CSMM

PhD Positions & Topics

Academic Year 2017-2018

Deadline for applications

May 22, 2017

CYPRUS SCHOOL
of molecular medicine

THE CYPRUS INSTITUTE OF
NEUROLOGY & GENETICS

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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 2017-2018.

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 a competitive research and 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.

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 lecturers who will go the extra mile to mentor and guide you. Note, that you will have the opportunity to work alongside experienced scientists, who use state of the art techniques to solve real every day diagnostic problems, for the benefit of patients and our community. In November of 2016, we proudly graduated the very first PhD graduates from the CSMM and we are all determined to continue and build on this success.

This booklet is designed to provide you with useful information about the currently available PhD Positions and Topics, the Hosting Departments and the Research Supervisors.

We are all here to assist you in accomplishing your tasks and prepare you for a successful future career.

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

Deadline for applications: May 22nd, 2017

Warm Regards,

Professor Kyriacos Kyriacou, PhD, FRMSoc (UK)

T1: "INVESTIGATION OF THE EFFECT OF MUSCLE-DERIVED EXOSOMES IN THE PATHOLOGY OF MYOTONIC DYSTROPHY"

Hosting Department/Clinic/Group:
Molecular Genetics, Function & Therapy Department (http://www.cing.ac.cy/mgft/)

Contact Persons:
Prof. Leonidas A. Phylactou (laphylac@cing.ac.cy)
Dr Andrie Koutsoulidou (andriek@cing.ac.cy)

Abstract:
Myotonic dystrophy type 1 (DM1) is a multi-systemic disorder that is characterised by progressive skeletal muscle wasting. DM1 patients face severe secondary complications including cardiac conduction abnormalities and diabetes. This multi-systemic character of the disease remains unclear. Our team was the first to identify some muscle constituents to be encapsulated within exosomes isolated from the serum of DM1 patients [1,2]. Exosomes are extracellular microvesicles, which carry molecular constituents and were identified to play a role in intercellular communication by directing their cargoes to specific cell types thus altering the cellular phenotype of target cells. The proposed project aims to investigate the role of muscle-derived exosome biogenesis, secretion, distribution into secondary tissues and effect in DM1 pathogenesis. Specifically, this project aims to identify and characterize the regulation of molecular mechanisms that are responsible for exosome biogenesis and secretion in DM1. The target tissues of the muscle-derived exosomes will be identified, and the effect of their cargoes to target tissues will be investigated. Several state-of-the-art molecular biology and biochemical techniques will be applied. Exosomes will be isolated from human, animal models and muscle cells. Experiments will be performed both in-vivo and in-vitro. In-vivo techniques include intravenous and intramuscular injections, tissue isolations and live imaging analysis. Molecular studies such as RNA and protein isolation, Real-Time PCR and western blot will also be conducted. The completion of the project will provide novel information regarding the abnormal exosomal release of muscle constituents and their biodistribution that possibly affect the development of secondary complications in DM1 patients.

References:

1. Koutsoulidou A, Kyriakides TC, Papadimas GK, Christou Y, Kararizou E, Papanicolaou EZ, Phylactou LA (2015). Elevated Muscle-Specific miRNAs in Serum of Myotonic Dystrophy Patients Relate to Muscle Disease Progress. PLoS One. 10, e0125341.

2. Koutsoulidou A, Photiades, M, Kyriakides TC, Georgiou, K, Papadimas GK, Christou Y, Łusakowska, A, Kararizou E, Papanicolaou EZ, Phylactou LA. Exosomal Muscle-Specific miRNAs in Serum of Myotonic Dystrophy Patients Relate to Muscle Disease Progress. Under preparation.

T2: "AETIOLOGY OF COMMUNITY ACQUIRED PNEUMONIA IN CYPRUS AND THE ROLE OF VIRAL/BACTERIAL CO-INFECTIONS"

Hosting Department/Clinic:
Molecular Virology Department (http://www.cing.ac.cy/easyconsole.cfm/id/373)

Contact Person:
Dr Jan Richter (richter@cing.ac.cy)

Abstract:
Community-acquired pneumonia remains the leading cause of hospitalisation for infectious disease in Europe, and a major cause of morbidity and mortality. Its causes include bacteria, viruses, fungi and parasites. The rapid diagnosis of the etiologic agent of CAP is the basis for the appropriate management of patients with respiratory infections, the rational use of antivirals and antibiotics, as well as to obtain epidemiological data, which are a prerequisite for vaccine development, the design of interventional studies and the development of prevention and treatment strategies, however, published data on aetiology and frequency of antimicrobial resistance of CAP in Cyprus are not available.

