THE CYPRUS INSTITUTE OF
NEUROLOGY & GENETICS

PhD&DNA DAY

2023

WELCOME ADDRESS

Welcome to our joint event for PhD Day and DNA Day 2023!

DNA Day in 2023 commemorates both the 20th anniversary of the Human Genome Project's completion (2003) and the 70th anniversary of the discovery of the DNA double helix (1953), both milestones in scientific history and important for all researchers. PhD Day is dedicated to our own next generation of researchers, their Advisors and hosting departments.

During the morning sessions, PhD students will present their work to date. For the second segment of our event after lunch, we will hold a press conference focused on informing the media and general public of our activities and contribution to our patients and society, during which we will be honoured to host the Minister of Health, Dr. Popi Kanari.

PhD Day – DNA Day will culminate with a highly esteemed Keynote speaker, Prof. Kypros Nicolaides, Professor of Fetal Medicine at King's College, London University and Director at Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London.

Poster presentations will take place in the CING Foyer throughout the day.

Please refer to the schedule in this booklet for a full breakdown of the event.

BIOGRAPHY
KYPROS HERODOTOU NICOLAIDES

Date and place of birth: 9th April, 1953; Paphos, Cyprus

Qualifications:

1974 Biochemistry and Physiology BSc (1st class honours), King's College, London University.

1978 Medicine, MBBS, King's College Hospital, London University.

1984 Obstetrics and Gynaecology, MRCOG.

2014 Obstetrics and Gynaecology, FRCOG.

1992- Present:

Professor of Fetal Medicine, King's College, London University.
Director Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London

Awards:

1 Ian Donald Gold Award for Highest Contribution in Ultrasound, International Society Ultrasound in Obstetrics & Gynecology, 1999

2 Eric Saling Award for Highest Scientific Contribution in Perinatal Medicine, World Association of Perinatal Medicine, 2001

3 Excellence in Letters, Culture and Science, Government of Cyprus, 2004

4 Honorary Fellowship of the American Institute of Ultrasound in Medicine, USA 2004

5 Membership of the International Academy of Perinatal Medicine, Barcelona, Spain 2005

6 Spinoza Chair, University of Amsterdam, The Netherlands 2010

7 European Maternity Prize for distinction in professional career and dedication to the field of Perinatal Medicine, European Association of Perinatal Medicine, 2014

8 Eardley Holland Gold Medal for outstanding contribution to the science, practice and/or teaching of Obstetrics and Gynaecology, Royal College of Obstetricians and Gynaecologists, 2015

9 Naguib Pasha Mahfouz Award, Cairo University, Egypt, 2018

10 Medal of exceptional services to the Republic of Cyprus, 2018

11 Member of the USA National Academy of Medicine in recognition for outstanding professional achievement and commitment to health and medicine, USA 2020

12 Argo award given to distinguished figures of the Greek diaspora for excelling in their field and promoting Greece abroad, Athens Greece 2021

13 Grand Cross of the Order of Makarios III, Highest award of the Republic of Cyprus, 2021

14 Gold Cross of The Order of the Phoenix, Republic of Greece, 2022

15 Fellowship of Kings College, London University, UK, 2022

16 Chesley award. Highest award of the International Society for the Study of Hypertension in Pregnancy in recognition of sustained and substantial contribution to research in pre-eclampsia. 2022

17 Honorary Doctorates in Medicine

- National and Kapodistrian University of Athens, Greece 2005
- University of Warsaw, Poland 2009
- University of Bucharest, Romania 2009
- University of Jinan, China 2010
- Palacky University of Olomouc, Czeck Republic 2011
- University of Ioannina, Greece 2012
- European University of Cyprus, Cyprus 2013
- University of Thessaly, Greece 2016
- Aristotelion University of Thessaloniki, Greece 2017
- Medical University of Preven, Bulgaria2017
- University of Cyprus, Cyprus 2017
- University of Leuven, Belgium 2019
- University of Sao Paolo, Argentina 2019

Scientific Activities:

- Published 1,603 peer-review papers in Scientific Journals. His h-index is 184 (highest of all obstetricians and gynaecologists in the world) and his work has been cited more than 135 thousand times
- Edited or wrote 14 books. Introduced internet based courses for health care professionals and patients
- Supervised 66 doctors to undertake research leading to postgraduate qualifications and has provided training in Fetal Medicine to 600 doctors from 50 countries.

Professional Activities:

1 ***Director of the Research Centre for Fetal Medicine, King's College Hospital***
This centre, which was opened in 1984 by Princess Diana, was the first Fetal Medicine Centre in Britain and is the biggest one in the world. More than 20,000 patients are examined each year and many of these patients are referred from other hospitals in Britain and other countries because of serious complications of pregnancy. In addition, more than 200 doctors from all over the world visit the centre to observe and receive training.

2 ***Founder and Chairman of the Fetal Medicine Foundation***
This charity was set up in 1995. The main source of income is a private clinic which donates all its profits to the charity. The aims are to promote research and training in Fetal Medicine throughout the World. More than £45,000,000 have been donated to finance the training of many doctors from all over the world and to carry out major multicentre studies on screening and prevention of preterm delivery and preeclampsia, which are the leading causes of perinatal mortality. The Foundation has also introduced the yearly World Congress in Fetal Medicine and has implemented a series of educational courses throughout the World.

3 Has been a member of several study groups of the Royal College of Obstetricians and Gynaecologists, the chairman of the Educational Committee of the World Association of Perinatal Medicine and the chairman of the Scientific Committee of the International Society Ultrasound in Obstetrics and Gynecology.

AREAS OF RESEARCH

1 Screening for chromosomal defects

Developed a new method based on a combination of ultrasound findings and maternal serum biochemistry at 11-13 weeks. This method has evolved over the last 26 years from: (i) observational phase to (ii) development of a model, to (iii) controlled clinical implementation, to (iv) establishment of an international network of centres in more than 60 countries for continuing audit and development. It has also evolved by the introduction of new sonographic and biochemical markers into the most effective method of early screening worldwide. Recently, several studies have examined the effectiveness of cfDNA analysis of maternal blood and a proposal that this method should be offered contingent on the results of the combined test.

2 Screening for fetal abnormalities

Described a sonographic method for the prenatal diagnosis of spina bifida, which is now performed world wide. Continuing research arising from the ultrasound screening programme at 11-13 weeks has established the effectiveness of this scan in firstly, detecting a wide range of fetal defects, with emphasis on cardiac abnormalities, which are the commonest cause of neonatal and infant mortality from congenital defects, and secondly, describing more than 50 congenital defects and genetic syndromes in association with increased nuchal translucency thickness.

3 Screening for and prevention of preterm birth

Preterm birth is the main cause of perinatal death and handicap in survivors. A series of studies over the last 15 years have established that first, effective screening can be provided by sonographic measurement of cervical length at 20 weeks, second, a major multicentre RCT has shown that cervical cerclage does not prevent preterm birth, third, a major multicentre RCT has shown that the prophylactic use of progesterone in women with a short cervix can reduce the rate of preterm birth by about 40%, fourth, two major multicentre RCTs have shown that the use of a cervical pessary in singleton and twin pregnancies does not prevent preterm birth.

