenes* cells to be phenotypically pre-adapted to survive optimal or adverse environmental conditions. The stochastic model showed that heterogeneity in growth dynamics is increased when cells are recovering from sublethal stresses, leading to inefficient detection of *L. monocytogenes*.

Conclusions: The present study provides insights into how low levels of contamination, VBNC induction and heterogeneity in outgrowth kinetics of *L. monocytogenes* cells, due to prior exposure to sublethal stresses, may affect the efficient detection of this pathogen.

OP.13

Exosomes as a vaccine candidate against visceral leishmaniasis

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Introduction: Leishmania, a protozoan parasite of the trypanosomatid family, is the causative agent of leishmaniasis which has wide range of clinical manifestations. Annually, 0.9–1.6 million new infections are reported and 20–50 thousand deaths occur due to Leishmania infection. Current chemotherapy for treating leishmaniasis exhibits numerous drawbacks while there are no effective human vaccines with protective immune responses which could help on elimination of the disease. To this end, exosomes play a new role in the vaccination approach of a variety of diseases such as cancer, Toxoplasma gondii etc.

Methods: In the current study, exosomes derived from Leishmania infantum promastigotes were isolated by density gradient ultracentrifugation and were characterized. Subsequently, BALB/c mice were immunized with the isolated exosomes with two intramuscular immunizations with 15-day interval in the presence and absence of Addavax adjuvant. The mice were then infected with L. infantum parasites and the effect of the regimen was evaluated on the acute and chronic phase of the disease.

Results: Vaccination with exosomes in the presence of adjuvant resulted in a dramatic reduction of the parasitic burden in both the acute and the chronic phase of the infection in BALB/c mice, accompanied by an immunomodulatory effect on the host. More specifically, the parasitic burden was reduced by 93% in both liver and spleen during the acute phase and 80% in spleen and 90% in liver during the chronic phase of the disease. The reduction in parasite load was accompanied by an increase in antigen-specific CD4+IFNγ+ T-lymphocytes in the acute phase. In the chronic phase, increase of CD8+INFγ+ T -lymphocytes and of CD4+ T- cells expressing IL-17 was also observed.

Conclusion: According to the above data, an exosome-based vaccine regimen emerges as a new potent vaccination approach and promising tool in the fight against the parasitic disease leishmaniasis.

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OP.14

Qualitative and quantitative detection of Non *Helicobacter pylori* Helicobacter (NHPH) species in human gastric biopsies

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Introduction: Non-*Helicobacter pylori* Helicobacter (NHPH) species, which are difficult to culture, can naturally colonize the stomach of animals and humans potentially contributing to human gastric pathology. Our prime objective was to develop and validate a SYBR-Green-based qPCR strategy for the rapid and accurate determination of the most prevalent NHPH in humans namely, *H. heilmannii, H. bizzozeronii, H. salomonis, H. ailurogastricus, H. felis* and *H. suis* and used it to screen gastric biopsies from Greek symptomatic adults and children.

Materials & Methods: Genomic DNA from reference NHPH strains (donated by Prof. Freddy Haesebrouck, Ghent University) and specific primers for *ureA, ureB, ureAB and IpsA* genes were utilized. Respective internal control standards were prepared by TOPO cloning. Optimization and validation for accuracy and precision was assessed in gastric biopsies from symptomatic, *H. pylori*-positive and -negative Greek adults (N=102) and children (N=149) from collaborating gastroenterology clinics in Athens. No cross-reactivity was detected and bacterial load was determined at copy number level.

Results: *Helicobacter pylori* (*Hp*) was present in 21.5% of samples, 43.1% in adults and 6.7% in children. *H. salomonis* was detected in 15.7% of adults and 14.8% children, while 18,5% were cases of co-infection with *Hp*. *H. ailurogastricus* was detected at 12.7% of adult and 10.1% of children biopsies, with 16.6% *Hp* co-infection, whereas, *H. felis* and *H. bizzozeronni* were detected in just 2 *Hp*-negative children cases. *H. salomonis* & *H. ailurogastricus* co-infection was determined in 3.2%, 37.5% of which were actually a triple co-infection with *Hp*. One case of *H. felis* and *H. salomonis* co-infection was identified.

Conclusion: Our assay has proved to be rapid, accurate and reliable and highlighted the significant presence of NHPH in gastric biopsies in adults and children, underlining the need for further investigation with reference to age-related differences, occupational exposure to animals or divergent exposure routes in NHPH colonization.

OP.15

Human Endogenous Retro Vi ruses (HERVs) and Multiple Sclerosis

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Viruses are believed to be the most abundant and diverse biological entities worldwide, with an estimation of 10^{31} particles. The human virome is similarly vast and heterogeneous, consisting of approximately 10^{13} particles per human body. Human endogenous retroviruses (HERVs) occupy approximately 8% of our genomic DNA. HERVs are mainly formed via two major mechanisms: 1) horizontal transmission, in which exogenous retroviral RNA is integrated into the host genome, thus becoming a provirus that will produce infectious virus; 2) vertical transmission, in which the past retroviral infection of germline cells results in a provirus with Mendelian heritability.

Over time the host tries to restrict the capacity of these particles to reinsert anywhere in the genome (jumping genes), to produce viral-like particles with limited effect or even proteins with distinct functions to the host. Moreover, the host controls the activity of endogenous retroviruses via epigenetic modulation, a mechanism that declines with age. Upon acute exogenous viral infection the equilibrium of human host and endogenous retroviruses can be disturbed. The interplay of viruses and endogenous retroviruses may lead to sustained immune system dysregulation long after the infection (chronic inflammation). Recent findings have demonstrated many implications of a role of viruses and endogenous retroviruses in diseases. The latest years, studies of the human virome using metagenomic sequencing, bioinformatics and other methods have clarified aspects of human virome diversity at different body sites, the relationships to disease states and mechanisms of establishment of the human virome during early life. Since many human viral genetic, immunologic related pathways are involved in disease establishment many new anti HERV therapies are being in research focus the latest years.

In this chapter multiple sclerosis will be discussed.

OP.16

Utilizing powerful molecular tools to understand environment-threatening viruses: the case of viral encephalopathy

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Introduction: Fish viral infections have great environmental and economic implications in aquaculture. The most significant disease in terms of severity, economic impact and spread, is viral nervous necrosis (VNN). VNN is a devastating disease, which induces cell necrosis accompanied by vacuolation in fish retina and brain. The disease is caused by nervous necrosis virus (NNV), which is a pathogen affecting more than 120 different species worldwide, causing high mortality and morbidity. The first step forward on the battle against VNN is to fully understand its progression and its effects on the host.

Methods: Our aim is to study how NNV infection affects the transcriptome of European sea bass (*Dicentrarchus labrax, L.*), in one disease resistant and one disease susceptible family. For that reason, we performed RNA-sequencing analysis to assess differential gene expression in *D. labrax* brain and head-kidney tissues. Fish were experimentally infected by the virus, and the surviving fish were sampled 3 hours and 14 days post infection. A wide range of genes were de-regulated in both *D. labrax* head-kidney and brain, on both fish families.

Results: Genes de-regulation due to infection was more intense for the resistant family than the susceptible family. For both families, the enriched gene ontology (GO) categories included signaling and immune processes revealing their critical role in virus defense. Also, the resistant family utilized mobility implicating genes to be protected. Pathway analysis revealed a more intense pathways enrichment in resistant family in most KEGG categories. Only a few pathways were commonly enriched in the two families further indicating that the resistant and susceptible families utilize completely different mechanisms to overcome the NNV infection.

Conclusion: NNV resistant and sensitive sea bass transcriptomes analysis offers a glimpse on how host attempts to control the infection depending on its genetic background in relation with virus resistance.

OP.17

COVID-19 Infection Reactivates Epstein-Barr Virus and Precipitates the First Clinical Episode in a Greek Cohort of Multiple Sclerosis Patients

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Background: Multiple sclerosis (MS) is a chronic autoimmune disorder strongly associated with Epstein-Barr virus (EBV) infection. The hallmark event of viral infection is double-stranded RNA (dsRNA) representing a signature of specific virus genomes, viral replication, and transcription intermediates. All DNA viruses, including EBV, and positive-strand RNA viruses like SARS-CoV-2 produce dsRNA upon replication. Here we investigate the interplay between viral infections and autoimmunity, particularly how COVID-19 reactivates EBV and results in the first MS clinical episode.

Methods: Plasma and cerebrospinal fluid (CSF) from 78 MS patients were analyzed for: 1) dsRNA using an in-house Sandwich-ELISA with the J2 and K2 antibodies, 2) antiviral cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-α2, IFN-β, IFN-λ1, IFN-λ2/3, IFN-γ, TNF-α, CXCL10, GM-CSF), 3) EBV-specific antibodies, and 4) SARS-CoV-2-specific antibodies (anti-Spike and anti-Nucleocapsid IgG and IgM), by in-house ELISAs.

Results: Plasma dsRNA levels significantly correlated with all 13 measured antiviral cytokines in the plasma of MS patients. Patients with a recent COVID-19 infection (anti-Nucleocapsid IgM+, n=8) demonstrated higher levels of dsRNA and antiviral cytokines. Patients with evidence of anomalous EBV reactivation (anti-EBNA-1 IgG+ and anti-EBNA-1 IgM+, n=9) also displayed elevated dsRNA and antiviral cytokine levels. A strong positive correlation was observed between anti-Nucleocapsid IgM and both anti-VCA EBV IgM and anti-EBNA-1 EBV IgM, indicating concurrent EBV reactivation. In contrast, vaccination against COVID-19, performed in some patients more than five months prior to hospitalization, did not appear to initiate MS. Furthermore, 71% (35/49) of the CSF samples were positive for anti-Spike IgG, potentially due to blood-brain barrier permeability.

Conclusions: This study provides the first evidence that COVID-19 infection can reactivate EBV, possibly triggering the first MS clinical episode in some patients. These findings underscore the

Oral Session 4: Bioinformatics II

OP.18

In Silico Identification and Analysis of Proteins Containing the Phox Homology Phosphoinositide-Binding Domain in Kinetoplastea Protists

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Background: Kinetoplastea are a group of protists that includes both free-living and parasitic species. Pathogenic Kinetoplastea parasites, such as *Trypanosoma* and *Leishmania* species, undergo multiple developmental transitions critical for their survival within their hosts. These transitions require extensive membrane and cytoskeleton reorganizations, processes that rely heavily on physiological functions of acidic phospholipids named phosphoinositides (PIs). PIs, serve as pivotal regulators of eukaryotic essential functions such as signal transduction, protein transport and sorting, membrane trafficking and remodeling, and cytoskeleton remodeling. In pathogenic Kinetoplastea, various PI-metabolizing enzymes and PI-binding proteins or PI-effectors, which contain specific PI-binding structural modules like the phox homology (PX) domain, play significant roles in facilitating these processes and contribute to the organisms' life cycles and virulence.

Methods: PX-domain-containing proteins (PX-proteins) from Kinetoplastea organisms were retrieved using Pfam, Uniprot databases and HMMER through a three steps approach. Proteins from *Homo sapiens* were also retrieved from Uniprot for reasons of comparison. Sequence alignments and phylogenetic tree construction aided the identification of domain architectures of these proteins while phylogenetic relationships among Kinetoplastea PX-proteins were also explored using phylogenetic trees and a.a. conservation logos.

Results: The search process in Uniprot and Pfam databases and HMMER tools uncovered 170 protein sequences containing PX domains. Four structural domains (i.e. PX, Pkinase, Lipocalin_5, and Vps5/BAR3-WASP) were also identified in these sequences and the proteins were classified into five subfamilies according to their domain architectures. All species have at least one protein with only one PX domain while only *Trypanosoma* spp. contain proteins with two PX domains as well as proteins with domain architecture PX-Pkinase. High evolutionary conservation was revealed among Kinetoplastea and *Homo sapiens*, particularly in residues critical for PtdIns3P recognition.

Conclusions: This study identified the PX-Pkinase domain architecture as unique to *Trypanosoma* spp., providing a possible candidate for development of anti-parasitic drugs.

OP.19

Machine Learning-Based Prediction of Antimicrobial Resistance in ESCAPEE Pathogens Using Genomic Data

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Background: Antimicrobial resistance (AMR) is a global health issue that poses significant threats to modern medicine. Traditional methods for antimicrobial susceptibility testing are often slow, low-throughput, and require microbial cultures. Advances in next-generation sequencing (NGS), bioinformatics tools, and machine learning (ML) models offer potential solutions for efficient AMR identification. In this study, we analyzed the genomes of 18,916 assemblies of ESCAPEE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp, and Escherichia coli) from the NDARO, BV-BRC, and CDC-NARMS databases, along with their corresponding antibiograms, to develop ML models capable of predicting AMR phenotypes from genomic data.

