THE CYPRUS INSTITUTE OF NEUROLOGY & GENETICS

PHD TOPICS

APPLICATION DEADLINE: 10/05/2024 12:00 NOON

ACADEMIC YEAR 2024-2025

Contents

Message from the Dean

I am pleased to announce the PhD Research Projects offered by the Cyprus Institute of Neurology and Genetics (CING) for 2024-25.

At the CING, we are committed to producing a high calibre research output that contributes to improving 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 curricula.

Our programmes aim to train and expose you to competitive research and a stimulating scientific environment. We will provide you with the knowledge and experience needed to enable you to cope with future demands and set you on a promising career path, considering how competitive the employment market has become. Our PhD graduates have successfully entered the labour market, acquiring positions in Cyprus and abroad.

As you explore science and learn with us, you will have many opportunities to make new friends and acquire life-long skills. You will meet dedicated and experienced scientists who will mentor and guide you. CING departments headed by highly accomplished scientists and doctors will host you. 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 problems, which benefit patients and our community. The COVID-19 pandemic showed us that we need to intensify our efforts to advance knowledge through scientific discovery and innovation. Join us in this quest and experience the exciting promise that molecular biology and genetics hold for advancing the frontiers of both science and medicine.

We designed this booklet to provide helpful information about the currently available PhD positions and topics, the hosting departments and the research supervisors. We are all here to assist you in developing critical thinking and accomplishing your tasks, to challenge and support you to prepare for a prosperous professional 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!

Prof Kyproula Christodoulou

PhD Topics

Deadline for PhD applications: 10/05/2024 (12noon, Cyprus Time)

T1: Mining the data landscape from single-cell omics to imaging and clinical data with advanced bioinformatics methods for biomarker discovery and repurposing.

Hosting Department/Clinic/Group/Unit
Bioinformatics Department (C-BIG)

Contact persons:
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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Abstract:
A pillar of the Precision Medicine vision is the provision of accurate prognosis, diagnosis, monitoring, risk assessment, prediction of treatment efficiency. Various types of biomarkers serve these purposes. A biomarker can be defined as a characteristic of a therapeutic response or a normal or pathological process that can be objectively and reproducibly assessed and measured. According to the World Health Organization (WHO) a biomarker is “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease”. However, biomarker discovery is a difficult task and currently is supported by advanced bioinformatics methods that analyze a large spectrum of existing data from single cell omics to signaling, imaging, and clinical data as well as data from the interaction with our environment (exposome). A step beyond the state-of-the-art in this matter is biomarker repurposing, that is to find new applications for already existing biomarkers. The wide data spectrum that is currently publicly available as well as specific data collections from biobanks and data from existing well-studied cohorts offers the opportunity and the challenge for multiscale and multi-source analysis towards building a comprehensive profile of the human organism in the condition under study. These challenges can be tackled with state-of-the-art computational methodologies and techniques, such as advanced data mining, modelling and simulation, network reconstruction and visualization, complex network analysis, data integration, machine learning/deep learning, text mining/semantics and association analysis. In this thesis, the PhD candidate will have the opportunity in collaboration with her/his supervisors and the rest of the Bioinformatics Department at CING to learn how to mine the data landscape from single-cell omics to imaging and clinical data and to develop or apply advanced bioinformatics methods for biomarker discovery and repurposing for a range of conditions. It is expected that the computational findings will be followed by a verification testing procedure and by a

small-scale experimental validation through our collaboration with other related CING Clinics and Labs.

Project plan (years 2, 3 & 4):
In year 2, extended data collection (including data from UKBiobank where we have licensed access) will take place, and computational analytics will define what is expected to be measured from normal samples from various omics levels (normomics) as well as from other data types to generate a reference framework regarding normal conditions. In year 3, advanced computational methods will be applied to integrate data, construct condition-related profiles, and perform network-rewiring/network differentiation studies to reveal simple and complex patterns of biomarkers, as well as to perform biomarker repurposing. In year 4, the highlighted sets of candidate biomarkers will be tested with advanced AI systems using machine learning/deep learning models to measure their possible contribution to computer-aided diagnosis/prognosis and monitoring systems. Our computational findings will be followed by a verification testing procedure and by a small-scale experimental validation through our collaboration with other related CING Clinics and Labs.