4 Screening for and prevention of pre-eclampsia and fetal growth restriction

This is an important cause of maternal mortality and morbidity and fetal mortality as well as possible causes of adult cardiovascular and endocrine disease. A series of studies over the last 20 years have established that: first, growth restricted fetuses demonstrate fetal hypoxemia and impaired biochemistry, metabolism, hematology and immunology, second, fetal hypoxemia can be predicted non-invasively by Doppler ultrasound demonstrating a redistribution in the fetal circulation, third, effective screening for pre-eclampsia and fetal growth restriction can be provided by Doppler sonographic measurement of blood flow in the uterine arteries at 20 weeks of pregnancy and fourth screening for pre-eclampsia and fetal growth restriction can be provided by a combination of Doppler measurement of blood flow in the uterine arteries, mean arterial pressure and serum placental growth factor at 11-13 weeks' gestation. We performed major multicentre RCTs which showed that the use of low dose aspirin after 20 weeks in high-risk pregnancies does not prevent preeclampsia, but treatment starting from 12 weeks is highly effective.

5 Fetal therapy

Research in our centre over the last 35 years has established a series of effective intrauterine therapeutic interventions including fetal blood transfusions for red cell isoimmunized pregnancies, fetal platelet transfusions for alloimmune thrombocytopenia, pleuro-amniotic shunting for fetal pleural effusions, endoscopic laser separation of communicating placental vessels in monochorionic twins with severe twin transfusion syndrome or selective growth restriction and in collaboration with professors Jan Deprest and Eduard Gratacos endoscopic placement of a balloon in the fetal trachea in fetuses with diaphragmatic hernia.

PHD & DNA DAY SCHEDULE

9:20–9:30 **Prof. Kyproula Christodoulou**
Opening Address

9:30–9:50 **SESSION 1 – Christodoulos Messios**
Molecular Genetics, Function and Therapy Department
Investigation of a novel muscle communication pathway

9:50–10:10 **SESSION 2 – Androniki Chrysanthou**
Neurogenetics Department
Identifying the biological role of anoctamin 10 protein

10:10–10:30 **SESSION 3 – Ellie Mitsi**
Neurogenetics Department, Neuroepidemiology Department
Genetic and environmental risk factors contributing to ALS in Cyprus

10:30-11:00 **COFFEE BREAK & POSTER PRESENTATIONS**

11:00–11:20 **SESSION 4 – Efi Athieniti**
Bioinformatics Department
Multi-omics data analysis to obtain markers and mechanisms of disease progression

11:20–11:40 **SESSION 5 – Petros Ladas**
Molecular Virology Department
Investigation of the aetiology of community acquired pneumonia in Cyprus and characterization of host factors in viral/bacterial co-infections

11:40–12:00 **SESSION 6 – Sevgi Özkaramehmet**
Molecular Genetics Thalassaemia Department
Identification of novel γ-globin repressors through a custom CRISPR knockout screen and validation studies for the treatment of β-hemoglobinopathies

12:00–12:20 **SESSION 7 – Venetia Tragkola**
Cancer Genetics Therapeutics & Ultrastructural Pathology Department
Phytochemicals as Novel Epigenetic Modulators in Skin Cancer Therapeutics

12.20–13.45 **LUNCH BREAK & POSTER PRESENTATIONS**

13.45 **DNA Day**

Address by the Minister of Health Dr Popi Kanari

Address by Prof. Leonidas Phylactou

Members of the Media - Q&A

14.15 **KEYNOTE SPEAKER - Prof. Kypros Nicolaides**
ZOOM
Fetal Medicine

CLOSING REMARKS & DISCUSSION

POSTER PRESENTATIONS

SELECTED ABSTRACTS

SA1 INVESTIGATION OF A NOVEL MUSCLE COMMUNICATION PATHWAY

CHRISTODOULOS MESSIOS
Molecular Genetics, Function and Therapy Department
Research Advisor/s: Prof. Leonidas Phylactou

Background: MicroRNAs (miRNAs) are small non-coding RNA molecules that have a regulatory role in multiple cellular processes. They negatively regulate gene expression through miRNA-mRNA interactions that lead to inhibition of translation. MiRNAs are involved in muscle cell communication, especially during development. Previously reported data suggested that muscles communicate locally by transferring small RNAs. The aim of this study is to investigate for the first time whether muscles communicate with other distant tissues.

Methods: To achieve our aim we designed oligonucleotides and administered them intramuscularly in mice. An antagomiR against miR-133b was initially used to assess the downregulation of miR-133b in distant tissues. Three different versions of the antagomiR were designed, a 3′ cholesterol conjugation, a 5′ cholesterol conjugation and one without a cholesterol conjugation. Differences of the conjugate effect on the communication pathway were assessed by examining the efficacy and biodistribution of each antagomiR. Intramuscular and intravenous administration methods were also compared. Moreover, fluorescently labelled miR-133b mimic with similar chemistry to the antagomiR was designed in order to assess its biodistribution.

Results: Intramuscular injections in the tibialis anterior (TA) of mice showed remarkable systemic delivery. Interestingly, the 5′ modification showed enhanced downregulation of miR-133b in various skeletal muscles, over the 3′ cholesterol. Intramuscular administration of 5′-antagomiR showed enhanced inhibition of target miRNAs in skeletal muscles compared to intravenous. The miR-133b mimic showed similar biodistribution to the distant tissues where the antagomiR showed miR-133b downregulation.

Conclusion: Our data shows that intramuscular administration of antagomiR and mimic is transported to other muscles suggesting that skeletal muscles possibly communicate distantly through molecular cargo. This pathway could help us understand more about how muscles develop and function and might prove useful in the delivery of therapeutics for muscle diseases.

SA2 IDENTIFYING THE BIOLOGICAL ROLE OF ANOCTAMIN 10 PROTEIN

ANDRONIKI CHRYSANTHOU
Neurogenetics Department
Research Advisor/s: Prof. Kyproula Christodoulou, Dr Antonis Ververis

Introduction and Objectives

Anoctamin 10 (ANO10), also known as TMEM16K, is a member of a broader family of dual function proteins exhibiting phospholipid scrambling and ion channel activity. Endosomal sorting, spindle assembly, calcium signalling, cell volume regulation, and apoptosis are other biological processes that have been associated with ANO10. ANO10 was also found to be associated with acetylated tubulin of spindles in mouse macrophages, while defects in the ANO10 ortholog in Drosophila, Axs, were found to cause abnormal spindle assembly and chromosome segregation. These findings suggest implication of ANO10 in spindle formation and cell cycle progression. Variants in the ANO10 gene are linked to a rare type of autosomal recessive spinocerebellar ataxia (SCAR10), probably mediated by degeneration of Purkinje cells in the cerebellum due to ANO10 defects. The aim of this study is to further uncover the biological role of human ANO10, and especially, to investigate the effects of ANO10 depletion at the cell division level.

