ACADEMIC YEAR 2021-2022

PHD TOPICS

APPLICATION DEADLINE: 10/05/21 12:00 NOUN

THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

CYPRUS SCHOOL of molecular medicine

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Message from the CSMM Dean

Dear Prospective PhD Candidates,

I am in the pleasant position to announce the PhD Research Projects offered by the Cyprus School of Molecular Medicine (CSMM) for the Academic Year 2021-2022.

At the CSMM, we are committed to producing a high calibre research output that contributes towards the improvement of the quality of human life in Cyprus and worldwide. We aim to challenge our students with a wide variety of research projects and concepts and we enforce international standards of excellence throughout our exceptional curricula.

Our programs are designed to train and expose you to competitive research and a stimulating scientific environment. We strive to provide you with the knowledge and experience that is needed, to enable you to cope with future demands and set you on a career path, taking into account how competitive the employment market has become. Indeed, our graduate PhD students have successfully entered the labour market acquiring positions in both Cyprus and abroad.

As you explore science and learning with us, there will be many opportunities for you to make new friends and pick up life-long skills. You will meet dedicated and experienced scientists who will go the extra mile to mentor and guide you. You will be hosted in CING departments and clinics who are headed by highly accomplished scientists and doctors. You will have the opportunity to work in a professional environment, learn state-of-the-art techniques and how these are applied to solve real everyday diagnostic problems, which benefit patients and our community. The present pandemic shows us that as scientists, we need to intensify our efforts to advance knowledge, through scientific discovery and innovation. Join us in this quest and experience with us the exciting promise that molecular biology and genetics hold, for advancing the frontiers of both science and medicine.

This booklet is designed to provide you with useful information about the currently available PhD positions and topics, the Hosting Departments/Clinics and the Research Supervisors. We are all here to assist you in accomplishing your tasks, challenge and support you in order to prepare you for a successful professional career.

We are looking forward to receiving your applications and to joining hands, in the fight to reduce the suffering caused by human diseases, in an effort to create a better tomorrow, especially for our patients!

Deadline for PhD applications: May 10th, 2021 (12pm, Cyprus Time).

Warm Regards,

Professor Kyriacos Kyriacou, PhD, FRMSoc (UK)

The topic is eligible for the following Program(s):

- ✓ PhD in Medical Genetics
- ✓ PhD in Molecular Medicine
- ✓ PhD in Neuroscience

T1: *Exploring novel gene therapy approaches for Amyotrophic Lateral Sclerosis*

Hosting Department/Clinic/Group:
Molecular Virology Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/mv)

Contact Persons:
Prof Christina Christodoulou (cchristo@cing.ac.cy)
Dr Jan Richter (richter@cing.ac.cy)

Abstract:
With a lifetime risk of 1 in 400, Amyotrophic Lateral Sclerosis is a fatal, progressive, mostly adult-onset disorder of both lower and upper motor neurons. Neurodegenerative diseases like ALS represent a particular devastating health problem for which there is significant unmet medical need. Gene therapy is an alternative to traditional pharmacological approaches that has made important advances over the last decade in treating the nervous system. A tremendous amount of preclinical data has demonstrated the relevance and feasibility of AAV-based gene therapy to treat CNS disorders.

In previous studies a dramatic neuroprotective effect of recombinant Hsp70 in mouse models of neurodegeneration by intranasal administration was shown previously where multiple Alzheimer's disease-like morphological and cognitive abnormalities were mitigated. It was also shown that rhHsp70 injections can delay symptom onset and increase lifespan in the SOD1 mouse model of ALS. In addition, it was shown that a recently developed transgenic FUS 1-359 mouse model of proteinopathy could recapitulate several key features of human ALS, which allows for more a more rapid analysis of treatment effects.

For this PhD project we propose to investigate the neuroprotective properties of human recombinant heat shock protein 70 (hrHsp70) following an AAV-mediated gene delivery in different mouse models such as the TDP-43A31ST , the h(G4C2)37-500 model or the FUS(1-359) proteinopathy model of ALS with the overall aim to develop a novel neuroprotective gene therapy approach.