T2: Deciphering the genetic architecture of breast cancer

Hosting Department/Clinic/Group/Unit
Biostatistics Unit

Contact persons
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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time

Abstract:
Different variants have been identified to date that influence breast cancer susceptibility, and more than 200 breast cancer susceptibility regions have been identified through genome-wide association studies (GWAS). Although these variants mainly fall in non-coding regions, one challenge with GWAS is its potential to produce variant associations that might either be directly linked to the phenotype or merely due to genetic linkage (linkage disequilibrium, LD). Post-GWAS methodologies, with fine-mapping being prominent, combine regression analyses and functional experiments to differentiate true causal associations from LD correlations.

Project plan (years 2, 3 & 4):
Year 2: Analyses using different fine mapping tools and identification of the best-performing pipelines for real datasets.
Year 3: Identification of breast cancer-specific functional annotations/in silico tools that can complement the fine mapping efforts.
Year 4: Final fine mapping analyses and identification of candidate causal variants in each of the loci.

T3: The use of multi-omics for understanding pathological commonalities between malignant melanoma and skin psoriasis towards discovery and development of drug targets and therapeutic agents respectively

Hosting Department/Clinic/Group/Unit
Cancer Genetics, Therapeutics & Ultrastructural Pathology (CGTUP)

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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time

Abstract:
Psoriasis and melanoma are the two major skin diseases in which their underlying pathological mechanisms have not yet been fully elucidated. In both diseases, their characteristic pathological feature is excessive cellular proliferation suggesting a certain degree of commonality between them. The aim of this project is to explore cellular proliferation further as a common pathological pathway and its relation to apoptotic deregulation as well as modulation of immune microenvironment and epigenetic response pathway(s). For this reason, multi-omics approaches will be used to identify synergies between these cellular pathways as potentially common drug targets for therapeutic action. To these ends, in vitro and reconstituted tissue models of psoriasis and malignant melanoma will be utilized with or without treatment with current common clinical and/or experimental therapeutic agents (e.g., cell cycle regulators, immune and epigenetic modulators, etc.,). Last, but not least, drug repurposing will be used as a research strategy where both computational and experimental work will highlight drugs already approved for other diseases as possible candidates for melanoma and psoriasis. In this context, advanced computational drug repurposing methods will be applied to construct a shortlist of repurposed drugs (that will be further validated experimentally) against these skin disease models regarding their toxicity and efficacy profiles. Finally, natural compounds (similar to the shortlist of the repurposed drugs) will be tested against these major skin diseases.

Project plan (years 2, 3 & 4):
In year 2, during the first half, a detailed experimental approach will be followed to utilize the in vitro and reconstituted tissue models of psoriasis and malignant melanoma with or without treatment with current common clinical and/or experimental therapeutic agents. During the second half, multi-omics approaches will be used to identify synergies between the above-mentioned cellular pathways as potentially common drug targets for therapeutic action. In year 3, advanced computational drug repurposing methods will be applied to construct a shortlist of repurposed drugs (that

will be further validated experimentally) against these skin disease models regarding their toxicity and efficacy profiles. In year 4, various natural compounds (similar to the shortlist of the repurposed drugs) will be computationally and experimentally tested against these major skin diseases.

T4: Charcot-Marie-Tooth type 1 biomarkers discovery

Hosting Department/Clinic/Group/Unit
Neurogenetics

Contact persons
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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Abstract:
Charcot Marie Tooth (CMT) is the most common inherited peripheral neuropathy, with a prevalence of 1 per 2.500 individuals, and it is characterized by a variable age of onset and a variable phenotype. CMT is grouped into demyelinating (CMT1), axonal (CMT2) and intermediate (CMTI) forms according to electrophysiological and pathological findings and inheritance can be autosomal dominant, X-linked or autosomal recessive. Pathogenic variants in more than 80 genes have thus far been associated with CMT. Due to the large number of rare diseases and the difficulty in diagnosing them early, developing specific disease biomarkers is crucial for providing accurate and early diagnoses and treatments to patients, which is essential for improving patient care. Effective biomarkers can also be a powerful tool for monitoring disease progression.
This project aims to discover possible CMT1 microRNA-based biomarkers for CMT1 patients with a PMP22 pathogenic variant. More specifically, through this project, CMT1 patients with a PMP22 mutation will be recruited, and blood will be collected. Total RNA will be extracted for miRNA profile using high throughput NGS techniques. Possible differential miRNA will be selected using bioinformatic data analysis for further validation.