Methodology

Immunofluorescence microscopy was performed to assess ANO10 localization in SH-SY5Y and U2OS cells. ANO10 silencing using RNAi technology, followed by validation, was employed to resemble and study the effects of a pathogenic deletion (c.289del [p.Thr96_Met97ins*]) identified in three Cypriots with SCAR10 phenotype.

Results

ANO10 was found to localize at the centrosomes of mitotic and non-mitotic cells, and at the ER in agreement with previous studies. Transfection of cells with siRNA targeting ANO10 mRNA resulted in a significant reduction in the expression of both gene and protein levels.

Discussion

Centrosomic localization of ANO10 indicates a potential role of the protein in cell division. The effects of ANO10 silencing are currently being investigated to further characterize the protein and delineate its role in the cell cycle, cell growth and ciliogenesis.

SA3 GENETIC AND ENVIRONMENTAL RISK FACTORS CONTRIBUTING TO ALS IN CYPRUS

ELLIE MITSI
Neurogenetics Department
Neuroepidemiology Department
Research Advisor/s: Dr Eleni Papanicolaou Zamba, Prof. Kyproula Christodoulou, Dr Paschalis Nicolaou

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons, presenting with relentlessly progressive muscle atrophy and weakness. Since the identification of the first causative gene SOD1 in the 1990s and with recent advances in genetics, more than 50 potential causative or disease-modifying genes have been identified, with SOD1, TARDBP, FUS and C9orf72 being the most common. However, the etiology of ALS remains unexplained for over 85% of all cases, suggesting that various environmental risk factors are implicated in the pathogenesis of the disease. This study aimed to conduct a detailed genetic epidemiological investigation and to detect potential exogenous risk factors of ALS in the Cypriot population. A total of 82 ALS patients including 21 fALS (26%) and 61 sALS (74%), provided the cohort for the variant screening in the most common causative genes of ALS including C9of72, SOD1, TARDBP, FUS, ATXN2, and SMN1. In addition, a case-control study was conducted with a total of 56 ALS patients and 56 healthy controls of Greek-Cypriot ethnicity, to investigate the contribution of known environmental risk factors in ALS. One patient with the pathogenic c.800A>G (p.Asn267Ser) genetic variant in the TARDBP (1.25%) and 16 additional patients with a pathogenic hexanucleotide repeat expansion in C9orf72 (20%) have been identified. Furthermore, statistical analysis of the case-control study showed that head trauma, electric injury and exposure to chemicals were increasing the risk for ALS, whereas no significant difference in the demographic characteristics between the two groups were identified. Collectively, findings indicate that C9orf72 repeat expansions are indeed causative for ALS in the Cypriot population, but genetic clusters of pathogenic variants in the remaining genes are not present. Finally, the case-control study will shed some light on the nature of ALS epidemiology in Cyprus, by demonstrating a number of environmental determinants of ALS in the Cypriot population

SA4 MULTI-OMICS DATA ANALYSIS TO OBTAIN MARKERS AND MECHANISMS OF DISEASE PROGRESSION

EFI ATHIENITI
Bioinformatics Department
Research Advisor/s: Prof George Spyrou

The heterogeneity in the progression and outcome of treatment in neurodegenerative disease makes it challenging to develop and assess the efficacy of new therapeutic interventions. Multi-omics datasets are increasingly being collected to enable the construction of molecular profiles of patients, and thus their separation into more coherent subtypes. The vast amount of multi-omics datasets and the increasing realisation to understand complex phenotypes demand new computational methods to integrate these datasets.

The aim of this project is to develop methodologies to extract molecular markers and mechanisms of disease from the analysis of multi-omics datasets. We utilise the Parkinson's Progression Marker Initiative dataset which includes multi-omics datasets for Parkinson's Disease (PD) patients across different time points within 5 years. We used omics (blood RNA, miRNA and plasma proteomics) and clinical data, from patients and controls across four time points (years 0, 1, 2, 4).

Differential expression (DE) analysis of the three datasets is performed to obtain DE RNA, miRNA and proteins between the two groups. Gene Set Enrichment Analysis (GSEA) is used to obtain enriched molecular processes in PD patients. We then propose methodologies to integrate the three omic layers, blood RNA, miRNAs and plasma proteomics using the tool Multi-Omics Factor analysis (MOFA), to establish markers of covariation within the cohorts and enriched molecular processes.

For the following steps, we also outline the proposed methodology to obtain markers associated with the progression of the disease by utilising the datasets at different time points simultaneously.

SA5 INVESTIGATION OF THE AETIOLOGY OF COMMUNITY ACQUIRED PNEUMONIA IN CYPRUS AND CHARACTERIZATION OF HOST FACTORS IN VIRAL/BACTERIAL CO-INFECTIONS

PETROS LADAS
Molecular Virology Department
Research Advisor/s: Dr Jan Richter

Community-acquired pneumonia remains the leading cause of hospitalisation for infectious disease in Europe and a major cause of morbidity and mortality.
We have designed a prospective observational study in collaboration with the Nicosia General Hospital, which aims to determine and characterize for the first time the aetiology of CAP in hospitalized adults in Cyprus as well as to assess genetic host factors associated with CAP severity and progression.
Real-time and RT-PCR were employed for the detection of bacterial and viral pathogens respectively. Next generation using the respiratory pathogen ID/AMR enrichment panel (RPIP) analysis was used to elaborate on the presence of respiratory pathogens as well as to investigate the antibiotic resistance genes of bacterial pathogens. Multiplex real-time PCR was employed for probe based allelic discrimination in order to investigate host factors associated with CAP.
We identified the most prevalent viral pathogens in CAP patients as Influenza A, human Rhinovirus and Sars-COV-2, while the most prevalent bacterial pathogens were Streptococcus pneumoniae, Staphylococcus aureus and Haemophilus influenzae. Antibiotic resistance genes were detected in 20% of the samples for six pathogens S.pneumoniae, S.aureus, E.coli, M.tuberculosis and A.baumani with macrolides and penicillin being the most resisted drug classFor host factors we detected a positive correlation between bacterial infections and the NOS3 G allele (rs1799983) and the FCGR2A G allele (rs1801274). A positive correlation was also detected between the TNF A allele (rs1800629) and sepsis while a negative correlation was identified with the ACE insertion genotype (rs1799752) and severity of pneumonia.
In conclusion this is the first study conducted in Cyprus that characterizes microbial pathogens in hospitalized adults. It identified the most common pathogens in CAP patients as Streptococcus pneumoniae and Influenza A. Associated 36% of bacterial infections with antimicrobial resistance genes. Created an initial profile of genetic host factors associated with CAP in hospitalized patients.