Several promising AAV serotypes will be produced containing a novel, promising neuroprotective factor and then subsequently tested in randomized treatment trials in vivo. An intrathecal as well as intravenous injection route will be investigated for their efficiency and feasibility of vector delivery to target tissues. To assess outcomes, we will perform detailed behavioural testing, biochemical and histological analysis of nerve tissues as well as morphometric examination of nerve pathology in treated mice compared to controls. We expect that the human recombinant Hsp70 will be successfully expressed in neurons and/or glial cells comparing both, intrathecal and

intravenous AAV vector delivery. Hsp70 expression will hopefully lead to a significant improvement in mouse life expectancy, enhanced motor neuron functions that is detected via behavioural testing. On the molecular level, we expect that Hsp70 would act as a neuroprotector and inhibit pathological aggregation of aberrant protein in nerve tissues of transgenic animals.

The topic is eligible for the following Program(s):

- ✓ PhD in Medical Genetics
- ✓ PhD in Molecular Medicine

T2: Development of comprehensive Preimplantation Genetic Testing for Monogenic Diseases; the road towards an all-embracing universal application.

Hosting Department/Clinic/Group/Unit:
Molecular Genetics Thalassaemia Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/mgt)

Contact Persons:
Dr Thessalia Papasavva (thesalia@cing.ac.cy)

Abstract:
Preimplantation genetic testing (PGT) has evolved into a well-established alternative for couples at high risk of having an affected child, with the advantage that the genetic testing is performed at the embryo stage and the couple can thereby avoid a pregnancy termination of an affected foetus. The main indications for PGT are monogenic disorders and chromosome abnormalities and there is an increasing demand for PGT each year. PGT for single-gene defects has been carried out worldwide since 1990. Since then, specific mutation tests have been developed for an ever-widening range of diseases. The development of PGT tests for single-gene disorders is challenging and can be time-consuming. The development and application of these specific tests requires customized preclinical workup design and optimisation of protocols that require a substantial waiting time for the couples.

The aim of this PhD thesis is the development, optimization, evaluation and comparison of Single-Cell Whole Genome Amplification Methods with ultimate aim the implementation of a robust uniform PGT approach covering a wide range of monogenic disorders.

For this purpose, three state-of-the-art approaches will be evaluated. We will compare the suitability of the different WGA methods for the detection for the detection of single-nucleotide polymorphisms and for de-novo genome assembly. The proposed methods are:

- WGA followed by genome-wide methods based on single nucleotide polymorphism (SNP) array. The SNP array platform is especially powerful for double indications, for instance two monogenic disorders or a monogenic disorder plus HLA matching as whole genome haplotyping is accomplished from a single data set.
- WGA followed by different versions of Multiple Displacement Amplification (MDA)
- Non-invasive PGT (niPGT), a revolution in reproductive genetics, will be evaluated by analyzing cell-free DNA (cfDNA) from blastocoel and spent blastocyst medium (SBM)

WGA methods introduce representation bias and nucleotide changes during amplification. This hampers the applicability of these methods in PGT. Non-uniform amplification of regions across the genome may result in over- or underrepresentation of such genomic regions. Detection of mutations, more specifically SNPs, in insufficiently amplified (i.e. underrepresented) regions, can introduce genotyping errors. Additionally, the introduction of errors in the sequence during amplification could result in mutations being obscured or introduced. Numerous metrics of interest, will be compared including the specificity, the uniformity of genome coverage, de-novo genome assembly quality and the performance of each method for the identification of single nucleotide variants (SNVs). The bias in amplification that results from the different chemistries will also be analyzed.

The optimal approach will be selected based on the above parameters, evaluated and validated for implementation.

The topic is eligible for the following Program(s):

- ✓ PhD in Molecular Medicine
- ✓ PhD in Medical Genetics

T3: Spatial modelling for haemoglobinopathy epidemiology

Desirable background: *BSc or MSc in Mathematical Sciences, Computer Science, Bioinformatics or closely related field.*

Hosting Department/Clinic/Group/Unit:
Molecular Genetics Thalassaemia Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/mgt)

Contact Persons:
Dr Petros Kountouris (petrosk@cing.ac.cy)

Abstract:
Haemoglobinopathies represent the commonest monogenic disorders in the world. Nevertheless, in many cases, available data on the the epidemiology of haemoglobinopathies are sparse and, often, outdated. Computational and statistical methods can provide valuable insights on the current distribution and frequency of hemoglobinopathies in the world.