Results: We established a pipeline to identify all proteins associated with antimicrobial resistance within each assembly. We also extracted the 300 bp DNA region upstream of the transcription start site for each of these proteins, as well as the rRNA genes (5s, 16s, and 23s) from each assembly. By integrating data from protein and DNA sequences, we constructed various ML models to predict the AMR phenotype for the antibiotic gentamicin and Amoxicillin-Clavulanic Acid. We employed multiple encoding techniques (k-mers, one-hot encoding, and position-specific scoring matrix [PSSM]) alongside several ML algorithms (SVM, Logistic Regression, Random Forest, XGBoost, and multilayer perceptron [MLP]). Random Forest algorithm using k-mer encoding of size 3 achieved the highest performance, with an Accuracy of 98.39% for gentamicin and 97.18% for Amoxicillin-Clavulanic Acid, surpassing previous efforts documented in the literature for phenotype prediction.

Conclusions: Leveraging extensive online datasets and advanced ML algorithms, we can develop models for rapid and accurate prediction of AMR phenotypes across a wide range of antibiotics. While this abstract presents results only for gentamicin and Amoxicillin-Clavulanic Acid, our goal is to create models for 90 additional antibiotics and develop a comprehensive suite for predicting AMR phenotypes from bacterial genomic data.

OP.20

HBV Integrations: How Does Viral Load Affect Their Frequency and Genomic Distribution?

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Background: HBV integrations in the genome of hepatitis B patients play a critical role in the progression of chronic hepatitis B (CHB) and liver damage, and are present in over 85% of HBV-associated hepatocellular carcinoma (HBV-HCC) cases. These integrations can lead to genome instability, alter host gene expression, activate oncogenes, and provide a continual source of HBV subviral particles. However, the challenges they pose to achieving a functional cure remain unclear, and the dynamics of integration under varying viral replication levels are poorly understood.

Methods: We analyzed 4,564 NGS datasets from liver biopsies of CHB and HBV-HCC cases to identify integration hotspots in transcriptionally active loci. To explore integration dynamics further, we developed a long PCR-based target enrichment method to capture HBV integration events. Using MinION long-read sequencing, we applied this method to the HepDE19 Tet-off cell line, conducting triplicate experiments with and without tetracycline to modulate HBV replication. An in-house bioinformatics pipeline was designed to analyze the sequencing data using a combination of R and Python programming languages, BASH scripting and other bioinformatics tools.

Results: HBV integrations frequently occurred within genes vital to liver function, particularly in transcriptionally active regions. Fifteen genes were significantly associated with HBV-HCC development ($p<0.01$), potentially serving as prognostic markers. In the HepDE19 cell line, HBV integrations were identified both in presence and in absence of tetracycline, with no significant difference, suggesting they can occur during low replication phases in CHB.

Conclusions: This study highlights the frequent occurrence of HBV integrations in essential liver genes, even under low viral replication conditions, with implications for therapeutic strategies aimed at functional cures. Our novel PCR-based enrichment and MinION sequencing method is a powerful tool for further investigation of HBV integrations and could be useful for screening at the point of care.

OP.21 BEST ORAL PRESENTATION B' AWARD

LncRNA EPB41L4A-AS1 encodes a novel mitochondrial microprotein with tumor suppressive effects.

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Introduction: Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides and lack protein-coding capacity. They have recently emerged as pivotal players in cellular physiology and disease. Recent studies have shown that a subset of lncRNAs contain small open reading frames (lncORFs) encoding microproteins involved in diverse biological processes, expanding our understanding of the coding and non-coding genome. However, their small size and lack of conservation make the identification and functional characterization of lncORFs challenging.

Results: We focused on discovering novel lncORFs encoding microproteins in carcinogenesis. Specifically, we conducted meta-analysis of ribosome profiling experiments to identify lncORFs with the potential to translate microproteins in HCT116 cells. By integrating ribosome profiling, transcriptomics, and mass spectrometry, we compiled a list of candidate translatable lncORFs. Notably, we discovered that EPB41L4A-AS1 lncRNA encodes a previously unknown microprotein localized in mitochondria. To explore its function, we cloned the lncRNA 5' UTR and open reading frame fused to a flag epitope. We found out that the overexpression of the microprotein reduced mitochondrial membrane potential. Additionally, its overexpression induced G2 arrest, resulting in decreased cancer cell clonogenicity, while shRNA-mediated downregulation of EPB41L4A-AS1 induced cancer cell proliferation. Proteomic profiling revealed that overexpression of the microprotein led to the downregulation of multiple mitochondrial proteins, primarily localized to the mitochondrial inner membrane or matrix, as well as critical mitotic components. Immunoprecipitation followed by mass spectrometry revealed several mitochondrial components as interaction partners of the microprotein.

Conclusions: Overall, we identified multiple lncRNAs with protein-coding potential, suggesting that this 'hidden' class of biomolecules remains overlooked. Moreover, we unveiled the role of a novel lncRNA-encoded microprotein in modulating cellular bioenergetics and mitochondrial physiology, thereby exerting growth-suppressing effects in cancer cells.

OP.22

Development of an analytical NGS workflow for the clinical interpretation of whole exome sequencing data from patients with Multiple Myeloma

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Introduction: Multiple Myeloma (MM) is a clonal B-plasma cell malignancy. Is an incurable disease as, despite temporary achievement of deep responses, the majority of patients will eventually relapse. As a consequence, there remains an urgent, unmet medical need to more precisely understand the biology of MM and the relevant drug resistance and therapy response mechanisms, in the era of Precision Medicine.

Methods: We obtained gene data from 37 Multiple Myeloma studies with the use of multi-criteria optimization-based data mining techniques. The collected genes were filtered by relevance to prognosis and progression of MM patients, creating a signature gene database in vcf-format. Regarding data analysis, we used publicly available datasets derived from MM patients. We applied in-house developed R scripts to filter the sequencing output variant calling file (VCF) by different allele frequencies, as well as by the created signature gene database. Finally, we developed an in-house R script for the clinical annotation of the called variants.

Results: The in-house panel of prognostic and progression signature genes, including 264 genes and 1319 variants, was developed and used during sequencing data analysis of all exons of patients with MM to include only the variant data with greater relevance to the disease. Also, the variant annotation pipeline, allowed us to simultaneously communicate with several databases, such as: GNOMAD, SIFT, ClinVar, dbSNP, COSMIC, DANN, through the Ensembl database. This tool provides useful information and clinical annotation filters for each recorded variant during the analysis of MM sample data.

Conclusion: This study and the resulting optimized tools aim to clarify the molecular base of Multiple Myeloma, through predictive gene signatures. In the frame of “EDIMO” (Hellenic Precision Medicine Network in Molecular Oncology) this work will also enrich the publicly available oncology databases with sequencing data and metadata of patients with multiple myeloma in Greece.

Oral Session 5: Structural Biology Biotechnology & Public Health

OP.23

Towards the development of novel allosteric activators for human angiotensin-converting enzyme 2

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Background: Human angiotensin-converting enzyme 2 (ACE2) is expressed in the heart, lungs, kidneys and arteries. As part of the renin-angiotensin-aldosterone system, ACE2 plays a critical role in blood pressure homeostasis, by hydrolyzing Angiotensin II (AngII) to Angiotensin 1-7, a vasodilator with anti-proliferative and anti-inflammatory properties. The deficiency of ACE2 is proved to enhance thrombotic and inflammatory processes. Hence, ACE2 is a pharmacological target for the development of activators for the treatment of hypertension and related deceases. However, only few activators are reported, exhibiting low potency and controversial effects. This study aims at discovering ACE2 binding sites for allosteric activators. Notably, there is no experimental data for such ACE2 sites, apart from in silico studies. To seek for the existence of such internal cavities, available upon substrate binding, a phosphinic transition-state analog of the natural substrate AngII (p-AngII) was synthesized and co-crystallized with the catalytic domain (CD) of ACE2.

Methods: The CD of human ACE2 was expressed in P. pastoris and purified via IMAC and SEC after deglycosylation. Kinetic and thermostability assays were performed. Enzymatic assays were conducted using an ACE2-specific fluorogenic substrate. ACE2-CD was co-crystallized with p-AngII, followed by X-ray crystallography. Screening of a small FDA-approved drugs library for identifying ACE2 activators was performed.

Results: The expressed ACE2-CD was catalytically active, while binding of p-AngII exhibited nanomolar affinity and thermostabilizing effect on the enzyme. High-resolution crystal structure of ACE2-CD in complex with p-AngII was obtained. At least one chemical compound exhibiting activating properties was identified through initial screening.

Conclusions: The obtained crystal structure of ACE2-CD with bound p-AngII is a valuable tool for the discovery of allosteric activating binding sites. Soaking of the obtained co-crystals with the identified activator and in crystallo fragment screening of small molecules libraries will be performed for discovering such allosteric sites and additional activators, respectively.

OP.24

Expression, purification and characterization of human tyrosinase and crystallographic studies of a bacterial structural surrogate

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Background: Melanin is the primary pigment responsible for skin, hair and retina color in mammals1. Dysregulation of the melanin biosynthesis process (melanosynthesis) leads to pigmentation disorders, such as hyperchromias or hypochromias2. Tyrosinase catalyzes the conversion of L-tyrosine to dopaquinone, the first step in melanosynthesis3, being a key target for treating hyperpigmentation disorders. In our study, we focus on human tyrosinase (hTYR) and its ortholog from Priestia (Bacillus) megaterium (BmTYR), sharing 44% similarity with hTYR, thus serving as its optimal structural surrogate. Major goals are expression and purification of hTYR for studying its kinetics and interactions with potential inhibitors and its crystallization, aiming to solve the first structure of human tyrosinase.

Methods: BmTYR was cloned into a pET-9a plasmid and overexpressed in BL21 (DE3) cells. The enzyme was purified via affinity and size exclusion chromatography and its kinetics and inhibitor IC50 values were determined. For hTYR, the catalytic domain (1-456) was transiently expressed in HEK293T cells using a pcDNA-3.1 vector. Both enzymes were subjected to crystallization trials, aiming at obtaining high-resolution structures.

Results: The structure of BmTYR was successfully solved at 2.2-Å resolution and we were also able to consolidate the crystallization process for ligand soaking experiments. Subsequently, ligand screening identified some potent inhibitors that significantly reduced enzyme activity, but also some unexpected activators. Additionally, large yields of high purity hTYR were obtained, allowing kinetic characterization and crystallization trials for future structural studies.

Conclusions: To date, the closest structural surrogate of hTYR is BmTYR. In our studies, we observed that ligands that acted as inhibitors or activators on BmTYR showed similar effect on hTYR. This observation indicates that studying the crystallization-prone BmTYR might provide valuable insights into the structural and functional characteristics of hTYR. In parallel, the recently accomplished high-yield expression of hTYR paves the way for its crystallization trials.

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OP.25

Comparing different lysis conditions for proteomics analysis of amyloid deposits in biopsies for diagnostics

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Amyloidosis refers to a group of heterogeneous clinical conditions caused by the deposition of misfolded proteins in the extracellular matrix of tissues and organs. A key feature of these proteins is their tendency to form antiparallel beta-pleated sheets, which then become non-branching fibrils resistant to degradation. These fibrils cause mechanical damage and oxidative stress in organs like the heart, liver, kidneys, nervous system, and gastrointestinal tract. The gold standard for diagnosing amyloidosis is Congo red staining of sections from formalin fixed paraffin embedded biopsies, showing amyloid deposits as green birefringent areas under polarized light. More than 40 precursor proteins are known to misfold and self-assemble as amyloids. Therefore, upon histological confirmation of the amyloid deposits, the identity of the amyloidogenic (precursor) protein must be determined, given that several amyloidoses have disease-specific therapies. This is called amyloid typing. Mass spectrometry-based bottom-up proteomic analysis of laser microdissected biopsy areas is currently the most reliable method to type amyloid. But sample preparation, as developed 10-15 years ago, is somewhat tedious as the fibrils are not easily lysed. We conducted experiments to compare the state-of-the-art sample preparation used for proteomic typing of amyloid to a simpler procedure inspired from the latest developments in other domains of proteomics. More specifically, we compared a protocol involving lysis with TrisBase, EDTA, Zwittergent (TEZ) buffer to a more user-friendly protocol featuring lysis with trifluoroacetic acid (TFA). Tryptic peptides were analysed on a timsTOF fleX (Bruker) using Data Independent Acquisition - Parallel Accumulation–Serial Fragmentation (DIA-PASEF). Raw data were processed with the DIA-NN library-free software. This study discusses the differences between the two methods in terms of 1) practicalities, cost and time, 2) number of protein groups detected and overlapping profiles, 3) capacity to conclude on the diagnosis.