SA6 IDENTIFICATION OF NOVEL γ-GLOBIN REPRESSORS THROUGH A CUSTOM CRISPR KNOCKOUT SCREEN AND VALIDATION STUDIES FOR THE TREATMENT OF β-HEMOGLOBINOPATHIES

SEVGI ÖZKARAMEHMET
Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Marios Phylactides

Reactivation of γ-globin for the production of HbF can ameliorate β-thalassemia and sickle cell disease. Although therapeutic strategies involving addition of a functional β-globin gene or genome editing for γ-globin reactivation are promising, the high cost and limited availability together with safety and efficacy issues constrain such therapies to younger patients with access to sophisticated clinical care. Hence, we want to identify and validate novel γ-globin repressors as potential druggable targets.

We have identified several candidates based on a custom CRISPR/Cas9 knockout screen targeting 293 genes selected from previously published literature. The three most promising candidate genes have been selected for further validation and functional studies. One gene encodes for a protein involved in ion transport and iron homeostasis, the other gene is a transcriptional regulator and the last gene plays a central role in chromatin remodeling and acts as a transcription regulator. These genes are being validated individually through CRISPR/Cas-mediated knockouts based on lentiviral transduction as well as nucleofection with ribonucleoproteins in the HUDEP-2 cell line. Editing efficiency is tested using ICE (Inference of CRISPR Edits), while loss of candidate gene expression is tested using western blotting. RP-HPLC and western blotting are used to investigate the effect of the knock out on γ-globin expression levels.

The CRISPR knockout screen has identified seven potential γ-globin repressor genes, which scored as highly during the screening process as some of the well-known γ-globin regulators. So far, we have been unable to validate these results. Multiple repetitions of the screening protocol and validation experiments point away from experimental error. A number of reasons could contribute to the validation inconsistencies we experience, which we need to investigate. These include exon skipping caused by the CRISPR-generated mutations, alternative splicing of the targeted mRNA or on-target mRNA misregulation.

Our work could potentially identify new HbF regulators, which may provide novel therapeutic targets for the treatment of β-hemoglobinopathies.

SA7 PHYTOCHEMICALS AS NOVEL EPIGENETIC MODULATORS IN SKIN CANCER THERAPEUTICS

VENETIA TRAGKOLA
Cancer Genetics, Therapeutics & Ultrastructural Pathology Department
Research Advisor/s: Prof. Mihalis Panagiotidis, Dr Ioannis Anestopoulos, Dr Maria Loizidou

Malignant melanoma is an aggressive type of skin cancer. Despite the progression made in its clinical management, the development of new therapeutic approaches is of utmost importance. On the other hand, a lot of research has shown the use of several phytochemicals as preventive and/or therapeutic agents in a variety of cancer types. Such a phytochemical is phenethyl isothiocyanate (PEITC), an enriched source of Watercress (an aquatic flowering plant that belongs to the family of Brassicaceae). Finally, several studies have documented the anti-oxidative and anti-cancer effects of PEITC. To this end, the aim of this project is to chemically and biologically characterize a naturally-derived watercress extract in an in vitro model of malignant melanoma consisting of human malignant melanoma (A375, COLO-679), non-melanoma epidermoid carcinoma (A431) and no-tumorigenic keratinocyte (HaCaT) cells. Initially, UPLC MS/MS and HPLC-PDA methodologies were employed to identify the chemical composition of the watercress extract derived from edible and non-edible parts of the plant. Additionally, the Alamar Blue assay was utilized to evaluate the cytotoxic profile of the extract on the in vitro melanoma model. Exposure to various concentrations of the extract revealed a time- and dose-dependent cytotoxicity in both melanoma cell lines, while non-malignant cells exhibited increased resistance. Such increased cytotoxicity was associated with the activation of apoptosis by means of a commercially available multiplex kit quantitating activation of caspases - 3, - 8 and -9. To ensure apoptotic activation as the mode of cell death, RT-PCR methodologies were used aiming toward identifying major anti- and pro-apoptotic gene candidates involved in extrinsic and/or intrinsic apoptotic pathways. Out of 32 apoptosis-related genes tested, 18 were selected as the most significant for further investigation. Finally, our group and others have shown a potential role of PEITC in inducing the epigenetic response perhaps as a means of leading to apoptotic cell death. Thus, current attempts aim to evaluate any potential involvement of watercress in inducing specific epigenetic alterations capable of leading to apoptotic activation.

POSTER PRESENTATIONS

PA1 **Fetal genetic factors associated with sonographic abnormalities and pregnancy loss**
Andrea Hadjipanteli

PA2 **Genome Editing for Beta-Haemoglobinopathies without Double-Strand DNA Cleavage**
Nikoletta Papaioannou

PA3 **$HBB^{IVSI-110(G>A)}$-specific gene editing as advanced therapy for β-Thalassemia**
Basma Naiisseh

PA4 **Global spatial epidemiology of haemoglobinopathies**
Mikaella Kontopoulou

PA5 **Bespoke PGT-M: From in-silico to in-vivo.**
Antri Florentia Romanou

PA6 **Whole exome sequencing and functional studies to characterize novel/rare genetic causes of CMT**
Feride Cinarli Yuksel

PA7 **The role of oligodendrocytic connexin 47 in de- and re-myelination studied in mouse models of multiple sclerosis**
Styliani Theophanous

PA8 **Evaluation of specific pathways related to lipid rafts in acid ceramidase depleted SH-SY5Y stable cells**
Sereen Abbara

PA9 **Enhanced ER-mitochondria association and its implications in mitochondrial function, glycogen metabolism and insulin resistance**
Andria Theodoulou

PA10 **Polygenic burden of epilepsy and high-risk association with abnormal EEG oscillations and structural MRI phenotypic characteristics**
Aristotelis Karamousoulakis

PA11 **Metabolic Disease related medication repurposing in murine models of Alzheimer's Disease**
Demos Kynigopoulos

PA12 **Genetic Study of Early Onset Parkinson's Disease in Cyprus**
Rana Abu Manneh

PA13 **Developing a computational framework to exploit synergies between signalling/imaging data and molecular data/omics towards more precise diagnostic and therapeutic approaches**
Sotiroula Afxenti

PA14 **Network-based analysis of multiple source information towards efficient drug repositioning**
Kyriaki Savva

PA1 FETAL GENETIC FACTORS ASSOCIATED WITH SONOGRAPHIC ABNORMALITIES AND PREGNANCY LOSS

ANDREA HADJIPANTELI

Cytogenetics and Genomics
Research Advisor/s: Prof. Carolina Sismani, Dr Ludmila Kousoulidou

Introduction: Spontaneous pregnancy loss (SPL) is common during the first trimester of pregnancy and can be caused by various factors including large-scale chromosomal abnormalities and submicroscopic aberrations. However, in most SPLs that occur after the first trimester the aetiology remains undetermined. This study aims to resolve SPL cases of unknown aetiology by investigating the fetal genome and its effect on pregnancy outcome.

Methods: Twenty-nine samples were collected from fetuses that were spontaneously aborted, terminated or died neonatally. All fetuses had abnormal ultrasounds and no findings after karyotype and array-CGH. Trio-based whole-exome sequencing (WES) was performed to identify causative fetal variants.