Project Plan for years 2, 3 and 4:
In the 2nd year, consenting patients and controls will be recruited, blood will be collected, and total RNA will be extracted. Then, Next Generation sequencing (NGS) based miRNA analysis of CMT patients and matched controls will be performed.
During year 3, data analysis will be employed to compare CMT patient and matched control groups, and differentially expressed miRNAs will be selected through computational analysis.
In year 4, validation of the selected miRNAs in additional CMT1 patient and control samples will result in discovering possible biomarker(s).

T5: Gene therapy for Charcot-Marie-Tooth Type 4D

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

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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Abstract:
Charcot-Marie-Tooth Type 4D (CMT4D) is a rare, recessively inherited severe childhood onset demyelinating neuropathy, characterized by distal muscle weakness and atrophy, foot deformities, and sensory loss affecting all modalities. It is caused by loss-of-function mutations in N-myc downstream-regulated gene-1 (NDRG-1) gene. Although *NDRG1* is ubiquitously expressed and has been proposed to play a role in growth arrest and cell differentiation, possibly as a signaling protein shuttling between the cytoplasm and the nucleus, a particular high expression level is detected in Schwann cells and peripheral neuropathy remains the main manifestation of the disease. There is currently no effective treatment for CMT4D. Therefore, we propose to develop a gene replacement therapy by replacing the human *NDRG1* gene specifically in Schwann cells throughout the peripheral nervous system. This approach will be tested at first in the *Ndrg1* knockout mouse model of the disease. A cell-specific myelin protein zero (MPZ) promoter will be used in order to target expression of the human *NDRG1* coding sequence in myelinating Schwann cells. The stretcher mouse model, with total Ndrg1 deficiency which displays normal initial myelination and a transition to overt pathology between weeks 3 and 5 will be used. Overall, it represents an authentic model of CMT4D that recapitulates the major pathological aspects of the disease and provides the opportunity to test therapeutic approaches. The AAV9 viral vector, which is currently used in clinical trials for other disorders, will be used to deliver the gene by the clinically translatable route of lumbar intrathecal injection. The level of pathology rescue will be evaluated both following pre- as well as with post-onset intervention by motor behavioral, electrophysiological, and morphological analysis 2 months after treatment, in order to provide a proof of principle for clinical translation.

T6: Longitudinal, prospective, case-control study for the investigation and integration of the epidemiology, risk factors and multi-omics landscape of abdominal aortic aneurysms for risk stratification in the Greek Cypriot population

Hosting Department
Department of Cardiovascular Genetics & The Laboratory of Forensic Genetics

Primary Supervisor and contact person:
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Collaborators:
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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Abstract:
Abdominal Aortic Aneurysms (AAA) constitute a silent clinical condition of the vascular system where the abdominal aorta undergoes gradual dilatation and if it evades detection can lead to rupture with internal haemorrhage in the affected adult population. The condition has a progressive nature with the need for surgical intervention when the aneurysm approaches or exceeds 5.5 cm in diameter (depending on other risk factors) in order to avoid the catastrophic sequelae of rupture once it exceeds this threshold. Based on the European Society of Cardiology (Vol. 18, 2020), there is a 4-6 times higher prevalence in men than women, and the risk of disease increases after 60 years old whereas for women it develops approximately 10 years later. Prominent risk factors for AAA development are male gender, smoking, hypertension, inflammation of the aortic wall and atherosclerosis, while risk factors for AAA rupture include female gender, arterial blood pressure, smoking, reduced forced expiratory volume, and aneurysm diameter >5cm and >5.5cm for females and males, respectively. Two main types of such aneurysms exist, i.e., saccular and fusiform, with saccular being eccentric (involving only a portion of the vessel) and fusiform being concentric (involving the entire circumference of the vessel wall). Saccular aneurysms are less common but may lead to rupture (thus requiring urgent surgical intervention), whereas fusiform aneurysms, which can result from layer injury or atherosclerosis, are more frequent but with lower rapture rates. In countries where there are no national screening programmes the majority of patients with AAA are identified when examined for other medical reasons. There is therefore a need to identify biomarkers for