OP.26

Development of a molecular assay for the inedntification of *Streptococcus spp.* directly in clinical samples and the impact on an accurate diagnosis in public health measures

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Background: Accurate identification of streptococcal species other than S. pneumoniae, S. agalactiae (GBS) and S. pyogenes (GAS) causing meningitis and septicemia, still remains a challenge in clinical microbiology, due to the limitations in sensitivity and time of conventional methods. The present study aims at the development of a PCR/ sequencing-based methodology for the rapid and accurate identification of Streptococcus spp. directly in clinical samples, from patients with meningitis and septicemia which had not been identified neither as S. pneumoniae nor GAS and GBS.

Methods: This method utilizes PCR amplicons, from positive DNA patient's samples for Streptococcus spp. with meningitis and septicemia, to enhance the detection of tuf gene in the bacterial genome. Key steps in this assay include DNA extraction from biological samples, amplification of the targeted DNA and sequencing, which decodes the PCR amplicons to identify different streptococcal species. Results from sequencing were visualized with CHROMAS software and sequences were edited using CLUSTALW software. Finally, the edited sequences were input in BLAST for their comparison with reference sequences.

Results: Out of 145 clinical samples tested, PCR amplification was successful in 92.4% (134/145) and sequencing allowed accurate species level identification in 88.8% (119/134) of PCR (+) samples. This method detected a wide range of streptococcal species, including S. dysgalactiae, S. salivarius, S. mitis/ pseudopneumoniae, as well as other bacteria which included tuf gene in their genome, and previously were identified as streptococcal species. Moreover, the turnaround time of diagnosis was significantly reduced compared to culture- based methods.

Conclusions: This PCR/ sequencing- based approach provides a fast, accurate and culture- independent method for identifying streptococcal species directly in clinical samples with an increased distinctive power. This approach is very important to the clinicians, for the epidemiology and for public health measures.

OP.27

Subtyping of *L. infantum* strains from Greece by using genomic DNA isolated from cultivated parasites and different PCR-based techniques

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Background: *Leishmania (L.) infantum* is a species endemic in Greece, with wide geographical distribution but small genetic variability. *L. infantum* is zoonotic and causes mainly visceral leishmaniasis in humans. Recently, new monophyletic groups have appeared in the Mediterranean, and hence the difficult task of low-cost *L. infantum* subtyping could be important for leishmaniasis surveillance.

Methods: Clinical samples were obtained from six *Leishmania*-infected patients, diagnosed in the Diagnostic Department of HPI or other Public Health Facilities. Parasites were cultivated from biological samples, and DNA was extracted using commercial kits. *Leishmania* typing was performed by sequencing of the amplified Internal Transcribed Spacer-1 (ITS-1) product of PCR. In addition, deeper discriminating capacity was achieved by applying PCR assays targeting *HSP70-I* and *K26* genes.

Results: The ITS-1 sequences from clinical isolates were aligned by ClustalOmega to ITS-1 sequences of different *Leishmania* species. The alignments were visualized with BioEdit software and the causative *Leishmania* species for all six clinical samples, was identified as *Leishmania infantum*. Then, the typing tools based on the amplification of the *HSP70-I* and *K26* genes were validated. The size of the *HSP-70-I* UTR amplification products was detected at 730-bp for all the analysed strains. The PCR assay targeting the *K26* gene resulted in a main 940-bp amplicon as the predominant product and, occasionally, in a second 870-bp amplification product, for 5 out of 6 studied strains. However, one isolate from a possibly imported case of cutaneous leishmaniasis who had recently travelled in Spain, produced a different amplicon size of 830-bp.

Conclusions: The use of *Leishmania* genomic DNA as a PCR template, isolated from cultivated parasites versus DNA from infected tissue samples, is essential to abrogate sensitivity pitfalls of “multimarker” PCR. Combination of the aforementioned PCRs, is sufficient to subtype *L. infantum* isolates, even from a relatively small number of samples.

Oral Session 6: Neurobiology

OP.28

Deciphering and targeting early synaptic dysfunction in pre-clinical models of familial Parkinson's disease

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Alpha-synuclein (αSyn) is a highly expressed and conserved presynaptic protein, which has been tightly linked to Parkinson's disease (PD). Synaptic dysfunction is considered to be an early yet progressive pathological feature in PD, with the triggering mechanisms remaining undefined. This study analyzes how the G209S pathogenic mutation in the synuclein gene, and its aberrant protein product p. A53TαSyn protein disrupts synapse formation and function, even at early stages of neuronal differentiation. Herein, a transgenic mouse model expressing the human p.A53T-αSyn in brain neurons under the control of the PrP promoter (Prnp-SNCA*A53T), alongside with human-derived neurons bearing the p.A53T mutation are employed. Proteomic analysis of synaptosomes from different brain regions of 6-month-old mice reveals dysregulation of presynaptic proteins primarily associated with synaptic vesicle trafficking while imaging analysis of the p.A53T synapse structure demonstrates diminished number of synaptic vesicles and impairment of PSD formation, prior to αSyn aggregation. In compliance, the cortical and hippocampal expression of the vesicular glutamate transporter protein vGLUT1 and the neurotransmitter GABA is impaired long before the reduction of dopaminergic neurons in the substantia nigra. Moreover, immunocytochemical assessment of mouse and human p.A53T-αSyn neurons unveils aberrant connectivity, alterations in the numbers of excitatory and inhibitory synaptic contacts, and a largely compromised network. The early appearance of these defects is further reinforced by the partial inability of p.A53T neurons to form artificial synapses. Notably, the administration of dual-allosteric NMDAR antagonists, Memantine and Nitrosynapsin, potentially reverses the observed synaptic dysfunction. The cellular and molecular analyses of the in vitro p.A53T-αSyn pre-clinical systems are complemented by longitudinal electrophysiological studies using a multi-electrode array system to record neural network activity and synaptic functionality. Altogether, our approach provides spatiotemporal evidence of early synaptic dysfunction as a key aspect of p.A53T-αSyn pathology, which can be reversed by the use of neuromodulatory agents.

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OP.29

Connecting Connectomes to Physiology via optogenetics and complex network theory: an ex vivo Investigation of Spontaneous Oscillations in the Mouse Motor Cortex

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Introduction: The advent of genetically encoded voltage indicators (GEVIs) has long been praised as a major breakthrough in the field of neuroscience, yet the impact on our understanding of circuit physiology remains elusive. Such ambiguity is driven by both technical limitations regarding indicator performance and imaging technology, as well as the absence of a broadly accepted framework for analysing the emerging imaging data.

Methods: Here, by expressing ArcLight, a potent GEVI for in vitro and in vivo imaging, we imaged the mouse primary motor cortex pan-neuronally and visualized both the neuronal excitation and inhibition during bath application of bicuculline. Using widefield epifluorescence microscopy, we were able to optically resolve spontaneous oscillations in brain slices of the mouse motor cortex ex vivo. Next, we resorted to complex network theory techniques in order to document the functional dependencies among recording sites, aiming to identify discernible patterns in the functional networks across various recording conditions that could serve as reliable predictors of oscillatory behaviour.

Results: While preliminary results indicated clearly that ripple anticipation is associated with increased coordination in the network, such hypothesis failed to generalize as additional experiments were conducted.

Discussion: Though network theory provides a powerful framework for the analysis of large datasets in general, the absence of a detectable network reorganization at times surrounding a ripple event suggests its limitations in being used as a data exploratory tool in spontaneous settings. Therefore, we conclude, alternative interdisciplinary intuitions about the functional interpretation of the data might be more appropriate, at least until technical challenges related to GEVIs are resolved.

OP.30

Control iPSC-derived astrocytes rescue P.A53T-αSyn patient-iPSC-derived neurons from Parkinson's disease-related pathology

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Introduction: Parkinson's disease (PD) is characterized by progressive loss of midbrain dopaminergic neurons while the major histopathological hallmark is the presence of α-synuclein (αSyn) inclusions, termed Lewy bodies and Lewy neurites1. Approximately 10% of PD cases are associated with mutations in specific genes, such as the p.A53T-αSyn mutation. Despite intensive research on neuronal dysfunction, the role of astrocytes in PD remains largely unexplored. Ongoing research in our lab suggest that PD-patient induced pluripotent stem cell (iPSC)-derived astrocytes display cell-intrinsic pathologies such as intracellular protein phospho(Ser129)αSyn+ aggregates, as well as disturbed autophagy, endocytosis, and lysosomal function. Our aim is to study the reciprocal interactions of astrocytes and neurons in PD pathology.

Methods: To investigate the contribution of PD astrocytes (PDa) in neuronal pathology, as well as the potential protective role of healthy astrocytes (Ha), we differentiated iPSC to ventral midbrain astrocytes and dopaminergic neurons (Hn and PDn) and studied them in a direct and indirect co-culture setup.

Results: We observed that the PDa introduced neuropathology in co-cultured Hn, including compromised viability, impaired neuritic outgrowth and neurodegeneration features. In contrast, the physiology of PDn was improved when co-cultured with Hn. Notably, neurodegeneration was reversed by Ha, at least partially, due to their capacity to uptake and resolve neuronal aSyn aggregates – a process impaired in PDa. Accordingly, the synaptic connectivity and calcium oscillations of Hn were disturbed on PDa, whereas Ha improved the connectivity and restored calcium homeostasis of PDn. Interestingly, most aforementioned phenotypes were also observed in a non-contact setup, in which Hn or PDn were treated with conditioned medium from healthy or PD astrocytes.

Conclusions: Our data underscore a critical impact of mutant astrocytes in PD pathology and a remarkable ability of healthy astrocytes in rescuing neurodegeneration. These effects are mediated, at least partially, in a paracrine manner.

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OP.31

In vivo study of the effect of microglial BIN1 deletion on mouse brain under homeostatic and neuroinflammatory conditions

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Introduction: Genome-Wide Association Studies have identified Single Nucleotide Polymorphisms (SNPs) strongly associated to increased risk of developing Alzheimer's Disease (AD). SNPs in the locus harboring Bridging Integrator 1 (Bin1) gene show the strongest association with AD, after Apolipoprotein E. BIN1 is a member of the Bin/Amphiphysin/Rvs (BAR) family of adaptor proteins that are implicated in cell membrane modelling dynamics and expressed in microglia. Although BIN1 implication in neuronal dysfunction has been studied both in vitro and in vivo, its role in microglial activation state and its contribution in AD pathology remains elusive.

Methods: To this end we have developed a conditional double transgenic $Cx3CR1^{Cre\text{-}ERT2}$//$Bin1^{fl/fl}$ mouse line, in which Bin1 is knocked-out in microglia and have challenged Bin1cKO mice with LPS, to investigate the effect of microglia-specific BIN1 deletion on mouse brain under homeostatic and inflammatory conditions. To study our model, we performed snRNA-Seq analysis as well as in vivo immunohistochemical analysis and molecular analysis.

Results: SnRNA-Seq analysis of adult mouse cortices indicated that a number of signaling pathways are differently impacted in Bin1cKO versus control microglia following LPS administration. More specifically, Bin1 deletion resulted in the enrichment of microglial cell subpopulations exhibiting enhanced proliferative capacity and IFN-type I-mediated inflammatory response following LPS treatment, findings that were confirmed by subsequent real time RT-PCR and immunohistochemical analysis. Moreover, FACs analysis indicated increased $CD11c^{+}$ microglia, while immunofluorescence/morphometric analysis revealed that microglia exhibits enhanced CD68 expression, along with appearance of a hyper-ramified morphology with increased number of intersections and convex hull volume.

Conclusions: Our study indicates that the deletion of microglial BIN1 promotes the enrichment of proliferative and proinflammatory microglial subpopulations following LPS-induced inflammation. Furthermore, upon LPS-induced inflammation, BIN1 knockout (KO) cortical microglia exhibit a more activated phenotype, characterized by a hyper-ramified morphology, increased expression of the inflammation surface marker CD11c and increased microglia-specific CD68 volume, indicative of heightened phagocytic activity.

OP.32

The Impact of Multisensory Strategies on Functionality in Adults with Neurological Disorders

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Introduction: Neurological disorders such as Alzheimer's disease,traumatic brain injuries (TBI),and spinal cord injuries (SCI) significantly affect daily functioning and quality of life.This study seeks to examine the extent to which the adoption of multisensory strategies,through the use of sensory enhancements,auditory stimulation,and visual cues,can aid in improving the functionality and daily life of adults.

Methods: Within this research, a mixed design including both quantitative and qualitative data was applied.To identify the multisensory strategies that have been utilized in occupational therapy for patients with AD,TBI and SCI,a systematic review of the literature has been performed.Empirical data were gathered from clinical trials,case studies,and observational studies with multisensory interventions. Such study included patients of different degrees of neurologic dysfunction covering both group and individual treatment.CAA measures of cognition and motor function were taken in the form of several standard assessments and improvement measures.