Results: Out of nineteen tested trios, causative/potentially causative variants were uncovered in six cases. A known de novo heterozygous missense variant within *SCN2A* was found in a fetus presenting Developmental and Epileptic Encephalopathy 11 phenotypes. Two inherited novel missense variants in *SCN4A* were found in a compound heterozygous fetus resulting in severe SCN4A-related congenital myopathy. A known homozygous nonsense variant in *KLHL40* was found in a fetus with Nemaline Myopathy 8. Potentially causative heterozygous variants were identified in three cases, in genes *USP18, CC2D2A* and *CPLANE1* with autosomal recessive inheritance.

Conclusions: We identified causative variants in 3/18 cases as well the possible involvement of heterozygous variants in genes *USP18, CC2D2A* and *CPLANE1* in fetal development. Further investigation is required to assess the clinical significance of the latter findings. Accurate identification of variants in such genes creates new genotype-in utero phenotype associations, leading to the prospect of new additions in preconception and prenatal diagnostic panels.

PA2 GENOME EDITING FOR BETA-HAEMOGLOBINOPATHIES WITHOUT DOUBLE-STRAND DNA CLEAVAGE

NIKOLETTA PAPAIOANNOU

Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Carsten Lederer, Prof. Marina Kleanthous

Background/Objectives: Haemoglobinopathies, such as sickle-cell disease and β-thalassaemia, are the commonest monogenic diseases. Of these, β-thalassaemia has high prevalence in Cyprus and is marked by low adult haemoglobin ($\alpha_2\beta_2$, HbA), owing to defective β-globin (HBB) expression. Increased levels of the foetal haemoglobin ($\alpha_2\gamma_2$, HbF) can ameliorate the severity of the disorder and may be achieved by erythroid reduction of γ-globin repressors, such as the transcription factor BCL11A. This can be accomplished by catalysing base editors (BEs) that are double-strand-break (DSB)-independent, which are safer and likely more efficient than traditional DSB-dependent CRISPR/Cas technology. The project aims to adopt the newest generation of genome editors (BEs) for application to targets of relevance for β-haemoglobinopathies for correspondingly increased clinical potential of base editing.

Methods: The current project performed *in silico* design of target- and platform-specific guide RNAs to apply BE technology by nucleofection in HUDEP-2 and patient-derived $CD34^+$ cells, to modify targets of relevance for β-haemoglobinopathies. The study compared different BEs against one another and against DSB-based technology targeting the well-known BCL11A erythroid enhancer. To achieve higher HbF levels, a duplex base editing strategy was established targeting both and *trans*-acting factors and cis-acting elements. Editing efficiency and functional studies at the DNA, RNA and protein level were carried.

Results: Initial plasmid-based delivery of BEs resulted in poor performance, prompting us to establish *in vitro* mRNA synthesis for mRNA/gRNA-based delivery of BEs instead. Resulting precision editing with up to 86% bulk efficiency in HUDEP-2 cells indicated differential same-target efficiency of different BEs for the clinically relevant BCL11A target. Duplex base editing of both, *trans*-acting factors (*BCL11A*) and corresponding cis-regulatory elements (*HBG*), resulted in elevated HbF induction compared to single edits, reaching up to 70% increase of HbF levels in duplex base edited patient-derived $CD34^+$cells.

Conclusions: The present study demonstrates high efficiency and low toxicity of RNA-based delivery for base editing technology compared to the clinically applied RNP standard, superior editing outcomes based on BEs compared to DSB-based editing for a clinically relevant target, and superior, therapeutically relevant HbF hemoglobin induction by duplex compared to simplex BE application.

PA3 HBB$^{IVSI-110(G>A)}$-SPECIFIC GENE EDITING AS ADVANCED THERAPY FOR β-THALASSEMIA

BASMA NAIISSEH

Molecular Genetics Thalassemia
Research Advisor/s: Dr Carsten W Lederer

β-Thalassemia is brought about by defective β-globin (HBB) formation and in severe cases requires regular blood transfusion and iron chelation for survival. Genome editing allows correction of underlying mutations as therapy. As potentially safer alternatives to double-strand break (DSB)-based editors, base editors (BEs) catalyse base transitions (cytosine BEs: C>T, adenine BEs: A>G) for precision editing of DNA target sites. Four recently published BEs with relaxed protospacer adjacent motif (PAM) requirements are being evaluated for their ability to correct the common Cypriot HBB[IVSI-110(G>A)] splice mutation.
BEs were obtained from Addgene and the T7 promoter inserted to allow in vitro mRNA transcription. Editors were delivered into primary hematopoietic cells by nucleofection. Additionally, HBB-deficient cell models were created by DSB-based editing and plate-sorted on a BD FACSAria III for clonal isolation. DecodeR and EditR were used to assess DSB-based and base editing efficiencies, respectively, at the DNA level. HPLC and immunoblot analyses after erythroid differentiation were used for measurements at the protein level.
BEs were designed for three strategies, i.e. editing of (i) the mutated A, (ii) the G of the aberrant AG splice motif, or (iii) upstream sequence elements critical for aberrant splicing. In the process, efficiency of the ABEs for HBB[IVSI-110(G>A)] target sites was confirmed, including by correction of β-globin expression, and removal of the GFP reporter doubled on-target efficiency for the SPRY BE. Finally, to facilitate further analyses, DSB-based precision editing of HUDEP-2 cells was applied to create HBB[IVSI-110(G>A)]-homozygous cell models, displaying characteristically decreased HBB, and same-site deletion models.

PA4 GLOBAL SPATIAL EPIDEMIOLOGY OF HAEMOGLOBINOPATHIES

MIKAELLA KONTOPOULOU

Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Petros Kountouris

Haemoglobinopathies are monogenic diseases that arise from pathogenic variants impacting the synthesis and structure of globin proteins. They encompass the thalassemia syndromes and structural haemoglobinopathies, such as haemoglobin S that causes sickle cell disease. Due to altering migration patterns and miscegenation, they have heterogeneously spread into non-endemic countries and contribute to the global health burden.

Although prior investigations have been conducted to measure the health burden caused by haemoglobinopathies, they have sparse coverage of the world and they are often outdated, incohesive or lack detailed annotation. Herein, a global georeferenced database of epidemiological factors was compiled to evaluate the geographical distribution of the health burden caused by haemoglobinopathies, utilizing a Bayesian geostatistical modelling framework approach. As a proof of concept, the epidemiological data gathered for China were utilized to simulate on ~10km x 10km resolution maps the country's haemoglobinopathy caused health burden. Burden was assessed by estimating the number of affected individuals and live births across China. In conjunction, genetic diversity data were categorized into subtypes and a continuous map estimating the relative frequency of each was computed to better comprehend the severity of the health burden caused by haemoglobinopathies.

Overall, the analysis has assessed the efficacy of operational health policies while highlighting the precise locations where the implementation or revamp of national and sub-national haemoglobinopathy management and prevention health policies ought to be considered.