For this reason we propose a prospective observational study to be conducted in collaboration with the Nicosia General Hospital with the aim to determine and characterize for the first time the aetiology of CAP in hospitalized adults in Cyprus. In addition to the aetiology, identified bacteria will be analysed with regard to their antibiotic resistance profile.

New and innovative detection methods will be developed and established including multiplex Real-Time RT-PCR, next generation sequencing and/or DNA chips in order to be able to determine the full spectrum of viral and bacterial pathogens and characterize them. State-of-the-art bio-statistical methods will be employed to investigate possible synergistic and/or antagonistic interactions in co-infections and their role in the prognosis of CAP.

The results from this study are expected to provide crucial information essential for physicians involved in CAP treatment, improving patient management outcome and improve our understanding of the complex pathogen host interactions in pneumonia.

T3: "IDENTIFICATION OF DRUG-ABLE TARGETS FOR THE TREATMENT OF HAEMOGLOBINOPATHIES"

Hosting Department/Clinic:
Molecular Genetics Thalassaemia Department
(http://www.cing.ac.cy/easyconsole.cfm/id/349)

Contact Person:
Dr Marios Phylactides (mphylact@cing.ac.cy)

Abstract:
Pharmacological reactivation of the γ-globin genes for the production of foetal haemoglobin (HbF) is a very promising therapeutic approach for β-thalassaemia and SCD, as HbF can substitute for the defective or reduced/absent adult haemoglobin. The overall aim of the project is to investigate in detail a small number of proteins for their involvement in the regulation of γ-globin gene expression with the intention of identifying suitable pharmacological intervention targets.

In an earlier proteomics-based project we identified 192 proteins in human erythroid progenitor (HEP) cultures whose expression was significantly up- or down-regulated following HbF induction by decitabine. The specific aims of this project are:

1. knockdown studies to investigate whether the changes in the expression levels of some of these proteins are implicated in the induction of HbF. The gene with the strongest effect will be selected for in-depth functional analyses.
2. Expression of the gene of interest will be knocked down using lentiviral shRNA transduction in HEP and HUDEPII cells. The transcriptome of the cells will then be fully characterized using high throughput approaches (RNA seq) and genes whose expression is altered by the knock down will be identified.
3. lentiviral shRNA transduction will be used to study the effect of downregulating the top 10 genes on globin gene expression in HUDEPII (and HEP cells).
4. Identification of functional associations of the gene of interest and investigation of its genome-wide binding will be studied.

From this study novel potential pharmacological targets that can be manipulated to elevate HbF levels in patients can be identified.

T4: "SYSTEM APPROACHES TOWARDS A DISEASE RISK PREDICTION COMPUTATIONAL MODEL INTEGRATING MULTI-SOURCE INFORMATION"

Hosting Department/Clinic/Group:
Bioinformatics Group (http://www.cing.ac.cy/easyconsole.cfm/id/1356)

Contact Persons:
Dr George Spyrou (georges@cing.ac.cy)

Abstract:
There is a plethora of molecular data from various sources and of various types that can tell a piece of the disease story as far as the related mechanisms are concerned. It is necessary to integrate this information in a comprehensive computational model that will give better insights on the causal mechanisms and on the way we can provide a unified risk score related with a disease. Network-based and systems-based approaches will be applied on publicly available omic-data from various sources to integrate and finally derive the best feature combination for a machine learning scheme that will provide informed risk prediction. Finally, there will be a validation scheme and an optimization of the diagnostic method.