Results: The study reached the conclusion that the performance of the reconstructed subsystem and the performance of the activities of daily living have been improved in significant measure by the use of multisensory approaches.Such therapies as sensory enrichment and stimulation of hearing and vision led to improvement of cognitive and motor functions in patients suffering from Alzheimer's,TBI,and SCI.

Conclusions: The study confirms the positive outcome of the application of multisensory strategies within the occupational therapy practice for patients with neurological disorders such as Alzheimer's disease post-traumatic brain injuries and spinal cord injury patients.Results of psychosocial impact suggest that these methods and techniques may be of value in terms of improving the functioning and the quality of life.

OP.33 BEST ORAL PRESENTATION A' AWARD "THANASIS LOUKERIS"

Microglia regulate cortical remyelination via TNFR1-dependent phenotypic polarization

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Introduction: Microglia are key mediators in CNS neurodegenerative demyelinating disorders, exacerbating neuroinflammation through secretion of neurotoxic mediators or promoting repair by acquiring regenerative phenotypes.

Methods: Here we combine pharmacological approaches and conditional gene-targeting in mice together with transcriptomics, 3D brain imaging, and behavioral monitoring tools, with in in vivo and in vitro experimental models of demyelination, to investigate mechanisms of microglia-mediated CNS remyelination.

Results: Selective blockade of soluble TNF by XPro1595, a brain penetrating pharmacological inhibitor, promoted cortical remyelination in a cuprizone-induced demyelination model by modulating microglia activation. Therapeutic microglia-specific TNF receptor 1 depletion, by spatial and temporal conditional gene editing, was sufficient to promote cortical remyelination and improve functional motor performance, demonstrating a beneficial cell-autonomous microglia role in the absence of TNFR1. In situ cellular analysis of cortical microglia showed enhanced phagocytosis of myelin debris and altered activation morphology in the absence of solTNF-TNFR1. Longitudinal brain RNA-Sequencing revealed distinct brain transcriptome signatures associate with disease stages, with earlier recovery upon therapeutic microglia TNFR1 deletion during remyelination. Single cell transcriptomics of early cortical microglia revealed that activated disease-associated microglia maintain a IL-10-responsive reparative inflammatory phenotype associated with later remyelination in the absence of solTNF, and escape switching to an IL-1-related damaging profile. In vivo validation by IL-1 receptor blockade was sufficient to reproduce the pro-myelinating effects of microglia. Furthermore, disease-state microglia producing downstream IL-1/IL-18/CASP1 targets are identified in human demyelinating lesions.

Conclusions: Our results demonstrate a critical role of the microglia solTNF-TNFR1-IL-1 axis in controlling the switch between reparative and damaging disease-associate microglia in cortical remyelination, and suggest that harnessing the beneficial functions of microglia by cytokine targeting represents a promising therapeutic strategy for promoting CNS repair.

POSTER PRESENTATIONS

Poster Session: Bioinformatics

PP.01

Impact of Chios Mastic on Oral Health: Reducing Inflammation and Promoting Beneficial Bacteria

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Introduction: Gingivitis, affecting over 50% of the global population, is caused by bacterial accumulation due to poor dental hygiene and can progress to periodontitis, the leading cause of tooth loss. Chios Mastic, a natural resin from mastic trees on Chios Island, Greece, has notable antimicrobial and therapeutic properties. Recent studies suggest that chewing Chios Mastic gum significantly reduces specific oral bacterial colonies.

Methods: This study investigated the effects of Chios Mastic on the oral microbiome and host immune response. Twenty-two patients with gingivitis or stage I periodontitis were divided into three groups: 0% Chios Mastic gum (placebo), 25% Chios Mastic gum, and 100% Chios Mastic oil, supplementing the Standard of Care over 21 days. Clinical assessments and sample collections were conducted on days 0, 7, and 21.

Results: Significant reductions in the Gingival Bleeding Index (GBI) were observed from day 0 to day 7 and day 21 in the Chios Mastic groups, but not in the placebo group. Patients using mastic oil showed a greater reduction in GBI after 21 days compared to those using placebo gum. The Plaque Index (PI) showed variability, with reductions from day 0 to day 7 in both the placebo gum and mastic oil groups, but only the latter showed significant reductions after 21 days. Shotgun metatranscriptomics revealed a reduction in Porphyromonas gingivalis in subgingival plaque samples in patients using Chios Mastic gum after 7 and 21 days. The mastic oil group also showed a reduction in Tannerella forsythia and an increase in beneficial bacteria such as Lautropia mirabilis and Rothia aeria on day 21.

Conclusions: Overall, Chios Mastic products, particularly mastic oil, were associated with significant reductions in gingival inflammation and pathogenic bacterial colonies, supporting their potential in promoting oral health. Future research should explore the long-term effects and optimal usage of Chios Mastic in diverse populations.

PP.02

Agnodice: indexing experimentally supported bacterial sRNA-RNA interactions

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Introduction: In the last decade, the rapid growth in the field of bacterial small RNAs (sRNAs), combined with biotechnological breakthroughs in Deep Sequencing, has allowed a deeper understanding of sRNA-RNA interactions. However, microbiology still lacks a thoroughly curated collection to organize this rapidly expanding area.

Methods: We present Agnodice, a systematic effort to catalog and annotate experimentally supported bacterial sRNA-RNA interactions. Agnodice integrates data from various experimental methodologies, including state-of-the-art Deep Sequencing-based interactome identification techniques. All interactions are annotated at strain-level resolution and include entries derived from both low-yield and high-throughput experimental methods.

Results: Agnodice contains 39,600 annotated entries, encompassing 399 sRNAs and 12,137 target RNAs from 71 bacterial strains. The data is exclusively experimentally supported and publicly accessible. Researchers can freely download the database for further analysis.

Conclusions: Agnodice is a valuable resource that enables microbiologists to formulate new hypotheses, identify potential sRNA-based drug targets, and explore the therapeutic potential of microbiomes through the lens of small regulatory RNAs.

PP.03

TarBase v9.0: Expanding Experimentally Validated miRNA–Gene Interactions to Virally Encoded miRNAs and Cell-Type Specific Contexts

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Introduction: TarBase v9.0 is a major update to the well-established database of experimentally validated microRNA (miRNA)–gene interactions. This latest version incorporates over 6 million interactions, including new data on virally encoded miRNAs and target-directed miRNA degradation (TDMD) events. TarBase v9.0 is designed to support researchers in exploring miRNA interactions across various cell types, tissues, and experimental conditions.

Methods: TarBase v9.0 integrates data from 37 experimental protocols across 172 tissues and cell types, providing a comprehensive view of miRNA–gene interactions. The platform includes interactions obtained from both high- and low-throughput experiments, annotated with rich metadata, including gene, transcript, miRNA, and experimental context information. A new interface offers advanced search options with filtering criteria for miRNA expression levels, cell lines, and experimental conditions.

Results: TarBase v9.0 represents the largest collection of experimentally supported miRNA–gene interactions, with over 2 million unique miRNA–gene pairs. New features such as cell-type resolution of miRNA binding sites and interactions with virally encoded miRNAs enhance the database's utility for diverse biological and clinical research applications. The redesigned platform facilitates efficient data retrieval and analysis, empowering researchers to perform complex queries.

Conclusions: TarBase v9.0 offers an unparalleled resource for the study of miRNA–gene interactions, with expanded content and enhanced functionality. Its integration of vast experimental data, combined with advanced search capabilities, provides researchers with a powerful tool to explore miRNA regulation in various biological systems and diseases.

PP.04

Biomedical Question Answering using a 'Farm' of Open Large Language Models

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Keywords: Biomedical Question Answering, BioASQ, Large Language Models, Retrieval-augmented generation

Abstract. Biomedical text mining and question-answering are essential and complex tasks driven by the need to access and process the ever-expanding volume of biomedical data. With the exponential growth of published biomedical literature, effective retrieval and accurate question-answering systems are crucial for researchers, clinicians, and medical experts to make well-informed decisions. The emergence of open-source Large Language Models (LLMs) marks a significant trend in the tech landscape, with these models increasingly tailored to address diverse tasks. In this work, we present our participation in the twelfth edition of the BioASQ challenge, which involves biomedical semantic questionanswering for task 12b and biomedical question answering for developing topics for the Synergy task. We deploy a selection of open-source LLMs for embedding and retrieval of documents and snippets, as well as retrieval-augmented generators to answer biomedical questions. Dense retrieval methods, leveraging distances between dense representations of documents and questions obtained from LLM embeddings, and hybrid sparse/dense approaches, outperform traditional sparse retrieval methods in terms of mean average precision. We also implement a 'farm' of open-source LLMs to provide exact answers to biomedical Yes/No type questions. A variety of models process the prompts, and a majority voting system combines their outputs to determine the final answer. Ideal answers, summarizing the most relevant information for each question type, are generated by the MIXTRAL LLM. In the four rounds of the 2024 BioASQ challenge, our system achieved notable results: 1st and 2nd place in two rounds for 'exact answers,' 2nd place in one round for 'documents,' 2nd place in one round for 'ideal answers,' and 1st place in one round for 'snippets'.

PP.05

Longitudinal whole brain RNA sequencing reveals characteristic transcriptomic signatures at hallmark timepoints in cuprizone demyelination and remyelination.

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Introduction: RNA sequencing has revolutionized the identification and characterization of pathogenic mechanisms in many diseases. NGS RNA-seq technologies are widely used in biomedical research for understanding gene expression signatures involved in brain pathology.

Materials and Methods: Here, we focus on multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS, using NGS whole brain RNA-seq in a pre-clinical animal model for MS induced by dietary cuprizone. Specifically, to document the transcriptomic profiles of mouse brains at key pathological timepoints of CPZ demyelination and remyelination, we performed whole brain RNA sequencing from young (2-month-old) wild type male C57BL/6 mice, isolated at hallmark disease stages, specifically naïve (CPZ0), peak of demyelination after 5 weeks of cuprizone (CPZ5), and remyelination after 6 weeks of cuprizone and 1 week of normal diet (CPZ6+1). Longitudinal differential gene expression analysis between the different timepoints was performed, using computational tools and bioinformatic visualization techniques, such as Protein-Protein interaction networks, Gene Ontology networks, gene expression heatmaps, volcano and balloon plots. Brain RNA-seq revealed distinct gene clusters and transcriptomic profile that were correlated with each disease state.

Results: The pairwise gene expression comparisons between the disease model stages revealed markedly different brain transcriptomic profiles during health, demyelination and remyelination. The genes altered during cuprizone demyelination were primarily associated with immune responses, phagocytosis, and inflammation, reflecting significant microglial activation. Remyelination transcriptome showed gene expression changes linked to processes like lipid metabolism, lysosomal activity, axon ensheathment, and myelin repair, indicating tissue recovery and new myelin production.

Conclusions: Overall, this study highlights the power of the RNA-Seq approach for identifying CNS transcriptomes in both health and disease states and for characterizing specific genes and transcriptional processes involved in the pathogenesis of brain diseases.

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PP.06

MilkExosOmics: A Comprehensive Relational Database for Analyzing Milk-Derived Exosomal Cargo

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Background: Milk-derived exosomes are extracellular vesicles (EVs) secreted by mammary epithelial cells during lactation, play a pivotal role in neonatal development by transferring bioactive molecules from mother to offspring. Recent research has illuminated the significance of their RNA and protein cargo in influencing health and development. Despite the increasing volume of data generated, comprehensive resources specifically dedicated to analyzing milkderived exosomes across species are limited. Existing databases provide foundational information on EVs but often lack the depth and integration necessary for detailed comparative analyses of milk-derived exosomes. To address this gap, we developed MilkExosOmics, a specialized relational database that consolidates multi-species data on the RNA and protein cargo of milk exosomes.

Methods: A systematic literature search was conducted using PubMed with the query '((exosome) OR (extracellular vesicle) OR (EV)) AND (milk)'. The search identified studies that utilized transcriptomics (Microarrays/RNA-seq/miRNA-seq) and proteomics (Microarrays/MS) techniques to analyze milk-derived exosomes. Meta-analyses and studies lacking original data were excluded. Relevant studies were manually curated and incorporated into an SQLbased relational database, designed for efficient data management and querying.

Results: The MilkExosOmics database incorporates data from 86 publications, covering 12 mammalian species, 114 datasets, and over 2000 individual milk-derived exosome samples. The database catalogues more than 1500 miRNAs, over 16,000 mRNAs, and more than 2000 proteins. This relational framework enables researchers to explore both species-specific and conserved molecular cargo, enhancing cross-species comparisons of milk exosome biology.