PA5 BESPOKE PGT-M: FROM IN-SILICO TO IN-VIVO

ANTRI FLORENTIA ROMANOU

Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Thessalia Papasavva

Introduction:
Preimplantation genetic testing for monogenic disorders (PGT-M) is a molecular prevention test provided to couples with high reproductive risks for a single-gene disorder. PGT-M combines molecular genetic diagnosis with assisted reproductive technology (ART) to ensure unaffected pregnancies after in vitro fertilization (IVF). Every PGT-M assay is designed or tailored to meet the unique profile of each family.

We present our strategy and methodology, for designing and implementing bespoke PGT-M assays.

Materials and Methods:
Couples with a family history and high genetic risks for a specific diagnosed monogenic disorder, seeking prevention by PGT-M.
Feasibility study for the evaluation of the pathogenic gene variation(s) responsible for the specific disorder. Initial assessment of the genomic and flanking sequences for potentially usable short tandem repeat (STR) polymorphic sites for linkage analysis.
In-silico design of a combined multiplex PCR assay for both direct mutation detection and linkage analysis.
Optimization of the designed in-silico assay by testing the suitability of the linkage and the direct mutation detection primers on the family DNA samples. Redesign if necessary. Perform trials to establish optimal sensitivity and specificity.
Validation by application of the optimized assay on 35-40 single-cell samples subjected in 5-6 separate experiments.

Results:
PGT-M methodology was introduced in CING in 2004; since then, more than 500 cases have been successfully tested, with no reported misdiagnosis. We have developed, validated, and applied PGT-M assays for more than 30 autosomal recessive, dominant, and X-linked monogenic disorders. A constantly increasing number of new PGT-M panels are designed in our laboratory every year.

Conclusion:
Our data demonstrates that Bespoke PGT-M is an efficient and reliable tool in reproductive care allowing couples with reproductive risks to achieve a healthy pregnancy avoiding the stress and risks of invasive prenatal testing.

PA6 WHOLE EXOME SEQUENCING AND FUNCTIONAL STUDIES TO CHARACTERIZE NOVEL/RARE GENETIC CAUSES OF CMT

FERIDE CINARLI YUKSEL

Neurogenetics Department
Research Advisor/s: Prof. Kyproula Christodoulou, Dr Paschalis Nicolaou

Charcot-Marie-Tooth (CMT) represents motor and sensory hereditary neuropathies characterized by high genetic and clinical heterogeneity. CMT is traditionally classified as demyelinating (CMT1), axonal (CMT2) and intermediate (I-CMT) based on neurophysiological findings. *ATP1A1* was recently associated with autosomal dominant CMT2 and I-CMT to date. *ATP1A1* encodes for the catalytic α1 subunit of the Na^+/K^+ATPase, an essential transmembrane protein with ubiquitous expression. The α subunit is required to assemble with an auxiliary β subunit expressed by an ATP1B gene (ATP1B1-ATP1B3) to form a functional pump protein, responsible for regulating sodium and potassium levels in the cytosol hence, maintaining the electrochemical gradient across the plasma membrane. We identified a novel c.1799 C>G (p.P600R) variant in *ATP1A1* in a Cypriot CMT1 patient using whole exome sequencing. The wild type proline is highly conserved and constrained for missense variations. The mRNA and the protein expression levels of ATP1A1 and ATP1B1 were significantly reduced by ~50% in the patient compared to controls. Genetic analysis confirmed that the reduced *ATP1A1* levels are not due to a splicing or a biosynthesis defect caused by the variant. Moreover, overexpression of the $ATP1A1^{p.P600R}$ in the human SH-SY5Y neuroblastoma cells confirmed that the mutant *ATP1A1* allele is successfully transcribed and translated. The results suggested that the reduced Na^+/K^+ATPase expression in peripheral nerves caused the pathology in this case. Previously described CMT2 variants p.P600A and p.P600T were reported to cause pathogenicity through different mechanisms such as, the reduced Na^+ currents. Thus, we show that ATP1A1 mutations can cause demyelinating phenotype through loss of function mechanism in addition to previously reported axonal and intermediate phenotypes. Our results further confirm the causative role of ATP1A1 variants in peripheral neuropathy and broaden the mutational and phenotypic spectrum of ATP1A1-CMT.

PA7 THE ROLE OF OLIGODENDROCYTIC CONNEXIN 47 IN DE- AND RE-MYELINATION STUDIED IN MOUSE MODELS OF MULTIPLE SCLEROSIS

STYLIANI THEOPHANOUS

Neuroscience Department
Research Advisor/s: Prof. Kleopas Kleopa, Dr Irene Sargiannidou

Multiple sclerosis is the most common demyelinating neurodegenerative disease that affects young adults. It is a primarily inflammatory disorder of the central nervous system, during which infiltrating lymphocytes lead to dysfunction and apoptosis of oligodendrocytes causing demyelination and axonal degeneration. Glial gap junction (GJ) coupling is vital for oligodendrocyte function. More specifically, connexin 47 (Cx47), which is present in most oligodendrocyte-astrocyte GJs, was shown to be crucial for the myelination process. Previous studies have demonstrated that Cx47 knock-out (KO) mice are resistant to cuprizone-induced demyelination, especially at the corpus callosum area. The present study aims to investigate whether cuprizone administration leads to demyelination earlier (3 weeks) or later (7 weeks) than the previously used time point of 5 weeks and whether other connexins compensate for the absence of Cx47 and lead to the non-significant phenotype of the KO mice. Frozen brain sections of cuprizone treated mice and vehicle-only controls were assessed for demyelination, astrogliosis and microgliosis in various areas of the brain, like corpus callosum, cortex, thalamus and hippocampus. Additionally, the change in oligodendrocyte precursor cell (OPC) proliferation was studied. Ablation of Cx47 seems to have a protective effect over cuprizone-induced demyelination, as the corpus callosum appeared unaffected both medially and laterally, while the cortex and thalamus showed slight demyelination although not statistically significant. Even though the demyelination was not evident, there was profound microgliosis, astrogliosis and increased OPC proliferation. Additionally, there was no difference in the expression of other connexins in the Cx47KO mice compared to the wild-type. These findings clearly indicated that there is an involvement of Cx47 in the myelination process that needs to be investigated further.