diagnostic and prognostic purposes that can be used to identify AAA patients and subsequently stratify them into low and high risk/slow and fast progression of the aortic wall expansion in order to allow for timely elective surgical intervention. In Cyprus there is a lack of screening programmes for AAA patients, as well as poor characterisation of risk factors. Preliminary data indicate that about 100 individuals are admitted to the Nicosia General Hospital Vascular Department for surgical intervention annually with about 10% requiring emergency surgical intervention. This project will for the first time explore the epidemiology, clinical risk factors, and microRNA signatures of AAAs in the Cypriot population (of Greek-Cypriot ancestry), where there is an indication for a significant AAA prevalence. A longitudinal, prospective case-control study will be initiated through a collaboration between The Cyprus Institute of Neurology & Genetics and the Nicosia General Hospital's Department of Vascular Surgery. After informed consent from eligible patients, blood samples will be collected at the annual scheduled visits of patients to the Department of Vascular Surgery along with clinical data to allow time series experiments. Using Next Generation Sequencing, real-time PCR and bioinformatic tools, miRNA signatures will be assessed to identify differentially expressed miRNAs between patients and age and sex matched controls as well as differences between patients that progress faster than others and culminate in surgery earlier. Through the data evaluation, other correlations will be sought and quantified for their effect that may be of clinical significance such as gender, the impact of other comorbidities including hypertension and metabolic disorders such as hypercholesteroleamia and diabetes.

Key objectives of the programme:

1) Identify lifestyle risk factors through questionnaires
2) Identify other comorbidities that adversely influence the outcome of the disease
3) Identify differentially expressed blood-borne miRNA biomarkers that can be exploited as easily accessible surrogate markers of the presence of AAA (diagnostic biomarkers)
4) Identify differentially expressed blood-borne miRNA biomarkers that can be exploited as easily accessible surrogate markers of the stage of the disease and outcome (prognostic biomarkers)
5) Integrate data from steps 1-4 to formulate a composite risk stratification profile (miRNA signature and other variables such as 3D morphology and comorbidities)
6) Integrate data from steps 1-4 with other omics data that will be generated simultaneously by other team members from the same patient and control blood samples (whole exome sequencing data, differential DNA methylation and metabolomics data) in order to set the foundations for the design of a population specific risk assessment model for development and progression and need for surgical intervention.

Project Plan:

Year 1:

1) Attend MG101 and other courses
2) Prepare inclusion and exclusion criteria for participants
3) Prepare information leaflet for participants
4) Prepare questionnaires
5) Prepare a database for the study data collection (demographic, clinical, miRNA)

Years 1-3:

6) Recruit participants (100 cases and 50 age and sex matched controls), completed consent forms, questionnaires and blood samples, update database.

Years 2-3:

7) Extract miRNA and sequence using Next Generation Sequencing.
8) Analyze data to identify differentially expressed miRNAs between cases and controls, and fast and slow progressing patients (stratified within 100 cases).
9) Validation using RT-PCR.

Year 3:

10) Integrate data to formulate a composite risk stratification profile (miRNA signature and other variables.

Year 4:

11) Prepare manuscript on findings from step 10.
12) Integrate data from step 10 with other omics data that will be generated simultaneously by other team members from the same patient and control blood samples (whole exome sequencing data, differential DNA methylation and metabolomics data) in order to set the foundations for the design of a population specific risk assessment model for development and progression and need for surgical intervention.
13) Prepare manuscript on findings from step 12.
14) Prepare PhD thesis.