The primary aim of this PhD project is to use spatial modelling to study the burden of haemoglobinopathies in the world. To achieve this goal, available evidence in the literature and in public databases, such as IthaMaps, will be compiled and curated. Subsequently, statistical approaches will be employed to provide insights into disease burden. Finally, web services will be developed to facilitate evidence-based decision and policy making.

The topic is eligible for the following Program(s):

- ✓ PhD in Molecular Medicine
- ✓ PhD in Medical Genetics

T4: Semantic data models for rare blood disorders.

Desirable background: *BSc or MSc in Computer Science, Mathematical Sciences, Bioinformatics or closely related field.*

Hosting Department/Clinic/Group/Unit:
Molecular Genetics Thalassaemia Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/mgt)

Contact Persons:
Dr Petros Kountouris (petrosk@cing.ac.cy)

Abstract:
Anotation of complex biological datasets strongly relies on the use of data standards, which can make them Findable, Accessible, Interoperable and Reusable (FAIR). Disease-specific data models have been used in the past to facilitate standardisation of disease-specific resources and their integration in large general purpose databases.

The primary aim of this PhD project is the development of data models that can provide semantic interoperability for datasets related to rare blood disorders. The project will built upon relevant research conduncted by the ITHANET portal, the ERN-EuroBloodNet and EJP RD. Subsequently, the resulting models will be integrated into existing resources, such as the ITHANET portal and pan-European registries for rare blood disorders developed by the MGTD, namely RADeep and ENROL.

The topic is eligible for the following Program(s):

- ✓ PhD in Neuroscience

T5: Targeting glial connexins to promote remyelination in multiple sclerosis

Hosting Department/Clinic/Group/Unit:
Neuroscience Department
(https://www.cing.ac.cy/en/about-us/clinical-sciences/nce)

Contact Persons:
Prof Kleopas Kleopas (kleopa@cing.ac.cy)
Dr Irene Sargiannidou (irenes@cing.ac.cy)

Abstract:
Several lines of evidence from our recent studies and the work of other groups indicate that glia connexins play important roles in the process of demyelination and remyelination in the CNS, both the multiple sclerosis brain in the experimental model EAE. Abnormalities in blood brain barrier function, immunological responses, astrocyte reactions, OPC differentiation and remyelination can be affected by defects in oligodendrocyte connexins, especially Cx47, which is prominent in all oligodendrocyte linage cells forming gap junctions. In this project we will examine the role of Cx47 in de- and remyelination using 2 different experimental models, the lysolecithin and cuprizone model induced in both wild type and Cx47 deficient mice. In vivo and ex vivo oligodendrocyte responses will be analysed, and Cx47-interacting proteins will be studied to dissect downstream pathways leading to exacerbated demyelination and impaired re-myelination observed in the EAE model. Molecular changes will be assessed by immunostaining, real time PCR and immunoblot analysis, and will be further evaluated using a bioinformatics approach to identify key effectors of dysregulated CNS responses. Emerging molecular targets will be validated in highly active as well as in chronic post-mortem human brain tissue from MS patients. This project will provided important insights into the role of glia connexins in inflammatory demyelination and will potentially identify druggable targets for promoting remyelination in MS.

The topic is eligible for the following Program(s):

- ✓ PhD in Medical Genetics
- ✓ PhD in Molecular Medicine

T6: Enhanced preimplantation assessment of embryos produced by in vitro fertilization

Hosting Department/Clinic/Group/Unit:
Cytogenetics and Genomics Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/cg)

Contact Persons:
Dr Carolina Sismani (csismani@cing.ac.cy)
Dr Ludmila Kousoulidou (kousouli@cing.ac.cy)

Abstract:
In vitro fertilization (IVF) is an assisted reproduction technique offered to couples that have difficulty conceiving naturally. One of the most important parameters affecting IVF success is the quality of the produced embryos; therefore, a thorough assessment of the embryo prior to implantation is of paramount importance. Preimplantation genetic testing for aneuploidies (PGT-A) is used to screen IVF embryos for chromosomal abnormalities aiming to improve the overall clinical outcome of the implantation by selecting only euploid embryos for transfer. Other factors that may affect embryo implantation potential are morphology, mitochondrial DNA quantity and miRNA expression. The main objective of the proposed project is to enhance embryo selection by investigating the above parameters as well as the impact of embryo mosaicism on embryo quality to facilitate more accurate interpretation of PGT-A, thereby improving its validity for embryo selection.