PA8 EVALUATION OF SPECIFIC PATHWAYS RELATED TO LIPID RAFTS IN ACID CERAMIDASE DEPLETED SH-SY5Y STABLE CELLS

SEREEN ABBARA

Biochemical Genetics Department
Research Advisor/s: Dr Anna Malekkou

Plasma membranes contain specific microdomains, the so-called lipids rafts. In neurons, lipid rafts are present in the axonal plasma membrane and are important for neuronal function. These domains are rich in sphingolipids, sphingomyelin and ceramide (Cer) and cholesterol and act as a platform for signal transduction molecules regulating actin cytoskeleton and vesicular trafficking. Acid ceramidase (AC) is a key regulatory enzyme of Cer metabolism. AC deficiency due to mutations in the *ASAH1* gene, leads to the Farber disease, a fatal lysosomal storage disorder and to Spinal Muscular Atrophy (SMA). SMA characterized by the degeneration of motor neurons in the spinal cord and it is mostly caused by a homozygous deletion of the *SMN1* gene. Our previous work, showed that AC-depletion causes phenotypic alterations that are commonly observed in neurodegenerative diseases, such as distribution of lysosomes towards the cell periphery and significantly shortened and less branched neurites upon differentiation. Lipidomic analysis showed alteration in various lipids, suggesting dysregulation of lipid rafts. The aim of this work was to investigate whether AC plays a role in particular pathways, maintaining the normal phenotype and function of neurons. Two stable neuroblastoma SH-SY5Y cell lines have been used; *ASAH1* knockdown (sh*ASAH1*) and ShScramble (control), to evaluate the mRNA and protein levels of particular selected targets in specific pathways [spliceosome action, multivesicular bodies (MVBs)–exosome formation/secretion and autophagy] by performing real-time PCR and WB analysis. Survival motor neuron protein (SMN), small nuclear ribonucleoprotein and multiple U2-related components of the spliceosome were found to be up-regulated in sh*ASAH1* cells, indicating a gain of toxic function effect causing dysregulation of splicing mechanism. Additionally, AC-depletion significantly increases the mRNA and protein levels of factors that are components of the ESCRT-III complex that drives the formation of intraluminal vesicles inside the MVBs. Lastly, the autophagic flux in AC-depleted cells was found to be altered.

PA9 ENHANCED ER-MITOCHONDRIA ASSOCIATION AND ITS IMPLICATIONS IN MITOCHONDRIAL FUNCTION, GLYCOGEN METABOLISM AND INSULIN RESISTANCE

ANDRIA THEODOULOU

Biochemical Genetics Department
Research Advisor/s: Dr Petros P. Petrou

Endoplasmic reticulum mitochondria contact sites (ERMCs) are highly dynamic contact regions implicated in several biological processes. Disrupting the endoplasmic reticulum (ER)-mitochondria communication has been linked to metabolic disorders including insulin resistance (IR). Nevertheless, because of contradicting findings, the precise role of ERMCs in IR remains elusive. Starch binding domain-containing protein 1 (Stbd1) is an ER-resident, glycogen-binding protein that was previously demonstrated to undergo co-translational N-myristoylation. A non-N-myristoylated form of the protein Stbd1(G2A) is preferentially targeted to the ERMCs and enhances ER-mitochondria association. Therefore, the Stbd1(G2A) variant can serve as a useful tool to address the effects of increased ER-mitochondria association in mitochondrial function, glycogen metabolism and IR in vitro. To this end, AML12 cell lines overexpressing either Stbd1(G2A) or Stbd1(WT) were generated. AML12 cells overexpressing the unrelated GFP protein served as controls. Overexpression of Stbd1(G2A) resulted in increased ER-mitochondria interactions, altered mitochondrial morphology and respiratory function. Moreover, cells overexpressing Stbd1(G2A) exhibited significantly reduced glycogen content compared to the Stbd1(WT) cells. ATP-stimulated calcium influx into mitochondria and calcium content within the ER, mitochondria and cytoplasm were determined. Cells overexpressing Stbd1(G2A) displayed significantly reduced mitochondrial calcium uptake and increased mitochondrial calcium content. Overexpression of Stbd1(WT) was found to significantly improve IR induced by insulin treatment in AML12 cells, more efficiently than Stbd1(G2A). Our findings indicate that enhancement of ER-mitochondria association induced by Stbd1(G2A) results in disturbed mitochondrial morphology and respiratory function. The above appears to impact on glycogen metabolism and the response to insulin and may indicate broader changes in cell metabolism.

PA10 POLYGENIC BURDEN OF EPILEPSY AND HIGH-RISK ASSOCIATION WITH ABNORMAL EEG OSCILLATIONS AND STRUCTURAL MRI PHENOTYPIC CHARACTERISTICS.

ARISTOTELIS KARAMOUSOULAKIS

Neurobiology Department
Research Advisor/s: Dr Andreas Koupparis, M.D., Dr Ioanna Kousiappa

Background
Several common variants may contribute to epilepsy risk through a cumulative effect. In this study we aim to examine the polygenic burden of epilepsy in the Cypriot Epi25 cohort by comparing and contrasting the appropriate Single Nucleotides Polymorphisms (SNPs) available from Polygenic Risk Scores (PRSs) as based upon the relevant Epi25-Consortium Gene Wide Association Studies (GWAS). We then aim to categorize and correlate the results with the potential disease phenotypes expressed in electroencephalographic (EEG) and Magnetic Resonance Imaging (MRI) data. As a final step we aim to construct and train an Artificial Neural Network (ANN) for the purpose of improving identification accuracy and diagnostic production time thus adding to and working under the scope of personalized medicine.

Materials and Methods
All epilepsy-associated SNPs from published peer-reviewed studies which contain PRSs based on the latest Epi25 GWAS, were collected and extracted from the Cyprus cohort. Once identified and compared, they will be sorted into two categories of high and low risk association; then EEG and MRI biomarkers will be analysed and correlated. By that point, all existing data (PRS and EEG/MRI biomarkers) will act as the initial nodes of ANN.

Results / Discussion
The 2022 GWAS studies revealed 31 variants of interest. A preliminary search prior to imputation showed that five of these variants are indeed expressed in the Cypriot cohort while the initial PRS results suggested ordinate values of high and low risk associated variants. Imputation analysis was performed using the 1000 Genomes Phase 3 as a reference panel and Eagle2 algorithm for phasing. Currently undergoing analysis on the imputed data aims to unveil a higher number of variants leading to the first stage of high and low risk categorization.

Conclusions
Main goals and methods aside, this study strives to bring together up-to-date genetic risk calculation methods with traditional neuro-physiological and structural imaging data in a manner that is practical and assistive to all involved in the clinical diagnostic process.

PA11 METABOLIC DISEASE RELATED MEDICATION REPURPOSING IN MURINE MODELS OF ALZHEIMER'S DISEASE

DEMOS KYNIGOPOULOS
Research Advisor/s: Dr Elena Panayiotou-Worth

Background
Alzheimer's Disease (AD) is a chronic neurodegenerative disorder of the central nervous system (CNS). Epidemiological studies have linked Metabolic Syndrome (MetS) to AD, specifically, obesity, hypertension, and type 2 diabetes (T2D) with the onset of dementia associated syndromes.

Thus, we tested two drugs; Diamicron® (Gliclazide) and Praluent® (Alirocumab) involved in the insulin- and cholesterol metabolism respectively on a murine model of familial AD (5XFAD).

Objectives
Diamicron® and Praluent® were administered to both wild type (WT) and 5xFAD mice to evaluate their effects on the phenotype of AD mice, and ultimately determine whether treatment with metabolic disease targeted medication could be useful in delaying the onset and progression of the disease.