T7: Developing efficient genetic tools for the therapy of Myotonic Dystrophy

Hosting Department/Clinic/Group/Unit
Molecular Genetics Function and Therapy Department

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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Abstract:
Genetic therapies in muscle has several challenges, one of which is the high abundance of tissue in the body and the high heterogeneity of the various types. In Myotonic Dystrophy type I (DM1), several muscles are being affected and only those approaches with efficient protocols will result in efficient treatments. Delivering short genetic sequences (DNA and RNA oligonucleotides) is one of the possible routes which might eventually treat the disease. DM1 is the most common of the group of muscular dystrophies in adults and the cause is an amplification of the CTG triplet repeat in the 3' UTR of the DMPK gene. In other words, the pathogenesis is developed at the RNA level and not in the protein, itself. The mutant RNA molecules are trapped in the cell nucleus and from there onwards, a cascade of molecular events leads to the complex pathology of the disease. Small antisense oligonucleotides against the mutant transcripts have shown promise towards an efficient therapy of the disease. More development, however, is needed, before these agents enter the clinical practice.

Our laboratory has long worked and specialized in the field of RNA, including the design and application of antisense oligonucleotides for therapeutic and other purposes. Several successful projects, including PhD student projects have been completed in this area over the years, in our laboratory with a good number of high-quality publications.

The project aims to develop oligonucleotides against novel targets which are implicated in the disease pathway and at the same time, it aims to improve their delivery to muscle through a novel approach.

In order to achieve this, several approaches will be followed aiming at making oligonucleotides more efficient in terms of delivery and targeting key molecules which are implicated in the disease pathway. State-of-the-art molecular biology and cell

biology technologies, which have been already mastered in our laboratory, will be used towards this direction.

This is a novel project with a high impact aiming to enhance the efficiency of genetic tools against DM1.

Relevant biobliography from our laboratory

1. Philippou S, Mastroyiannopoulos NP, Tomazou M, Oulas A, Ackers-Johnson M, Foo RS, Spyrou GM, **Phylactou LA.** Selective Delivery to Cardiac Muscle Cells Using Cell-Specific Aptamers. Pharmaceuticals (Basel). 2023 Sep 6;16(9):1264.

2. Koutsoulidou A, Koutalianos D, Georgiou K, Kakouri AC, Oulas A, Tomazou M, Kyriakides TC, Roos A, Papadimas GK, Papadopoulos C, Kararizou E, Spyrou GM, Zamba Papanicolaou E, Lochmüller H, Phylactou LA. Serum miRNAs as biomarkers for the rare types of muscular dystrophy. **Neuromuscul Disord**. 2022 Mar 11; S0960-8966(22)00067-0. doi: 10.1016/j.nmd.2022.03.003

3. Georgiadou M, Christou M, Sokratous K, Wengel J, Michailidou K, Kyriacou K, Koutsoulidou A, Mastroyiannopoulos NP, Phylactou LA. Intramuscular evaluation of chimeric Locked Nucleic Acid/2´OMethyl-modified antisense oligonucleotides for targeted exon 23 skipping in mdx mice. **Pharmaceuticals (Basel)**. 2021 Oct 30;14(11):1113. doi: 10.3390/ph14111113.

4. Koutalianos D, Koutsoulidou A, Mytidou C, Kakouri AC, Oulas A, Tomazou M, Kyriakides TC, Prokopi M, Kapnisis K, Nikolenko N, Turner C, Lusakowska A, Janiszewska K, Papadimas GK, Papadopoulos C, Kararizou E, Spyrou GM, Gourdon G, Zamba Papanicolaou E, Gorman G, Anayiotos A, Lochmüller H, Phylactou LA. miR-223-3p and miR-24 as novel serum-based biomarkers for Myotonic Dystrophy type 1. **Mol Ther Methods Clin Dev.** 2021 Sep 14; 23:169-183. doi: 10.1016/j.omtm.2021.09.007. eCollection 2021 Dec 10.