Conclusions: MilkExosOmics provides a valuable resource for the milk exosome research community, facilitating the exploration of the molecular diversity and conserved functions of milk exosomes. By offering a platform for the analysis and interpretation of complex datasets, the database advances our understanding of the biological roles of milk-derived exosomes, particularly in the context of maternal-infant interactions across species.

Poster Session: Immunology

PP.07

Brain region specific microglial dynamics in Experimental Autoimmune Encephalomyelitis

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Introduction: Microglia are cells of the CNS with pleiotropic functions in health and disease. In Multiple Sclerosis (MS), which is a chronic inflammatory demyelinating disease of the CNS, they are known to be involved in the early pathology by becoming activated and forming nodules in the brain even before any detectable pathology. However, the precise relationship between brain microglial dynamics with the progression of neuroinflammation, a key feature of MS, remains elusive.

Methodology: We used C57BL/6 and CX3XR1-GFP transgenic mice, in which microglia are genetically labelled with e-GFP. We subjected them to EAE, a model of neuroinflammation, and captured microglial dynamics by chronic intravital two-photon imaging and immunohistochemistry on fixed tissues. We additionally performed Golgi staining in brains of mice with EAE and analysed data in 3D using Fiji and Imaris.

Results: Chronic intravital imaging of microglia in two brain regions simultaneously, cortex and cerebellum, during EAE revealed that they acquire a hypertrophic phenotype, a sign of activation, in cerebellum and not in cortex, and this effect appears at the preclinical stages of the disease. Immunohistochemistry on fixed tissues validated that this effect is region specific and further revealed that the activation of the innate immunity in EAE, by immunizing mice only with the adjuvant (CFA) and injecting with Pertussis toxin, is sufficient to promote cerebellar microglial activation in preclinical EAE. Last, by double immunofluorescence and surface colocalization analysis, we show that cerebellar microglia increase their contacts with neurons at the pre-onset and onset of EAE, and we are now investigating whether these contacts may mediate dendritic and/or spine alterations that could be a cause of cognitive disabilities.

Conclusions: Microglia in cerebellum during EAE show unique dynamics that were unknown so far. They get activated in preclinical stages and increase their contacts with neurons that might mediate cognitive dysfunctions.

PP.08

Spontaneous human CD8 T cell and autoimmune encephalomyelitis-induced CD4/CD8 T cell lesions in the brain and spinal cord of HLA-DRB1*15-positive multiple sclerosis humanized immune system mice

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Autoimmune diseases of the central nervous system (CNS) such as multiple sclerosis (MS) are only partially represented in current experimental models and the development of humanized immune mice is crucial for better understanding of immunopathogenesis and testing of therapeutics. We describe a humanized mouse model with several key features of MS. Severely immunodeficient B2m-NOG mice were transplanted with peripheral blood mononuclear cells (PBMCs) from HLA-DRB1-typed MS and healthy (HI) donors and showed rapid engraftment by human T and B lymphocytes. Mice receiving cells from MS patients with recent/ongoing Epstein–Barr virus reactivation showed high B cell engraftment capacity. Both HLA-DRB1*15 (DR15) MS and DR15 HI mice, not HLA-DRB1*13 MS mice, developed human T cell infiltration of CNS borders and parenchyma. DR15 MS mice uniquely developed inflammatory lesions in brain and spinal cord gray matter, with spontaneous, hCD8 T cell lesions, and mixed hCD8/hCD4 T cell lesions in EAE immunized mice, with variation in localization and severity between different patient donors. Main limitations of this model for further development are poor monocyte engraftment and lack of demyelination, lymph node organization, and IgG responses. These results show that PBMC humanized mice represent promising research tools for investigating MS immunopathology in a patient-specific approach.

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PP.09

Investigation of Neutrophil subpopulations and NETs in the peripheral blood of MS patients

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Objectives: Multiple Sclerosis (MS) is a multifaceted demyelinating disease with strong autoimmune, neurodegenerative and neuroinflammatory features. Its treatment is mainly based on disease-modifying therapies, mainly targeting B and T cells. However, administered treatments appear to significantly affect the abundance and function of neutrophils, a key cell type in innate immunity. Recent reports exhibit elevated neutrophil-to-lymphocyte ratio (NLR) and increased neutrophil activation in MS patients, correlating with disease activity. Herein, we sought to investigate changes in specific neutrophil subpopulations and the presence of NETs in the peripheral blood between patients with a first clinical episode of MS, patients with MS in remission and controls.

Methods: 20 RRMS/CIS patients (14 with a first clinical episode of MS, 6 in remission) and 15 healthy donors were included. Whole peripheral blood samples were analyzed by FACS using a panel of immune cell marker antibodies and 4 neutrophil subpopulations were assessed: Mature neutrophils: $CD66b^{+}$ $CD14^{-}$ $CD11b^{+}CD10^{+}$, Immature neutrophils: $CD66b^{+}CD14^{-}CD11b^{+}CD10^{-}$, Aged neutrophils: $CD66b^{+}$ $CD14^{-}$ $CD11b^{+}CD10^{+}CXCR4^{high}$ and Suppressive neutrophils: $CD66b^{+}CD14^{-}CD11b^{+}CD33^{DIM}LOX\text{-}1^{+}$. Neutrophils were isolated from whole peripheral blood by ficoll-double gradient density centrifugation and NETs were detected by immunofluorescence for specific markers Myeloperoxidase (MPO) and citrullinated H3 and quantified by MPO-DNA complex ELISA.

Results: Aged neutrophils were found elevated in RRMS/CIS patients with a first clinical episode compared to the other two groups, whereas suppressive neutrophils were increased in RRMS/CIS patients in remission. Increased NET release was observed in *ex vivo* neutrophils from RRMS/CIS patients with a first clinical episode compared to RRMS/CIS patients in remission and healthy donors. Interestingly, a statistically significant positive correlation was observed between aged neutrophils and NET release ($r=0.764$, $p>0,005$).

Conclusion: These preliminary findings suggest that neutrophils could be involved in the pathophysiology of MS and they could provide a biomarker for the disease activity.

Poster Session: Microbiology

PP.10

Leishmania Dual-specificity Tyrosine-Regulated Kinases: Investigating the role of parasite-specific members and discovering small molecule inhibitors against LinDYRK1

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Background: Kinases play a critical role in the life cycle of Leishmania parasites, the causative agents of the global disease leishmaniasis. DYRKs (Dual-specificity Tyrosine-Regulated Kinases) are a family of kinases that are relatively understudied. They are characterized by self-phosphorylation at a tyrosine residue in the activation loop, which is essential for full activity, and by phosphorylation of their substrates at serine/threonine residues. Leishmania parasites possess an expanded family of DYRKs. In Leishmania infantum, LinDYRK1 (LINJ_15_0180) is implicated in metacyclogenesis, a key stage of parasite development, as well as in promastigote infectivity. LinDYRK14 (LINJ_14_1140) and LinDYRK21 (LinJ.21.2010) belong to a unique clade of parasitic DYRKs, with potential functional roles that remain to be elucidated.

Methods: To identify inhibitors of LinDYRK1 that could eventually disrupt Leishmania metacyclogenesis, a LinDYRK1-specific kinase assay for medium-throughput screening was developed. A set of 50 bis-indole kinase inhibitors (indirubins) was tested for inhibitory activity. Additionally, CRISPR/Cas9 technology was used to tag LinDYRK14 and LinDYRK21 with mNeon-Green fluorescence protein and to generate knockout (KO) parasites for each gene.

Results: Eight indirubin compounds with potent inhibitory activity (IC50 < 2,5 μM) were identified, with certain substitutions showing promise as lead compounds for targeting LinDYRK1. Localization studies revealed that LinDYRK14 is found in the basal body of the parasites, while LinDYRK21 resides in their cytoplasm. LinDYRK14 KO parasites could not be generated, indicating its essential role for viability. In contrast, LinDYRK21 KO showed no apparent phenotype in promastigotes.

Conclusion: LinDYRK1 is a potential target for disrupting Leishmania metacyclogenesis, with indirubins emerging as promising inhibitors. LinDYRK14 appears to be essential for parasite survival, while LinDYRK21's role is still unknown, particularly in the amastigote stage. Further research is needed to validate these kinases as therapeutic targets for leishmaniasis treatment.

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PP.11

Interaction of human neutrophils with Bartonella henselae in Cat Scratch Disease

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Introduction: Cat scratch disease (CSD) is the most common clinical manifestation of Bartonella henselae infection and is considered one of the most frequent bacterial etiological agent of benign lymphadenopathy in the young population worldwide [1]. In immunocompetent patients it usually presents as a mild but often long lasting swelling of the lymph nodes, with a median of 7 weeks, but it can persist for as long as 12 to 24 months [2]. It is suggested that bacteria are not viable in infected lymph nodes [2-4], and it is assumed that immunology plays a role in lymph node enlargement. However, there is very limited information and understanding on the immune responses that occur during a B. henselae infection or how lymphadenitis is orchestrated. Innate immune system is usually the main sentinel against bacterial infections, however, very little is known about the implication of innate cells in CSD, despite the fact that CSD granulomas are neutrophil rich.

Methods: In vitro studies were performed to examine the interaction of neutrophils with B. henselae. Immunolabeling was conducted to determine the ability of neutrophils to phagocytose B. henselae and create Neutrophil Extracellular Traps (NETs). Reactive Oxygen Species (ROS) were measured using flow cytometry. In addition, lymph node biopsies were immunostained to investigate the neutrophilic involvement in the lymph node enlargement.

Results: Purified neutrophils were infected in vitro with B. henselae and it was found that neutrophils phagocytose B. henselae after opsonization, in comparison with non opsonized bacteria that are phagocytosed only to a very small extend. Moreover, we observed that B. henselae induce NET release in neutrophils. Finally, CSD lymph node biopsy specimens were stained, and NETs were found on them.

Conclusion: It seems that neutrophils and NETs play an important role in the long lasting lymphadenopathy.

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PP.12

Implication of L-dopa decarboxylase in dengue virus-mediated inhibition of autophagy and induction of cell death

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Introduction: L-Dopa decarboxylase (DDC), the biosynthetic enzyme of dopamine, has been identified as a potential mitigating factor of hepatitis C (HCV) and dengue (DENV) viruses propagation, through its interaction with phosphatidylinositol 3-kinase (Frakolaki_et_al.,2019) and its biosynthetic function (Mpekoulis_et_al.,2021,2022). Nevertheless, the precise mechanism by which DDC impedes DENV replication requires further investigation. Interestingly, dopamine has been reported to regulate the autophagic process in neuronal cells. DENV exploits autophagy to favor its life cycle, by inhibiting the autophagosomes lysosomes fusion at advanced stages of infection.

Aim: Based on the preceding findings, we explored whether DDC hinders viral replication by modulating the autophagic flux in Huh7-Lunet and Huh7.5 cells.

Methodology: To this end, we performed DDC silencing and overexpression experiments, followed by analysis of autophagic markers after induction or inhibition of autophagy.

Results: Our findings exhibited that DDC promotes autophagy, at least partially through its biosynthetic role. DDC silencing or inhibition of its enzymatic activity disrupted autophagy completion, thereby enhancing DENV replication and infectivity. Conversely, regulation of the autophagic process seemed to affect DDC expression, as chemical induction of autophagy upregulated DDC, while its suppression, either by employment of chemical means (NH4CL) or by genetic knockout (ATG14L), reduced DDC mRNA and protein levels. Moreover, we demonstrated that DDC is implicated in the cellular energy balance and DENV-induced cell death. Specifically, DDC silencing dampened oxidative phosphorylation and DENV-related cytopathic effects.

Conclusion: These findings indicate a potential crosstalk between DDC and the autophagic process, which could be exploited by the virus. Furthermore, the same data elucidate the regulatory function of DDC on cellular homeostasis and energy metabolism, emphasizing its critical importance in the context of viral infections.

PP.13

The role of exosomal cargo as viral fingerprint in HCV infection before and after treatment with DAAs.

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Introduction: Hepatitis C Virus (HCV) is a single-stranded positive sense RNA virus of approximately 9.6 kb in length that belongs to the Flaviviridae family. Almost 75% of HCV patients develop chronic infection that induces hepatic inflammation leading in many cases to cirrhosis and hepatocellular carcinoma. Extracellular Vesicles (EVs), and more specifically exosomes, contribute to intercellular communication through transfer of nucleic acids, proteins and lipids from donor to recipient cells. HCV exploits exosomal cargo in order to establish viral infection, enhance viral replication and evade the immune response.

Aim: The purpose of this work was to isolate and characterize exosomes from the HCV in vitro cell culture system before and after treatment with Direct Acting Antivirals (DAAs) and to investigate their content in immunosuppressive factors capable of promoting viral escape and reduced immune surveillance.