T1: "INVESTIGATING THE ROLE OF MKRN3 IN PREMATURE PUBERTY USING INDUCED PLURIPOTENT STEM CELLS (iPSC) TECHNOLOGY"

Hosting Department/Clinic/Group:
Molecular Genetics, Function & Therapy Department (http://www.cing.ac.cy/mgft/)

Contact Persons:
Prof. Leonidas A. Phylactou (laphylac@cing.ac.cy)
Dr Pavlos Fanis (pavlosf@cing.ac.cy)

Abstract:
The timing of puberty is influenced by genetic factors, many of which are still unknown. Premature puberty caused by early activation of the hypothalamic-pituitary-gonadal axis through the activation of the gonadotropin-releasing hormone (**GnRH**). This effect leads to increases in the secretion of gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary gland. Recently in our lab, one of the few labs worldwide, we identified loss-of-function mutations in the maternally imprinted MKRN3 gene which is associated with premature puberty1,2. The proposed research, for the first time, involves the systematic and comprehensive analysis of specific MKRN3 mutations. As human GnRH neuron cell lines are not available, in this project we aim to create GnRH-expressing neurons from induced Pluripotent Stem Cells (iPSC) generated by reprogramming dermal fibroblasts from patients bearing MKRN3 mutations. In the selected MKRN3 mutant GnRH neurons a series of molecular approaches will be used to characterize the mutations and unravel their role in molecular and cellular level. With these studies it is aimed to determine the level and stability of the GnRH and GnRH related factors at the protein and mRNA level. State-of-the-art approaches will be used in this study such as generation and differentiation of iPSC, advanced microscopy, molecular and biochemical techniques.

The ultimate goal of the proposed study is to delineate the exact role of MKRN3 that plays in premature puberty and to contribute to appropriate treatment therapies.

References:

1. Christoforidis, A. *et al.* A novel MKRN3 nonsense mutation causing familial central precocious puberty. *Endocrine*, doi:10.1007/s12020-017-1232-6 (2017).

2. Neocleous, V. *et al.* In silico analysis of a novel MKRN3 missense mutation in familial central precocious puberty. *Clinical endocrinology* 84, 80-84, doi:10.1111/cen.12854 (2016).

T2: "TRANSCRIPTOMIC/ PROTEOMIC APPROACHES IN IDENTIFYING DISEASE CAUSING MOLECULES AND POTENTIAL BIOMARKERS FOR ASCENDING THORACIC AORTIC ANEURYSMS"

Hosting Department/Clinic:
Department of Cardiovascular Genetics and the Laboratory of Forensic Genetic (http://www.cing.ac.cy/easyconsole.cfm/id/301)

Contact Persons:
Prof Marios Cariolou (cariolou@cing.ac.cy)
Dr Evy Bashiardes (evyb@cing.ac.cy)

Abstract:
The aorta is the largest blood vessel of the body, carrying oxygenated blood from the heart to the rest of the body. An increase in the diameter of the thoracic aorta is defined as a thoracic aortic aneurysm (TAA). An aneurysm develops and increases in size without any obvious clinical symptoms, until the diameter leads to a point where a dissection or a rupture occurs. Almost all of the patients do not have any symptoms before an event, such as dissection or rupture, occurs. A large percentage of patients die within the first 24 hours. It is therefore essential to be able to identify individuals who are prone to TAA development, in order to reduce mortalities. Although work on circulating biomarkers has been published with respect to TAAs, currently no established biomarker(s) exist(s) for management purposes.

The main aim of our project is continue to interrogate serum samples from TAA patients as well as excised aneurysmal tissue samples through transcriptomic and proteomic analysis. Any unique patterns of vascular-derived molecules will be identified and these will be further studied in the context of serving as sensitive and specific markers of TAA development.

The selected PhD candidate is expected to apply transcriptomics using Next Generation Sequencing technologies, as well as a range of proteomics techniques to identify specific biomarkers for TAA development.

T3: "GENOME-EDITING-MEDIATED LINEAGE-SPECIFIC KNOCKDOWN OF DISEASE MODIFIERS AS THERAPY OF B-GLOBINOPATHIES"

Hosting Department/Clinic:
Molecular Genetics Thalassaemia Department
(http://www.cing.ac.cy/easyconsole.cfm/id/349)

Contact Persons:
Dr Carsten Lederer (lederer@cing.ac.cy)

Abstract:
Elevated expression of the predominantly fetal γ-globin in adult erythrocytes is therapeutic for major β-globinopathies. Knockout of γ-globin repressor BCL11A corrects the disease phenotype in erythroid cells, but is unsuitable for clinical translation owing to side effects on differentiation and survival in other lineages.

The current study aims to achieve erythroid-specific deactivation of γ-globin repressors, targeting BCL11A as proof of principle. Key methods will be lentiviral delivery of CRISPR/Cas9-based designer nucleases and efficient site-specific integration of phosphorothioate oligonucleotides by non-homologous end joining. Establishment of effective sites, donor templates and nucleases will first be performed in adult-erythroid HUDEP-2 cells before experimentation in patient-derived primary erythroid cells to test for correction of disease parameters of β-globinopathies.