5. Christou M, Wengel J, Sokratous K, Kyriacou K, Nikolaou G, Phylactou L.A. and Mastroyiannopoulos N.P. Systemic evaluation of chimeric LNA/2'OMe steric blockers for DM1 therapy. **Nucleic Acid Ther**. 2019 Dec 23. doi: 10.1089/nat.2019.0811. [Epub ahead of print].

6. Styliana Philippou, Nikolaos P. Mastroyiannopoulos, Neoklis Makrides, Carsten W Lederer, Marina Kleanthous, Leonidas A. Phylactou. Selection and identification of skeletal muscle-targeted RNA aptamers. **Molecular Therapy-Nucleic Acids.** 2018 Mar2;10:199-214. Doi: 10.1016/j.omtn.2017.12.004. Epub 2017 Dec 9.

7. Koutsoulidou A, Photiades, M, Kyriakides TC, Georgiou K, Prokopi M, Kapnisis K, Lusakowska A, Nearchou M, Christou Y, Papadimas GK, Anayiotos A, Kyriakou K, Kararizou E, Zamba Papanicolaou E, Phylactou, LA. Identification of Exosomal Muscle-Specific miRNAs in Serum of Myotonic Dystrophy Patients Relating to Muscle Disease Progress. **Hum Mol Genet.** 2017 Sep 1;26(17):3285-3302. doi: 10.1093/hmg/ddx212. [Epub 2017 Jun 16].

8. Demetris Koutalianos, Andrie Koutsoulidou, Nikolaos P. Mastroyiannopoulos, Denis Furling, and Leonidas A. Phylactou. MyoD transcription factor induces myogenesis by inhibiting Twist-1 through miR-206. **J Cell Sci.** 2015 Oct 1;128(19):3631-45. doi: 10.1242/jcs.172288. Epub 2015 Aug 13. **Editorial** about this article appeared in: In This Issue: MyoD gets rid of Twist-1 with miR-206. **J Cell Sci.** 2015 128:e1905

9. Andrie Koutsoulidou, Tassos Kyriakides, Yiolanda Christou, Eleni Zamba Papanicolaou, Leonidas A. Phylactou. Elevated muscle-specific miRNAs in serum of myotonic dystrophy patients relate to disease progress. **PLoS ONE.** 2015 Apr 27;10(4):e0125341.

10. Antonis Antoniou, Nikolaos P. Mastroyiannopoulos, James B. Uney, Leonidas A. Phylactou. miR-186 inhibits muscle cell differentiation through myogenin regulation. **J Biol Chem.** 2014 Feb 14;289(7):3923-35. doi: 10.1074/jbc.M113.507343. Epub 2014 Jan 2.

11. Mastroyiannopoulos NP, Nicolaou P, Anayasa M, Uney JB, Phylactou LA. Down-regulation of Myogenin can reverse terminal muscle cell differentiation. **PLoS ONE** 2012; 7(1): e29896. doi:10.1371/journal.pone.0029896

12. Koutsoulidou A, Mastroyiannopoulos NP, Furling D, Uney JB, Phylactou LA. Endogenous TWIST expression and differentiation are opposite during human muscle development. **Muscle Nerve.** 2011 Dec;44(6):984-6.

13. Koutsoulidou A, Mastroyiannopoulos NP, Furling D, Uney JB, Phylactou LA. Expression of miR-1, miR-133a, miR-133b and miR-206 increases during development of human skeletal muscle. **BMC Dev Biol**. 2011 Jun 7;11:34. PubMed PMID: 21645416; PubMed Central PMCID: PMC3132729.

14. Mastroyiannopoulos NP, Chrysanthou E, Kyriakides TC, Uney JB, Mahadevan MS, Phylactou LA. The effect of myotonic dystrophy transcript levels and location on muscle differentiation. **Biochem Biophys Res Commun**. 2008 Dec 12;377(2):526-31. Epub 2008 Oct 16. PubMed PMID: 18930030.

15. Mastroyiannopoulos NP, Feldman ML, Uney JB, Mahadevan MS, Phylactou LA. Woodchuck post-transcriptional element induces nuclear export of myotonic dystrophy 3' untranslated region transcripts. **EMBO Rep**. 2005 May;6(5):458-63. PubMed PMID: 15832171; PubMed Central PMCID: PMC1299300.