Materials and methods: For this purpose, in vitro HCV infection experiments were performed. Huh7.5 cells were infected with HCV and treated with DAAs at indicated time points (post-infection –p.i.). Cell extracts and cell culture supernatants were collected and used to detect viral expression levels and investigate the exosomal cargo, respectively.

Results and conclusions: The characterization of EVs was performed by Nanoparticle Tracking Analysis (NTA) and Western blot analysis by testing for the specific EV markers TSG101, CD81, CD63. Furthermore, the presence of the immunoregulatory factor Programmed death-ligand 1 (PD-L1) in the isolated exosomes was investigated by Western immunoblotting and ELISA. The NTA results showed that the size of isolated EVs ranged between 50-150nm confirming the presence of exosomes, which were the most abundant vesicles in our samples. Moreover, PD-L1 was detected in exosomes during HCV in vitro infection before and after DAA treatment. Having observed similar results from clinical samples, we propose that virus-driven loading of specific factors in the exosomal cargo, could sustain deregulation of the host immune response long after HCV eradication.

PP.14

Metabolic activity of vaginal microbiome

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According to WHO, adult infertility affects 17.5% of the global population. Recent studies have shown that both vaginal and semen microbiomes influence infertility for both genders and are a possible treatment target. In this study, the symbiosis of the different bacterial species and viruses will be tested based on the community state types classification. Differences in the vaginal microbiota of healthy and infertile individuals will be investigated. Anonymous vaginal samples are collected from RUC students which will be characterised in the 4 community state types. The classification of the samples will be conducted by microcalorimetry to identify the different bacterial species and viruses of the microbiome and by amplicon metagenomic sequence to investigate the composition of the microbiome. A fermentation model will be used to investigate the symbiosis/dysbiosis of the bacterial species for the 4 community state types as well as the effect of viruses on the microbiota. Finally, for this model, proteomics and metabolomic analyses will be conducted to detect any change in the protein and metabolic activity in the microbiomes. By integrating microbiota analysis into clinical infertility diagnostics, both the vaginal and semen microbiomes can be selected as possible treatment targets to solve any dysbiosis-related infertility issues.

PP.15

Effects of polyI:C and LPS on immune status and gene expression of European Sea Bass (*Dicentrarchus labrax, L.*)

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Introduction: Widespread aquaculture losses attributed to pathogen infections are frequently observed in fish farms, especially in the Mediterranean area, with high economic damage. In order to establish a sustainable aquaculture sector and reduce disease outbreaks, it is essential to understand the immune mechanisms in fish during infection. Aim of the present study was to evaluate immune response and gene expression of European sea bass challenged with bacterial and viral infection mimics.

Methods: Fish were challenged with bacterial and viral mimics. Blood and tissues were collected in specific time-points up to 28 days. Serum samples were used for immunological parameters assessment. Non-specific immune parameters (i.e. nitric oxide, myeloperoxidase, lysozyme, proteases and anti-proteases) were determined according to well established protocols and total antibodies levels were determined by ELISA. Head kidney samples were subjected to qPCR to assess the expression levels of genes related to interferon pathway, cytokines, immunoglobulin, T-cell markers and antimicrobial peptide.

Results: The analyzed immune parameters did not change significantly during the experiment. Proteases and anti-proteases of control-group slightly increased at the initial time-points. NO values of control- and polyI:C-groups increased 12hpi while those of LPS-group increased 14dpi. Both, LPS and polyI:C, induced expression of most genes between 3-12hpi. Some of the analysed genes of polyI:C-group showed a second increase 7dpi indicating a second round of immune system reaction. In polyI:C challenged fish total antibody levels were slightly increased 3-4dpi whereas in LPS challenged fish antibody levels were slightly increased 7-21dpi.

Conclusion: In the present study, we investigated and compared the levels of serum parameters and immune related genes that are expressed during bacterial and viral infections. The resulting datasets will be useful for preparation of future studies in aquaculture species, which can further deepen our understanding of specific fish immune functions against pathogens.
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Poster Session: Biotechnology & Structural Biology

PP.16

Unlocking the Mi-FAR-1 Structure: Expression and Purification of a Nematode Protein

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Background: Meloidogyne incognita is a root-knot nematode (RKN) that poses a significant threat to the global economy and agriculture1. It secretes a complex mixture of understudied proteins (ESPs), including fatty acid and retinol binding proteins (FARs). Unable to synthesize lipids de novo, nematodes obtain essential lipids from plant roots, weakening host defense mechanisms 1,2. Some of these lipids seem to be involved in jasmonic acid synthesis, an important molecule for activating plant defense genes1. Native Mi-FAR-1 protein is considered a key element in the nutrient acquisition process of the nematode. It is approximately 20 kDa and presumably rich in α-helical elements, having no orthologs in animals and plants1. To date, its crystal structure has not been determined3,4.

Methods: The catalytic domain of Mi-FAR-1 conjugated with a 6-histidine-sequence affinity tag at the N-terminus, was cloned into pET-20b(+) expression vector and transformed into Escherichia coli BL21 grown in LB medium at 37oC. Protein expression was induced with 1 mM IPTG at 25o C and cells were harvested 18 h post induction. Protein was purified by nickel-affinity chromatography, followed by a size-exclusion chromatography step using a Superdex 75 Increase column. Protein peak fractions were pooled and concentrated for crystallization trials.

Results: Protein yield was approximately 11 mg/L of culture. SDS-PAGE analysis of the elution and pooled peak fractions confirmed a high level of purity, with a prominent band corresponding to the expected molecular weight of the recombinant Mi-FAR-1 catalytic domain.

Conclusions: Mi-FAR-1 is an important nematode secreted protein, which is poorly understood. This study aims to clone, express this protein, solve its crystal structure and study its function, including its potential to bind plant nutrients or natural blockers. Achieving the above goals could lead to a better understanding of its role in nematode parasitism and weakening of plant defense.

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PP.17

Exploring Molecular Mechanisms of Resilience to Environmental Stress via Proteomic Analysis of the Mediterranean Plant *Cistus creticus*

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Cistus creticus is a Mediterranean shrub well-known for its resilience in post-wildfire environments, where its seeds exhibit increased germinability after exposure to high temperatures. *C.creticus* plants produce a portion of dormant seeds that are covered by a hard seed coat, which is waterproof and does not allow imbibition to initiate germination. High temperatures break the impermeable layer allowing for germination. We aim to compare the proteome of dormant and non-dormant seeds of *C. creticus* collected from plants growing in either post-fire recovering areas or in undisturbed ones. Fresh frozen seeds were separated into hard-coated and non-hard-coated seeds according to their ability to absorb water, and homogenized on dry ice. Proteins were extracted from seed powder using trifluoroacetic acid. After protein reduction, alkylation, and tryptic digestion, a standardized quantity of the resulting peptides were analyzed by nanoflow high-performance liquid chromatography coupled with high resolution mass spectrometry. Raw data obtained from Data Independent Acquisition and Parallel Accumulation Serial Fragmentation were processed using library-free DIA-NN software for protein identification and quantification. We used the reference genome of the closest phylogenetic plant relative to build our peptide library. The results were processed using R scripts. Hard-coated seeds contained higher levels of antioxidant enzymes and lower levels of cellulase and pectinase, as compared to non-hard-coated seeds. In addition, they exhibited a significantly higher concentration of proteasomal subunits and ubiquitin ligases. Seeds from post-fire areas contained proteins belonging to the Tudor complex, responsible for DNA damage repair. These molecular features provide clues to elucidate the mechanisms of heat tolerance of *C.creticus* seeds. Our future research will focus on validating these preliminary findings using enzymatic and biochemical methods, alongside conducting a metabolomic analysis. Understanding these mechanisms could lead to potential applications in the field of biotechnology, offering insights into seed resilience and adaptability in fire-prone ecosystems.

Poster Session: Public Health

PP.18

Reduction of Airborne Microbial Bioaerosols in areas of Public Interest, using novel Air filtration-UV Irradiation technology

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Introduction: Bio-aerosols are airborne collections of biological materials. Ubiquitous indoors and out, bio-aerosols in suspended, aerosolized, liquid droplets typically contain microbes and cell fragments combined with byproducts of cellular metabolism. The deposited bio-aerosols create contaminated surfaces which, if touched, can act as a path to introduce pathogens and cause disease. There are different validated methods for reducing airborne microbial bio-aerosols, but the most effectively used are Air Filtration and UVC (253,7 nm wavelength) irradiation.

Methods: In order to study the reduction of airborne microbes in indoor public areas, through Air Filtering-UV application, a series of experiments were conducted by the Quality Control Department of Hellenic Pasteur Institute, testing the capabilities of a prototype air sterilization system, UVC ULTRAPURE, that filtrates air and also applies UVC radiation. The air passes through HEPA 13 filters, which have the ability of retaining microbial particles. These particles are subsequently UVC irradiated, a procedure that can kill most of the retained microbes. All testing procedures were performed at places of public interest, such as a university's classroom, means of public transport and furthermore, inside a hospital's ICU. The common principle of all experiments was to measure the initial microbial count of each site and to compare it with its corresponding, after the application of the air sterilization system, at standard time points.

Results: The results from the experimental procedure, can confirm that the sterilization system is not only capable of reducing airborne bio-aerosols successfully (ranging from 45% up to 93%), but can also achieve this reduction rapidly (within 30 min of usage).

Conclusions: It is of interest to mention that, although cleaning protocols were applied daily, the microbial count at all sites was relatively loaded due to high and diverse number of people occupying them. Therefore, more effective means of air sterilization are needed, especially in crowded spaces, where ordinary cleaning is inadequate.

PP.19

Development of Photoactivate Surfaces with Antibacterial and Antiviral Activity for a Clean and Safe Environment - APOGEION –

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Introduction: The Covid-2019 pandemic has significantly changed global daily life and highlighted an increasing need for a clean/safe environment, good quality of inhaled air in workplaces and especially in hospitals. New innovative photocatalysts with semiconductor content of TiO_2, g-C_3N_3 and Ag_3PO_4 are emerging as ideal for a wide range of redox antimicrobial coatings. The aim of the study was to evaluate antiviral potential of such coated-surfaces following their activation by exposure to visible light.

Materials & Methods: We assessed antiviral activity by applying ISO 21702:2019 on enveloped DNA viruses such as HSV-1 (strain F) and Vaccinia (Modified Vaccinia Virus Ankara, MVA) and ISO 18071:2016 for determination of bactericidal activity of bacteriophage Qβ (DSM 13768) in *E. coli* (DSM 5310) cultures. The photocatalytic antiviral effect was measured following overnight inoculation of the viral suspensions under indoor light of constant intensity (1000 lux) on both coated- and uncoated-surfaces with a combination of coating materials. $TCID_{50}$ as well as, plaque assay and Double Layer Agar Method were employed to determine the infectious viral titer.

Results: No cytotoxic effect was observed in the cell cultures or bacterial cells tested. No antiviral effect on Vaccinia virus both under visible light illumination and dark conditions was observed. Against HSV-1 virus, the antiviral activity observed was at the acceptable limit ($\Delta V \simeq 3$) for some of the coatings. For the Qβ phage, a range of antiviral effects (ΔV) were observed, ranging from -0,15 in the plain coating to 0,63 in the formulation with TiO_2/g-C_3N_3/Ag_3PO_4.

Conclusion: Varying levels of antiviral activity following indoor-light illumination for 12-16h were observed, depending on the virus, with phage Qβ suffering the biggest reduction, followed by HSV-1 and then Vaccinia. More studies are required to elucidate precise mechanisms behind this viral inhibition and explore more possible viral targets.

Poster Session: Neurobiology

PP.20

Differential sleep-like deficits of Neurofibromatosis 1 mutations in *Drosophila melanogaster.*

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Introduction: Neurofibromatosis 1 (NF1) is a hereditary autosomal dominant disorder affecting 1 in 3500 individuals. It presents with skin abnormalities, Lisch nodules, and neurofibromas. Patients often exhibit macrocephaly, short stature, learning disabilities, and attention deficits. Children and adults with NF1 are also at increased risk for sleep disturbances. The human *NF1* gene (17q11.2) encodes neurofibromin, a 2818-amino-acid protein expressed in various nervous system cells throughout life. Nf1 best-understood and evolutionarily conserved role is as a GTPase Activating Protein (GAP) for Ras, which reduces Ras biological activity. The GRD domain comprises about 10% of the protein (229 amino acids), while the functions of other protein regions remain largely unidentified. Mutations outside the GAP domain are known to precipitate pathological effects in patients. To understand the disturbed mechanisms underlying sleep defects we use the genetic power of *Drosophila* (ortholog of the human, *dNf1*), to investigate locomotor activity and a sleep-like state in mutants and patient mimicking point mutations.

Methods: Using the Trikinetics automatic monitoring system, we report circadian and several sleep deficits, which also present mutation specificity. We are investigating the neuronal circuitry that drives these behaviors and is affected by *dNf1* mutations and the affected molecular pathways therein.