The project aims to produce patentable components, specifically the RGENs and artificial donor templates developed. Of note, through modular function of components, early achievement of the project can be built upon subsequently to speed up development of additional therapeutic targets and tools.

T1: "HSV1- OLIGODENDROCYTES INTERACTION - POSSIBLE ROLE ON DEMYELINATION IN MULTIPLE SCLEROSIS"

Hosting Department/Clinic:
Molecular Virology Department (http://www.cing.ac.cy/easyconsole.cfm/id/373)

Contact Person:
Dr Christina Christodoulou (cchristo@cing.ac.cy)

Abstract:
Several hypotheses have been formulated concerning the pathogenesis of Multiple Sclerosis

(MS) and especially the destruction of myeline (demyelination) from the axones of oligodendrocytes (OgDc). One of these, is the hypothesis of a viral infection which disrupt the production of cell proteins and trigger the inflammation and subsequently plaques formation. There are numerous reports on the possible correlation between infectious agents and MS. Considering that many demyelinating diseases have a viral etiology and multiple sclerosis is one of the most prominent demyelinating diseases, we would like to investigate the role of herpes viruses (highly neurotropic) in MS demyelination. We believe that oligodendrocytes, the myelin forming cells of the central nervous system, could be the target of herpes virus and its persistency. The hepresvirus may induce severe reduction of host cell proteins synthesis (shut-off), affecting the majority of the proteines synthesized within the infected oligodendrocyte and even stop protein production.

To investigate this hypothesis we propose studying the effect of herpes virus infection on two human oligodendroglial cell lines.

The target is to establish two persistently infected cell lines, analyze the multiplication of viruses and the proteins synthesis to determine viral influence on myelin synthesis. We shall also try to treat the persistence infection by eliminating the viral genome, from the cell genome and follow the recovery of proteins synthesis and the possibilities of remyelination.

Studying and understanding these mechanisms may prove to be crucial in developing therapeutic approaches by targeting the virus with the aim of stopping the shut-off and demyelination. This would lead to the re-initiation of cell protein production resulting in the synthesis of myelin and re-myelination after the elimination of the virus.

Methods to be used:
Cell culture, viral infection in vitro, DNA/RNA extraction, agarose gels, Southern blots, Northern Blots, Western Blots, sequencing, PCR, RT-PCR, qPCR, IFA, ELISA. Other molecular methods that the project could need for its realisation will be used upon necessity.

T2: "NETWORK-BASED ANALYSIS OF MULTIPLE-SOURCE INFORMATION TOWARDS EFFICIENT DRUG REPOSITIONING"

Hosting Department/Clinic/Group:
Bioinformatics Group (http://www.cing.ac.cy/easyconsole.cfm/id/1356)

Contact Persons:
Dr George Spyrou (georges@cing.ac.cy)

Abstract:
Drug repositioning is the discovery and use of existing drugs to diseases other than the ones that were initially applied. This is a cost-effective trend that can lead fast from the drug discovery to the patient. Computational drug repositioning requires molecular data such as expression profiles related to a disease phenotype. However, the input given to these algorithms is of major significance. This PhD is going to show that network-based analysis of multiple-source information is more efficient towards drug repositioning. Network-based and systems-based approaches will be applied on publicly available omic-data from various sources to conclude to a short list of candidate drugs that could pass to further experimental validation through proper assays and animal models. Furthermore, structural bioinformatics and chem-informatics will be used to conclude to natural compounds similar to the repurposed drugs.

T3: "THE TRANSMISSIBILITY HYPOTHESIS OF ALZHEIMER'S DISEASE"

Hosting Department/Clinic:
Neurology Clinic B (http://www.cing.ac.cy/easyconsole.cfm/id/229)

Contact Person:
Prof Savvas Papacostas (savvas@cing.ac.cy)
Dr Ioanna Kousiappa (ioannak@cing.ac.cy)

Abstract:
The purpose of this project will be to investigate β-amyloid transmissibility in animal models of AD and extrapolate results to humans by injecting brain homogenates taken from mutant APP transgenic (5XFAD) mice that express b-amyloid and have the phenotypic characteristics of the disease, into the brains of healthy mice. These injections will check for seeding of β-amyloid which resembles the transmission of the more aggressive prionopathies in whish transmissibility has been shown. Our hypothesis is based on a research article in 2012, where researchers have used transgenic mice and have noticed that the seeding of β-amyloid was more critical in the formation of aggregates rather than age (Hamaguchi et al., 2012) implying that the theory of prion-like transmissibility in AD could occur.