Patient recruitment will be performed upon approval of the project by the National Bioethics Committee. Eligible couples will be recruited and sign the appropriate consent forms. The first group (G1) will consist of couples that are already scheduled for IVF with PGT-A, and will already have available embryos for testing with the routine PGT-A procedure. Each couple will participate with all blastocyst stage embryos planned for analysis. 100-150 blastocysts of G1 will be biopsied using standard procedures and DNA samples will be collected from each couple. The second group of subjects (G2) will consist of 30-50 IVF embryos not used for implantation, but frozen at blastocyst stage and donated for research following informed written consent. Sample collection for G2 will be performed by four trophoectodern (TE) biopsies and one inner cell mass (ICM) biopsy from each embryo and DNA samples will be collected from each donor couple. For both G1 and G2, embryo morphology, maternal age, number of previous attempts and other clinical data will be registered. PGT-A procedure along with mtDNA quantification and miRNA expression studies will be performed of both G1 and G2.

For G2, PGT-A results from four TE biopsies and one ICM biopsy of the same embryo will be compared in order to address the issue of interpretation of mosaic PGT-A results.

Correlations established among all studied parameters, will enhance embryo assessment procedures to allow for more accurate predictions of the implantation potential of an embryo and a more accurate selection procedure when several embryos are available, ultimately improving overall IVF success rates.

The topic is eligible for the following Program(s):

- ✓ PhD in Molecular Medicine
- ✓ PhD in Neuroscience

T7: Investigating the controlling effects of antibodies involved in coagulation upon factor Xa, thrombin and complement interactions

Hosting Department/Clinic/Group/Unit:
Neuroimmunology Department
(https://www.cing.ac.cy/en/about-us/clinical-sciences/ncc)

Contact Persons:
Dr Nancy Lambrianides (nancyl@cing.ac.cy)

Abstract:
Multiple sclerosis is an autoimmune inflammatory disorder of the CNS, characterized by demyelination and variable degrees of axonal loss. Recent evidence has pointed out the crucial role of innate immunity and in particular, it is hypothesized that both inflammation and coagulation are the main effector processes of innate immunity that act synergistically through mutual regulation mediating MS. A number of studies focused on the role of either thrombin, fibrin(ogen), or other coagulation factors in MS due to the findings of both a link between perivascular fibrin(ogen) deposition and clinical manifestations in EAE and its rapid enhancement after the inhibition of thrombin generation by heparin and several anticoagulant agents. Serine protease enzymes play a critical role in both the coagulation and complement cascades. However, little is known about the effects of anti-serine protease antibodies on complement activation in MS. This project will investigate whether antibodies to factor Xa and thrombin alter the effects of these serine proteases on complement cleavage in the presence or absence of the physiological inhibitor anti-thrombin. A role for complement in MS was suggested initially, following the evidence of complement component C3 deposited in the brains of MS patients. There is more evidence about the involvement of complement in MS whereby antibodies against two complement regulatory molecules expressed in the membrane of human cells (CD46 and CD59) were present in sera from relapsing-remitting MS patients in the acute phase, providing a mechanism by which cells of the nervous system might be damaged in a complement-dependent fashion during the acute MS phase. Complement and coagulation/fibrinolytic systems are essential components of the host defense system with cross talk between the various systems being apparent, including interactions such as that between C4-binding protein (C4BP) that regulates complement by inhibiting the classical initiation pathway and protein S, the latter being a cofactor for activated protein C, a regulator of coagulation and inflammation. Most importantly, there are in vitro and in vivo data that suggest coagulation cascade enzymes such as thrombin could cleave C5. With all the above knowledge in mind, we will aim to investigate the controlling effects of antibodies involved in coagulation upon Factor Xa, thrombin and complement interactions.

The topic is eligible for the following Program(s):

- ✓ PhD in Neuroscience

T8: Diabetes related medication repurposing in murine models of Alzheimer's disease

Hosting Department/Clinic/Group/Unit:
Neuropathology Department
(https://www.cing.ac.cy/en/about-us/clinical-sciences/nca)

Contact Persons:
Dr Elena Panayiotou Worth (panagiot@cing.ac.cy)
Dr Eleni Fella (elenife@cing.ac.cy)

Abstract:
This project will make use of the 5xFAD and 3Tg Alzheimer's disease models to investigate the effects of various FDA approved lipid-metabolism and diabetes medications in the onset and progression of Alzheimer's disease.