Results
Post-treatment behaviour testing showed significant cognitive impairment in the untreated 5xFAD mice when compared to the untreated WT group. 5xFAD mice treated with Diamicron® showed a slight increase in their performance compared to the untreated 5xFAD. However, 5xFAD mice treated with Praluent® displayed significant improvement. 5xFAD mice treated with Praluent® performed significantly better than the 5xFAD-Diamicron mice.

Electrophysiological experiments in acute hippocampal brain slices were carried to assess the synaptic response and Long-Term Potentiation (LTP) in the CA1 area. 5XFAD mice have significantly reduced LTP compared to controls. However, after drug administration, 5XFAD mice have significantly enhanced LTP, compared to 5XFAD mice without treatment.
Macroscopically 5xFAD control brain slices appear to have hippocampal atrophy when compared to the WT control mice. Hippocampal atrophy was also present in the 5xFAD Diamicron® mice but to a lesser extent. 5xFAD-Praluent mice showed almost no atrophy. No hippocampal atrophy was observed in WT mice.

Discussion
Praluent® appears to slow down hippocampal atrophy and to increase LTP in 5XFAD mice. Brain tissue, visceral fat and blood serum from all mice will be used for further investigation.

PA12 GENETIC STUDY OF EARLY ONSET PARKINSON'S DISEASE IN CYPRUS

RANA ABU MANNEH

Neuroepidemiology Department
Cancer Genetics, Therapeutics & Ultrastructural Pathology Department
Research Advisor/s: Prof. Eleni Papanicolaou Zamba, Prof. Andreas Hadjisavvas

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

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

Results: One previously described frameshift variant in PINK1 (NM_032409.3:c.889del) was detected in five patients (10.4%)—the largest number to be detected to date. CNVs in the PRKN gene were identified in one homozygous and 3 compound heterozygous patients (8.3%). No pathogenic variants were detected in the other 4 genes (FBXO7, SNCA, PLA2G6, and DJ-1) under investigation in this study.

Conclusion: To date, the pathogenic variants identified in this study have explained the PD phenotype for 18.8% of the EOPD cases. Almost 1 in every 5 patients in our cohort has been identified as a carrier of either a PINK1 or PRKN pathogenic variant. Currently, the diagnosis of PD is clinical and based on the presence of motor features. Early onset patients have a challenging journey towards a PD diagnosis as their initial symptoms may vary and their young age of onset may lead to differential diagnosis. Hence, the results of this study may contribute to the genetic screening of EOPD in Cyprus. We are currently analyzing the mitochondrial epigenetic landscape of our EOPD cohort.

PA13 DEVELOPING A COMPUTATIONAL FRAMEWORK TO EXPLOIT SYNERGIES BETWEEN SIGNALLING/IMAGING DATA AND MOLECULAR DATA/OMICS TOWARDS MORE PRECISE DIAGNOSTIC AND THERAPEUTIC APPROACHES

SOTIROULA AFXENTI

Bioinformatics Department and Neuroimmunology Department
Research Advisor/s: Prof. George Spyrou, Prof. Marios Pantzaris, Dr Nancy Lambrianides

Nowadays, there is an abundance of available information for numerous diseases from multiple sources. The ability to integrate this multi-source information together is a great challenge yet, it can help understand better the underlying mechanisms of a disease and build more comprehensive profiles of either the disease of interest or the patients.

This project aims to develop a computational framework that hosts a pool of integration of multi-level/multi-source data methods/tools and network-based approaches to contribute in more precise diagnostic and therapeutic approaches. It aims to identify patterns in composite profiles built from imaging and molecular data in case-control cohort data and between different phenotypes in different diseases' cases.

Initial analyses for this project are conducted using Alzheimer's disease (AD) as a case study. Subjects with normal cognition, mild cognitive impairment and dementia due to AD are then collected. As an initial step, MRI measurements, protein expression data, biological markers and clinical assessments for the baseline visit are downloaded from the AD Neuroimaging Initiative (ADNI) database. Next, multi-omics factor analysis (MOFA) tool will be used to perform an integrative analysis of brain imaging and protein expression data to unravel the variance within the cohort of participants. In parallel, we will generate different integration profiles (MOFA models) using different pools of subjects each time to compare the disease profiles. Association analysis of the different factors and the clinical data will also be performed. Lastly, in order to investigate which biological pathways are represented in each factor for AD, pathway enrichment analysis using the identified analytes from the proteomic layer will be performed.

From these analyses, we aim to identify the major dimensions of heterogeneity that together explain the variance within the cohort, and are associated with the core AD pathology. In addition, we aim to identify new molecular patterns interrelated within each factor that combine imaging and proteomics features. The outcomes of these analyses will demonstrate the added value of integrative multi-omics analysis to uncover interrelated pathway alterations in AD and its ability to identify biomarker combinations.
Using these novel approaches, we aim to discover underlying molecular mechanisms of the disease of interest, candidate composite biomarkers and repurposed drugs, boosting a faster and more accurate diagnosis, prognosis, prediction of treatment response, leading to more personalised treatments.

PA14 NETWORK-BASED ANALYSIS OF MULTIPLE SOURCE INFORMATION TOWARDS EFFICIENT DRUG REPOSITIONING

KYRIAKI SAVVA

Bioinformatics Department
Research Advisor/s: Prof. George Spyrou, Dr Margarita Zachariou

Neurodegenerative diseases (NDs) are a group of diseases that are prevalent in a large proportion of the total population and involve a progressive loss of function and neurons in the central nervous system. With no disease-curing drugs available for many NDs and an ever-growing healthcare burden, novel approaches for identifying therapies are needed. Drug repurposing, is one such approach, which involves the identification of novel indications for currently-used drugs, in a cost- and time-effective way.
In this thesis, we proposed stage-specific candidate-repurposed drugs against AD by using a novel network-based method for drug repurposing against different stages of AD severity. We applied this method to highlight stage-specific candidate repurposed drugs against AD. For each AD stage, this approach a) ranks the candidate repurposed drugs based on a novel network-based score emerging from the weighted sum of connections in a network resembling the structural similarity with failed, approved or currently ongoing drugs, b) re-ranks the candidate drugs based on functional, structural and a priori information according to a recently developed method by our group and c) checks the permeability through the Blood Brain Barrier. The top 13 drugs proposed from this work underwent further experimental validation using an in vitro BACE1 assay, and the effect of a single drug from our list, tetrabenazine, in the 5XFAD AD mouse model.
In the sequel, we further developed a novel methodology, D^{R}e^{A}mocracy, which exploits previous studies on computational drug repurposing that have attempted to highlight candidate repurposed drugs, as well as clinical trial studies that tested drugs in different phases. Through a weight-modulated majority voting of the drugs' modes of action, initial indications and targeted pathways for four NDs, D^{R}e^{A}mocracy gives a disease suitability score for each drug under investigation. Overall, we contributed towards more efficient drug repurposing through the development of novel computational methods.

THE CYPRUS INSTITUTE OF
NEUROLOGY & GENETICS

PhD&DNA DAY

2023