T4: "A STUDY OF PHARMACOLOGIC STIMULATION OF PHAGOCYTOSIS BY A FULL C5A RECEPTOR AGONIST IN A TRANSGENIC MODEL OF ALZHEIMER DISEASE"

Hosting Department/Clinic:
Neurology Clinic A (http://www.cing.ac.cy/easyconsole.cfm/id/159)

Contact Person:
Prof Theodoros Kyriakides (theodore@cing.ac.cy)
Dr Elena Panayiotou Worth (panagiot@cing.ac.cy)

Abstract:
According to the amyloid hypothesis of Alzheimer Disease the deposition of prefibrillar and fibrillar αβ peptide sets off pathogenic cascades of neuroinflammation and neurodegeration that lead to synaptic and neuronal loss and cognitive decline. Various approaches to reduce amyloid load by reducing prefibrillar production of αβ peptide (secretase inhibition) or enhance amyloid clearance (anti-αβ peptide antibody mediated clearance) has proven unsuccessful in clinical trials.

Complement C1Q has been found to modulate disease and in particular neuronal loss in the Alzheimer mouse model but its mechanism of action is controversial and has been called a double edged flower. C1Q has been shown to opsonize prefibrillar αβ peptide aggregates and facilitate phagocytosis yet complement activation leads to neuroinflammation and more amyloid deposition due to the production of cytokines in an "inflammatory environment". Macrophages and neutrophils carry C5a receptors. Animal experiments in the Alzheimer mouse model show that complement component C5a (a product of complement activation) receptor inhibition reduces amyloid and attenuates pathology.

In the ATTR neuropathy mouse, an animal model of transthyretin amyloidosis, C5a receptor inhibition exacerbates amyloid deposition while full C5a agonist administration significantly reduces amyloid deposition. Full C5a receptor agonists have not been tested in the Alzheimer model and we hypothesize, based on preliminary data, that in fact it will have a beneficial effect by clearing amyloid.

The project will entail administration of a full C5a agonist by mouth to a transgenic mouse model of Alzheimer disease. Thioflavin S, immunofluorescence, western blot and proteomic analyses as well as functional testing will be carried out to compare treated and untreated animals.

T5: "DEVELOPMENT OF GENE THERAPY FOR TREATING INHERITED NEUROPATHIES"

Hosting Department/Clinic:
Neurology Clinic E (http://www.cing.ac.cy/easyconsole.cfm/id/265)

Contact Person:
Prof Kleopas Kleopa (kleopa@cing.ac.cy)

Abstract:
Inherited neuropathies are among the commonest neurogenetic disorders causing chronic disability and remain incurable. Different cellular mechanisms and inheritance patterns have been discovered, either causing gain or loss of function of the affected gene. Gene therapy and neuroprotective approaches using viral vectors hold promise for gene replacement as well as for gene silencing. In this project we will develop novel lentiviral viral vectors to edit or silence overexpressing and dominant neuropathy genes causing some of the commonest forms of inherited neuropathy using in vitro and in vivo experimental disease models. These aims are based on delivery methods and cell targeting approaches developed in our lab and a variety of techniques, including molecular, biochemical, morphological, behavioral, and electrophysiological analysis. Previous background in cellular-molecular neuroscience, gene therapy, neurophysiology, peripheral nerve pathology, molecular virology, or molecular pathology and imaging techniques will be considered an advantage.

Key Recent Publications:

1. Kagiava A, Sargiannidou I, Theophilidis G, Karaiskos C, Richter J, Bashiardes S, Schiza N, Nearchou M, Christodoulou C, Scherer SS, Kleopa KA (2016). Intrathecal gene therapy rescues a model of demyelinating peripheral neuropathy. *Proc Natl Acad Sci U S A,* 113 (17):e2421-9
2. Sargiannidou I, Kagiava A, Bashiardes S, Richter J, Christodoulou C, Scherer SS, Kleopa KA (2015) Intraneural GJB1 gene delivery improves nerve pathology in a model of CMT1X. Annals of Neurology. 78:303-316.

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