Results: Our data thus far reveal that different dNf1 mutations impact differentially locomotor activity and sleep processes, probably affecting distinct neurons and mechanisms in the fly central nervous system.

Conclusions: This work aligns with the observed diversity of patient phenotypes, especially when considering that various mutations can lead to distinct tissue-specific consequences, contingent upon the specific *Nf1* domain that is affected.

PP.21 BEST POSTER AWARD

A neuronal circuit approach to Habituation and Dishabituation Mechanisms in *Drosophila melanogaster*

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Introduction: Neuronal circuits universally recognize novel stimuli, but unpredicted features in a stimulus amplify its importance, shaping attentive reactions. Habituation, the gradual reduction of responses to repetitive stimuli and its counterpart, dishabituation, the reversal of habituated responses upon exposure to novel stimuli, are fundamental processes that organisms employ to navigate their environment efficiently. Although mechanisms driving Habituation/Dishabituation remain partly understood, the communication within neuronal circuits, which ostensibly underlies these choices is an emerging field. It is understood that when the nature of the habituator is different from the dishabituator, the two stimuli engage distinct, but likely converging circuitry and dishabituation is efficient. In contrast, when the habituator and the dishabituator are of the same nature (i.e. two odors), it is unclear whether this homo-sensory habituation/dishabituation engages distinct neuronal circuits, different than the ones driving hetero-sensory dishabituation.

Methods: We characterized the neural circuit by blocking neurotransmission from specific neuronal subsets with the temperature sensitive GTPase *shibire*, to investigate if they are necessary to drive habituation or dishabituation. Expressing Channel Rhodopsin in these neuronal subsets can photoactivate the neurons (pulse of blue light (470nm), pre and post exposure to the habituator stimulus and activate the neuronal circuit they are participating in, testing for sufficiency.

Results: In this work we show that dopaminergic reinforcement in the Mushroom Body (MB) neurons is essential to encode the identity of the dishabituator stimulus (yeast odor & electric shock). GABAergic neurotransmission from APL neurons to the MB is also required for homo-sensory dishabituation with yeast odor. The MB appears to act as the Executive Control Unit using the information to promote habituation through the α′/β′ neurons and dishabituation through the α/β & γ neurons.

Conclusions: We propose a multifaceted approach to investigate the neural circuits and mechanisms driving these phenomena, contributing to our understanding of basic behavioral plasticity.

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PP.22

RNA editing contributes to RNA metabolism in Parkinson's disease

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Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the gradual loss of dopaminergic neurons in the substantia nigra of the midbrain. A hallmark of PD pathology is the formation of Lewy bodies within neurons, which result from the misfolding of alpha-synuclein (a-Syn). One of the well-researched genetic variants linked to PD is a-Syn p.A53T mutation. RNA editing, a post-transcriptional modification process, alters RNA sequences, with the most common form in mammals being Adenosine-to-Inosine (A-to-I) editing, catalyzed by the ADAR enzymes.

Methods: In this study, we analyzed RNA sequencing data from dopaminergic neurons derived from induced pluripotent stem cells (iPSCs), which were generated from skin fibroblasts of both healthy individuals and patients carrying the p.A53T mutation. Our aim is to validate these bioinformatics findings using both *in vitro* and *in vivo* models.

Results: Using an in-house developed pipeline, we identified distinct RNA editing patterns between healthy and PD samples. RNA-binding protein RBM4 exhibited altered editing between control and PD samples. RBM4 is also abnormally upregulated in this patient-derived PD model and returns to lower expression levels following treatment with kinase inhibitor BX175, which is found to alleviate disease symptoms. These findings were further validated through targeted re-sequencing (MiSeq). Immunostaining showed nuclear localization of ADAR1, with similar fluorescence intensity levels in p.A53T and control (CTR) cells, while RBM4 accumulated in the cytoplasm of cells carrying the p.A53T mutation, compared to CTR where was observed mainly nuclear. ADAR1 was not found to interact directly with RBM4, while RBM4 was not found in known RNA-protein structures such as stress-granules or P-bodies.

Conclusions: Our study highlights distinct RNA editing patterns in healthy and pathological dopaminergic neurons, offering new insights into the molecular mechanisms of Parkinson's disease. Our findings provide a foundation for future research into RNA editing mechanisms and their contribution to neurodegenerative diseases.

Funding statement

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PP.23

Impaired uptake and clearance capacity of astrocytes in a IPSC-derived Parkinson's disease model from p.A53T a-synuclein patients

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Introduction: Astrocytes play important roles in sustaining neuronal health and are emerging as critical players in neurodegeneration processes. Yet, their contribution to Parkinson's disease (PD) pathology is understudied. The ability of astrocytes to internalize and degrade αSynuclein (αSyn) has been reported. Tunneling nanotubes (TNTs) are pivotal structures for intercellular communication, enabling the direct transfer of cargos, such as αSyn, across distant cells. We aimed to investigate astrocytes capacity to receive and degrade αSyn aggregates from neurons and whether TNTs contribute to this process.

Methods: We leveraged our induced pluripotent stem cell (iPSC)-based model from PD patients harboring the p.A53T mutation in αSyn, which causes an early and severe PD form, and generated ventral midbrain dopaminergic neurons and astrocytes.

Results: Our data showed that treatment of heathy astrocytes (Ha) with PD neuron-conditioned medium resulted in efficient uptake of neuron-derived αSyn, a process that was hampered in PD astrocytes (PDa), indicating their reduced internalization capacity. PDa exposed to αSyn-PFFs for long time points accumulated PFFs in their cytoplasm, as compared to Ha, reaching significance at 16h, pointing toward a retarded degradation process. In PFF-loaded SHSY-5Y cells co-cultured with Ha, we observed significantly enhanced clearance of αSyn PFFs as opposed to co-cultures with PDa. In PFF-loaded astrocytes co-cultured with SH-SY5Y cells, no transfer of PFFs observed neither from Ha nor PDa to SH-SY5Y (ongoing quantification). PDa formed more TNT-connections among them before and after treatment with αSyn PFFs, as compared to Ha, and the treatment itself increased the TNTs both in Ha and PDa.

Conclusions: Overall, these findings suggest that astrocytes carrying the p.A53T-αSyn mutation display inherent deficiencies in their uptake and protein aggregate degradation properties, and thus are less capable of relieving neuronal cells from accumulated αSyn. Further, p.A53T-αSyn mutation increase TNT-like structures biogenesis in astrocytes as well as PFFs treatment.

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PP.24

Mirk/Dyrk1B kinase as a potential drug target in ALS.

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neurons (MNs) degeneration.

Aim: Here, we used SOD1G93A mice to investigate the role of Mirk/Dyrk1B kinase in ALS and to evaluate the therapeutic effect of its specific inhibitor, AZ191. We previously demonstrated that in the embryonic chick spinal cord (SC), Mirk/Dyrk1B regulates the generation and survival of motor neurons (MNs) and V2a interneurons and additionally affects the LMCm motor column that innervates ventrally the muscles of the limbs. Notably, motor neurons (MNs) and V2a interneurons are firstly affected in the spinal cord of SOD1G93A mice.

Methods: To perform the preclinical evaluation of AZ191 inhibitor, we developed a primary culture protocol enriched about 90% for spinal motor neurons (SpMNs) derived from E12.5 mouse SC.

Results: We revealed that SpMNs derived from E12.5 SOD1G93A mice exhibit significantly shorter and fragmented axons compared to WT, indicating an early axonopathy starting from embryonic life. Pharmacological inhibition of Dyrk1B kinase by AZ191 small molecule, increased both the axonal length of E12.5 WT and SOD1G93A SpMNs respectively and rescued the axonal phenotype of SOD1G93A SpMNs. Moreover, AZ191 inhibitor reduced the apoptosis of SOD1G93A SpMNs, suggesting a neurotrophic and anti-apoptotic effect following Dyrk1B inhibition. In addition, AZ191 was found to enhance autophagy both in E12.5 WT and SOD1G93A SpMNs. The beneficial effect of AZ191 in SOD1G93A SpMNs will be also studied in terms of mitochondrial dysfunction, mitophagy, ER-stress, proteasome dysfunction and SOD1 protein aggregation. Moreover, we found that Dyrk1B is implicated in neuroinflammation, as in the lumbar SC of P140 SOD1G93A mice (late-stage of the disease), where neuroinflammation is prominent, Dyrk1B is expressed by an increased number of astrocytes and microglia by 3.84-fold and by 8.45-fold respectively.

Conclusions: Our results render Mirk/Dyrk1B a potential drug target in ALS.

PP.25

Integration of diverse platforms to define local mechanisms and long-range signals that mediate brain-heart interactions after injury and during regeneration

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Introduction: Clinical data show that damage to the CNS/brain is often accompanied by heart pathologies and vice versa. Similarly, injury induces local repair mechanisms but also elicits coordinated communication between different organs. Yet, the mechanisms that regulate such inter-organ communication in both homeostatic and post-injury conditions remain largely unknown, representing a major obstacle to effective disease prognosis and treatment.

Materials and Methods: To investigate the responses of brain and heart after injury of the distant organ, we utilize i) different models of brain (stab lesion) and heart injury (cryoinjury and chemogenetic ablation of cardiac valve cells), ii) transcriptome analysis (RNA- seq), and iii) diverse functional readouts, in zebrafish.

Results: Using a T-maze as a readout for cognitive status and social preference upon heart injury, we showed that the ability of injured fish to recognize their conspecifics is altered after injury. Further, bulk tissue cell type deconvolution using single-cell expression reference datasets, revealed that both brain and heart injury result in altered cell type composition in bulk RNA-seq samples of the distant organ, as compared to controls. To further identify mediators of intra-organ communication, we investigate brain and heart exosomes under homeostatic and post-injury conditions. The role of candidate signaling molecules that are currently identified via RNAsequencing and the characterisation of exosome cargo, are functionally verified in vitro in primary cultures of adult mouse cardiac fibroblasts and neonatal cardiomyocytes.

Discussion: We propose that identifying the mechanisms underlying brain-heart interactions in zebrafish could reveal targets for reprogramming homologous pathways in mammals with implications for regenerative medicine.

PP.26

Microglia-specific phenotypic and functional changes upon neuronal expression of familial Parkinson's disease p.A53T-alpha synuclein

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Introduction: Parkinson's disease (PD) is the second most common neurodegenerative disorder (ND) with multiple clinical manifestations. A common denominator of most NDs is synaptic dysfunction caused by changes in synaptic structure and function, which can be either the cause or the effect of the disease. Recent data suggest a neurodevelopmental origin for synaptic dysfunction and challenge the decades-old postulate that synaptic dysfunction is among the ultimate sequelae of NDs. These findings suggest that synaptic dysregulation is a result of inappropriate glial interactions, not only in adulthood, but as early as critical embryonic and postnatal developmental stages, where the core role of microglia is well established.

Methods: In this study, we use a mouse model (M83) that overexpresses the human pA53T-alpha Synuclein (p.A53TαSyn) in neurons, which is linked to autosomal dominant familial PD. We perform morphological analysis of microglia in multiple brain regions of M83 mice at early developmental stages (E14-17 & P7-10), in young adults (P30), older adults (4-5 months) and symptomatic animals (>1-year-old). In parallel, we study how the neuronal expression of pA53T-αSyn alters the microglial subpopulations and signaling pathways associated with PD, even before the onset of symptoms, by transcriptomic analysis of M83 and wild-type microglial cells of animals at the respective developmental stages.

Results: The present study uses the pA53T-αSyn familial PD model to identify interactions between microglia and neurons during the presymptomatic stages of Parkinson's disease. Using this non-neurocentric approach, we aim to elucidate the role of microglia in disease progression, disease-associated cellular subpopulations and potential therapeutic targets.

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PP.27

Elucidation of the miR-124/ISX9-mediated transcriptional mechanism instructing the cell fate switch of astrocytes to induced-neurons

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Direct astrocytic reprogramming holds therapeutic promise for the amelioration of neuronal loss following neurodegeneration or brain trauma. For this, many neurogenic factors, namely transcription factors (TFs), miRNAs or chemical compounds have been employed for the direct conversion of astrocytes to induced-neurons (iNs) in various *in vitro* and *in vivo* models. We have previously shown, that the brain-enriched miRNA, miR-124, is a potent driver of the reprogramming switch of mouse cortical astrocytes towards an immature neuronal-like phenotype both *in vitro* and *in vivo*, whereas supplementation with the neurogenic compound ISX9 strongly reinforces reprogramming and maturation of iNs.
Aim of this study is the deeper understanding of the transcriptional mechanism through which miR-124 alone or in synergy with ISX9 instructs astrocytic reprogramming towards the neuronal fate. For this, using the data from our previous RNA-seq experiment, we constructed the TF activity network, presenting the major TFs that orchestrate the reprogramming process at day7 of our protocol. In order to define the core TFs in this network we calculated the betweeness centrality score of each TF. Based on the above analyses, we identified the DNA demethylation factor Tet1, as a pioneer factor in the miR-124-mediated transcriptional network and the DNA/RNA binding protein Lin28a as a prominent target in the ISX9-mediated network.
Using siRNA technology, we knocked-down Tet1 and Lin28a in order to further elucidate their contribution in the reprogramming process. Our results indicate that silencing of Tet1 strongly downregulates the mRNA levels of neurogenic TFs, and the levels of the newborn neurons' marker doublecortin, verifying its importance in the early steps of the reprogramming procedure guided by miR-124. On the other hand, silencing of Lin28a reduced the mRNA levels of genes related to dendrite development and synaptic signaling, highlighting its role in the maturation of iNs mediated by the action of ISX9.