Techniques such as Western blots, immunohistochemistry, immunoassays and murine behavioural testing will be used.

The topic is eligible for the following Program(s):

- ✓ PhD in Molecular Medicine
- ✓ PhD in Medical Genetics
- ✓ PhD in Neuroscience

T9: Use of neuroinformatics tools to understand genetic conditions associated with epilepsy

Hosting Department/Clinic/Group/Unit:
Department of Neurobiology
(https://www.cing.ac.cy/en/about-us/clinical-sciences/ncb)

Contact Persons:
Dr Ioanna Kousiappa (ioannak@cing.ac.cy)
Dr Andreas Koupparis (andreask@cing.ac.cy)
Dr Yiolanda Christou (yiolandac@cing.ac.cy)

Abstract:
Epilepsy is a disease of the brain, characterized by recurrent epileptic seizures. The diagnosis of epilepsy is typically made based on clinical observations and the use of diagnostic methods, like electroencephalography (EEG or video-EEG) to find abnormal patterns of brain waves, neuroimaging (MRI) to look at the structure of the brain and genetic testing to detect variants in genes that are associated with epilepsy conditions. By combining all of the above neuroinformatics tools with the advances in genetics we may enable a deeper understanding of disease mechanisms and promote a shift to more personalized medicine in the epilepsies and improve clinical care. This project aims to explore clinical features, as well as specific brain structural phenomena and/or abnormal EEG oscillations, focusing on particular epilepsy syndromes or phenotypes and associate the findings with whole exome sequencing data. Multimodal analyses and bioinformatics tools will be used to interpreted the results.

The topic is eligible for the following Program(s):

- ✓ PhD in Molecular Medicine
- ✓ PhD in Medical Genetics
- ✓ PhD in Neuroscience

T10: Acid ceramidase regulation of exosome formation, release and function

Hosting Department/Clinic/Group/Unit:
Biochemical Genetics Department
(https://www.cing.ac.cy/en/about-us/biomedical-sciences-/bg)

Contact Persons:
Dr Anthi Drousiotou (anthidr@cing.ac.cy)
Dr Anna Malekkou (annama@cing.ac.cy)

Abstract:
Acid ceramidase (AC) is a key regulatory enzyme of ceramide (Cer) metabolism, which catalyzes the conversion of Cer into a fatty acid and sphingosine inside the lysosomes (acidic pH). However, AC is also able to synthesise Cer at alkaline pH. Mutations in the AC gene (ASAH1) have long been known to be responsible for Farber disease (FD), a fatal lysosomal storage disorder. Mutations in the same gene have also been reported to be associated with Spinal Muscular Atrophy (SMA) or with SMA with Progressive Myoclonic Epilepsy (SMA-PME), a rare variant of SMA.

In our previous work, we have established a stable knockdown SH-SY5Y cell line (ASAH1KD) expressing short hairpin RNA (shRNA) against ASAH1 by a lentiviral approach and used it to determine the functional significance of AC in neurons (Kyriakou et al., 2020). Phenotypic alterations that are commonly observed in neurodegenerative diseases were found in ASAH1KD including; distribution of lysosomes towards the cell periphery and significantly shortened and less branched neurites upon differentiation.

In an attempt to identify alterations of the proteome profile of neuronal cells resulting from AC depletion or overexpression, a proteomic analysis was performed using the ASAH1KD and AC-GFP stable cell lines, respectively. We have identified a number of proteins that were up- or down-regulated in the endocytic and multivesicular bodies (MVBs) formation pathway.

MVBs are a specialised subset of endosomes that contain membrane-bound intraluminal vesicles (ILVs). MVBs can either fuse with lysosomes to initiate the degradation of their contents or fuse with the plasma membrane, where ILVs are released into the extracellular space as exosomes (nanovesicles of 30-150nm in diameter). Exosomes contain certain types of proteins, lipids and RNAs and are implicated in intercellular communication. However, exosomes have also been implicated in intracellular communication in some diseases such as cancer and neurodegenerative disorders. Abnormalities in MVBs components have been shown to lead to neurological disorders such as hereditary spastic paraplegia.

The present project will test a central hypothesis that AC plays a crucial role in the control of lysosome trafficking or fusion to MVBs and subsequent exosome excretion, maintaining the normal phenotype and function of neurons.

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