16. Phylactou LA, Darrah C, Wood MJ. Ribozyme-mediated trans-splicing of a trinucleotide repeat. **Nat Genet**. 1998 Apr;18(4):378-81. PubMed PMID: 9537423. **Editorial** about this article appeared in: a). Wunch Hannah. Engineering a change for myotonic dystrophy. **Lancet** (351), 1036, 1998. b). John J Rossi. Ribozymes to the Rescue: repairing genetically defective mRNAs. **Trends in Genetics**, 14(8) 295-298, 1998.

Project Plan for years 2, 3 and 4

The project aims to develop novel oligonucleotides for enhanced delivery in the muscle and in parallel aims to target key molecules implicated in the pathogenesis of the disease.

At the first stages of the project, design will take place in order to produce novel genetic sequences with higher delivery properties. In parallel, different genetic sequences (e.g. antisense oligonucleotides, antagomiRs) will be designed to interfere with the disease pathway through interaction with key molecules. As a next step, the novel sequences will be tested first in patients' cells (*in vitro*). The efficiency and biodistribution will be determined by various molecular biology and cell biology techniques, which are mastered in our laboratory. The final stages of the project will involve testing and analyzing the efficacy of these novel sequences in experiment animal DM1 mice *(in vivo)*. The assessment will be carried out in a great detail, through molecular, cellular and physiology methodologies.

T8: Metabolites and the beneficial effect of the Mediterranean Diet in Cypriot ATTR amyloidosis patients

Hosting Department/Clinic/Group/Unit
Neuroepidemiology Department

Contact persons:
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The topic is eligible for the following Program(s):
PhD in Molecular Medicine, Full Time
PhD in Medical Genetics, Full Time
PhD in Neuroscience, Full Time

Additional Requirement: Proficiency in both spoken and written Greek and English languages.

Abstract:
Transthyretin amyloidosis (ATTR) is a rare polyneuropathy, inherited, and progressive disease caused by the build-up of amyloid fibrils made up of the protein transthyretin, which damage nerves and other tissues. The mutation found in the Cypriot population is the Val30Met mutation. Although, there is a single mutation in our population there is variety in the age of onset in patients, some patients develop disease onset before 50 years of age and others after 50 years of age. However, the reason for this variability remains unknown.

The following project will include ATTR carriers and ATTR patients, along with their gender-age, matched controls. Carriers, patients and controls upon signing an informed consent form, demographic and medical history data, and a validated Food Frequency Questionnaire (FFQ) will be administered and a blood sample will also be collected.

Metabolomics are small molecules and the intermediate end-products of metabolism. Metabolomics is the comprehensive measurement of metabolites within a biological fluid or tissue, organ or cell. Untargeted metabolomics, will be used to investigate as many metabolites within serum of ATTR carriers, patients and gender-age matched controls.

Year 2: Recruitment of ATTR carriers, patients and gender-age matched controls, demographic, lifestyle, medical history and a validated Food Frequency Questionnaire (FFQ) to determine the dietary intake of all study participants. A blood sample will be processed using a standard a protocol, following this serum and plasma will be collected and stored at -80°C until time of metabolomic analysis.

Year 3: Data entry of all data collected from participants (Demographics, lifestyle and FFQ). Untargeted metabolomics analysis will be performed using serum obtained from all study participants. Metabolomics data will be analysed to determine the statistically significant metabolites for i) ATTR carriers vs Controls and ii) ATTR patients vs Controls. Additionally, bioinformatics analysis will be performed using established tools to identify pathways which are over-represented/under-represented between ATTR carrier's vs Controls, and ATTR patient's vs Controls.

Year 4: Statistical analysis will be performed on the demographic data and dietary intake in regards to macro and micronutrient intake to identify statically significant associations between carriers, patients and controls. The Mediterranean Diet (MD) Adherence Score such as the Panagiotakos MD adherence score will be used to asses which groups, i) carriers vs controls, ii) patients vs controls and iii) carriers vs patients' adhere to the MD.

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