PP.28

Cerebral lateralization for writing in left- and right-handers: Attempting to disentangle a Gordian knot

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Introduction: This study investigates cerebral lateralization in written language, an inquiry that has received less attention compared to oral language, particularly in left-handed individuals. While writing involves both a linguistic and a motor component, past research has often neglected the distinct roles these components play in cerebral lateralization. The aim of this study was to isolate the cerebral lateralization for the linguistic component of writing in both left-handed and right-handed adults.

Methods: To do this, functional transcranial Doppler ultrasonography was applied on 30 participants from each handedness group. The participants completed three tasks: (a) oral word generation starting with the same letter, (b) written word generation starting with the same letter, and (c) repetitive letter copying, which served as a motor control task. The linguistic component of written language was isolated by subtracting the lateralization index for the motor task (letter copying) from that of the written word generation task. Handedness was determined using the Edinburgh Handedness Inventory, and Bayesian *t*-tests for independent groups were employed to assess the quality of evidence.

Results: The study had two preregistered hypotheses: (i) that left-handers would show weaker cerebral lateralization for the linguistic component of writing compared to right-handers, and (ii) that there would be no correlation between the lateralization for oral language and the lateralization for the linguistic component of written language. Both handedness groups showed left-lateralization for writing, but the results were inconclusive regarding whether this was weaker in left-handers. Additionally, there was insufficient evidence to confirm or reject a correlation between oral language and written language lateralization. However, handedness appeared to influence this correlation.

Conclusions: These findings highlight the complexity of language processing, particularly in relation to handedness.

PP.29

Failure of major proteostatic mechanisms in p.A53T-αSyn PD patient iPSC-derived astrocytes, due to lysosomal malfunction

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Background: Autophagy is a highly conserved process responsible for the degradation of damaged molecules and subcellular structures. Disturbances in autophagy have been linked to a variety of diseases, including neurodegenerative disorders (ND). Among those Parkinson's disease (PD), is characterized by the progressive loss of midbrain dopamine neurons. The major histopathological hallmark of PD is the presence of intracellular protein inclusions rich in αSynuclein (αSyn). Studies suggest that the disruption of autophagy in neurons leads to the accumulation of aggregated αSyn(1). However, the role of astrocytes in PD pathology remains understudied. Here, we aimed to investigate how the p.A53T-αSyn mutation affects proteostasis in astrocytes.

Methods: Using our previously established iPSC model from PD patients harboring the p.A53T-αSyn mutation(2), we generated ventral midbrain astrocytes. Proteomic Analysis of PD astrocytes (PDa) versus healthy astrocytes (Ha) and western blot assays were performed to identify the affected biological processes and cellular compartments. Immunofluorescence and confocal microscopy were employed to investigate protein aggregation, autophagic flux and lysosomal integrity.

Results: PDa displayed accumulation of protein aggregates, including the pathological form of αSyn, pSer(129)αSyn^{+}. Proteome profiling revealed that protein catabolic processes, including autophagy, were among the most affected pathways in PDa. Turnover of cytosolic LC3I to membrane-bound LC3II, indicative of autophagosome formation, was elevated in PDa, alongside with reduced autophagosome to autolysosome transition. Additionally, PDa exhibited decreased LAMP1 levels, reduced lysosomal acidity, and increased number of lysosomes. Altered lysosomal positioning, essential for proper autophagosome-lysosome fusion, coupled with reduced lysosomal enzymatic activity, further indicated dysregulated autophagy due to impaired lysosomal function in PDa. mTORC1-related DE proteins clustered into a significantly disturbed network in PDa, confirmed by increased levels of ULK1 phosphorylation.

Conclusion: Our data suggest that the p.A53T-αSyn mutation in astrocytes leads to pathological alterations in protein catabolic processes, particularly affecting autophagy, and largely associated with lysosomal dysfunction.

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PP.30

Morphological and transcriptomic characterization of cortical astrocytes during early experimental demyelination

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Introduction: Astrocytes, the most abundant glial cell type in the central nervous system (CNS), are important regulators of CNS homeostasis but also significant mediators of neuropathology with therapeutic potential. In multiple sclerosis, astrocytes are now considered to be critical in the development of active lesions, further to the previous perception that they contribute to pathology only at progressive stages. Consequently, astrocytes have been the focus of increasing research in animal MS models, including the cuprizone (CPZ) model of de/remyelination.

Methods: We used the cuprizone demyelination model of multiple sclerosis to explore the morphological and transcriptomic signature of astrocytes in the cortex of mice during early demyelination at week 3 (CPZ3), by applying detailed cell-morphometric analysis of cortical astrocytes and single-cell RNA sequencing in isolated cortical tissues from healthy and CPZ3-demyelination brains.

Results: Detailed morphometric analysis revealed the morphological phenotype of astrocytes in early demyelination, characterized by thick and long processes. Differential gene analysis revealed a significant number of regulated genes with enrichment of genes typical of pan-reactive astrogliosis, while markers widely-associated with neurotoxic or protective astrocytes appeared mostly unaffected. We further report two clusters, also present in healthy brains, that are regulated in disease. Both these clusters were characterized by regulation of genes involved in neuroinflammation and neuroprotection. One of the clusters was further characterized by robust upregulation of reactive astrocyte markers that have been recently attributed to distinct astrocyte subpopulations in a mouse model for Alzheimer's Disease, and downregulation of markers involved in physiological astrocyte functions.

Conclusions: Our preliminary data provides a phenotypic and transcriptomic characterization of grey matter astrocyte responses in early experimental demyelination that might provide a basis for investigation and understanding of astrocyte mechanisms involved in pathology and for the design of strategies aimed at regulating astrocyte responses for the treatment of neurodegenerative diseases.

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PP.31

Study of cellular senescence phenotypes in astrocytes derived from Parkinson's Disease patient-iPSCs

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Background: Cellular senescence, a hallmark of aging, is characterized by altered phenotypical features, including irreversible cell cycle arrest, resistance to cell death, and a pro-inflammatory secretory phenotype (1). While a normal feature of aging, senescence can also be triggered by intracellular stressors. Increasing evidence suggests that senescent astrocytes may play a role in neurodegenerative disorders (2), including Parkinson's Disease (PD). PD, the second most common neurodegenerative disorder, is marked by α-synuclein (αSyn) aggregation and progressive loss of dopaminergic neurons in the substantia nigra pars compacta, leading to motor and non-motor symptoms. A major genetic risk factor for developing PD is the p.A53T mutation in αSyn. Previous studies provide evidence of senescence in both PD patient post-mortem brains and experimental models of PD (3). The aim of this study is to explore the link between the p.A53T mutation in astrocytes and senescence, improving our understanding of astrocytic contributions to PD and the potential application of senotherapeutics for treatment.

Methods: We differentiated induced pluripotent stem cells (iPSCs) from p.A53T-αSyn PD patient into ventral midbrain astrocytes. Senescence-related gene expression (p16, p21) was assessed by RT-qPCR. Immunofluorescence for the astrocytic membrane marker CD44 with confocal microscopy and image analysis were used to examine cell size. Lysotracker dye was used for lysosome tracing.

Results: PD astrocytes showed elevated mRNA levels of cell cycle inhibitors (p16, p21). Increased cell size and a higher number of lysosomes were also observed. Additionally, PD astrocytes exhibited significant autofluorescence levels suggesting accumulation of lipofuscins, structures observed due to senescence.

Conclusions: Our findings suggest that the p.A53T mutation in αSyn drives astrocytic senescence, which may contribute to PD pathology. Our ongoing experiments aim to identify additional senescence features, such as the senescent-associated secretory phenotype (SASP) in PD astrocytes that may impact neuronal health.

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PP.32 BEST POSTER AWARD

In vivo microglial Bin1 deletion regulates neuroinflammation and Adult Neurogenesis in the mouse hippocampus

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Introduction: Bridging Integrator 1 (BIN1) has been identified as the second major genetic risk factor for Late-Onset Alzheimer's Disease (LOAD) through GWAS analysis. While BIN1's involvement in neuronal functions is well-documented, its role in microglia has been less explored. Given the central role of microglia in neuroinflammation and Adult Hippocampal Neurogenesis (AHN)—both critical aspects of Alzheimer's Disease pathology—examining BIN1 in this context could provide valuable insights into disease progression.

Methods: We utilized a conditional double transgenic mouse model with microglia-specific BIN1 knockout to assess its function in the hippocampus, under homeostatic and inflammatory conditions. To evaluate the effects of microglial Bin1 loss, we performed FACS analysis alongside with immunohistochemical and molecular analyses.

Results: In alignment with snRNA-Seq data from the cortex of this mouse model, real-time qPCR showed upregulation of type I interferon pathway genes in LPS-treated hippocampi lacking microglial BIN1. Our analysis further revealed that neuroinflammation in microglial BIN1cKO mice increase proliferating microglial cells, as indicated by Ki67 staining, despite no overall microglial population expansion. Immunophenotyping of BIN1cKO microglia via flow cytometry for the CD11c activation marker did not reveal a disease-associated signature, possibly due to a potential regional heterogeneity, even though there was an increase in microglial reactivity following LPS treatment. Additionally, the lysosomal marker CD68 was elevated in hippocampal microglia under homeostatic conditions in BIN1cKO mice and further increased after LPS treatment. Interestingly, the loss of microglial BIN1 alone led to an increase in DCX+ neuroblasts in the Subgranular Zone, indicating that BIN1 plays a role in regulating AHN.

Conclusions: Our findings indicate that the loss of BIN1 in hippocampal microglia during inflammatory conditions drives the expansion of proliferative and pro-inflammatory microglial subpopulations. Additionally, microglial BIN1 deficiency independently modulates both phagocytic capacity of microglial cells and neurogenesis, although the molecular mechanisms underlying these effects are still under investigation.

PP.33

Metabolic impairment in PD-patient iPSC-derived p.A53T-αSyn astrocytes

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Introduction: Parkinson's Disease (PD) is the second most common neurodegenerative disorder, characterized by progressive loss of midbrain dopaminergic neurons and by the presence of intraneuronal protein inclusions of α-synuclein (αSyn), known as Lewy bodies. Astrocytes, the most abundant glial cells in the human brain, also play a role in PD. Astrocytes are involved in various homeostatic functions in the central nervous system, particularly in energy metabolism[1]. Recent studies have highlighted mitochondrial abnormalities in astrocytes derived from PD-patient induced pluripotent stem cells (iPSC) carrying the $LRRK2^{G2019S}$ mutation[2]. In this study, we focused on the effects of the p.A53T αSyn mutation ($SNCA^{G209A}$) in PD-patient iPSC-derived astrocytes, with regards to their metabolism and mitochondrial function, to better understand their contribution to PD neuropathology.

Methods: iPSCs derived from PD patients carrying the p.A53T mutation in αSyn were differentiated to ventral midbrain astrocytes. Immunocytochemistry, confocal imaging, live imaging, advanced image analysis and functional assays, including Seahorse analysis were employed to assess astrocytic metabolism, particularly mitochondrial morphology and function.

Results: Astrocytes derived from p.A53T-α-syn iPSCs exhibited increased lactate dehydrogenase release, indicative of elevated cytotoxicity. This was accompanied by abnormal mitochondrial morphology, including fragmented mitochondria and impaired mitophagy. Additionally, increased reactive oxygen species (ROS) levels were observed, with a particular rise in mitochondrial ROS. Lipid droplets, which serve as fat depots and are involved in lipid metabolism regulation, were also found to be accumulated in the p.A53T-αSyn iPSC-derived astrocytes.

Conclusions: Astrocytes derived from p.A53T-αSyn iPSCs exhibit dysfunctional mitochondrial morphology and impaired function, along with indications of disrupted lipid metabolism. These finding suggest that astrocytic metabolic deregulation may contribute to PD neuropathology. Further in-depth analyses are underway to validate and expand upon these observations.

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