THE CYPRUS INSTITUTE OF
NEUROLOGY & GENETICS

PHD TOPICS

APPLICATION DEADLINE: 10/05/22 12:00 NOON

ACADEMIC YEAR 2022-2023

Further to the approvals received from the relevant authorities, as of September 2022, the Cyprus School of Molecular Medicine (CSMM), will undergo a change in name and will be known as the postgraduate School of The Cyprus Institute of Neurology & Genetics. All programmes and operational information will remain the same. All degrees from September 2022 onwards, will be issued under the name "The Cyprus Institute of Neurology & Genetics".

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Message from the CSMM Dean

Dear Prospective PhD Candidates,

I am pleased to announce the PhD Research Projects offered by the postgraduate School of the Cyprus Institute of Neurology and Genetics (CING) for 2021-2022.

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 programs 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 graduate PhD students 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 present pandemic shows 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 Chistodoulou

Deadline for PhD applications: May 10th, 2022 (12pm, Cyprus Time).

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

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

T1: Exploring the therapeutic potential of current and experimental epigenetic drug compounds in human malignant melanoma

Hosting Department/Clinic/Group:
Cancer Genetics, Therapeutics & Ultrastructural Pathology
(https://www.cing.ac.cy/easyconsole.cfm?id=334)

Contact Persons:
Prof Michail Panagiotidis - Principal Supervisor (mihalisp@cing.ac.cy)
Dr Ioannis Anestopoulos - Co-Supervisor (ioannisa@cing.ac.cy)
Dr Sotiris Kyriakou - Co-Supervisor (sotirisk@cing.ac.cy)

Abstract:
Only recently the epigenetic response pathway(s) have been identified as key determinants capable of modulating health as well as disease outcomes. Deficiency to maintain the normal epigenetic state of cells results in a deregulated gene expression profile involved in signalling cascades leading to disease development. Thus, an extensive deregulation of normal epigenetic marks (beyond genetic alterations) can be related with disease onset and progression, including carcinogenesis. Given the well-established association between epigenetic modifications and alterations in gene expression, together with the reversible nature of such modifications, it becomes apparent that they have attracted major pharmaceutical interest as potential therapeutic targets. In the context of the proposed project, various epigenetic drug compounds will be screened (as single and/or combinational therapeutic protocols) for their ability to induce different epigenetic responses ranging from inhibition of aberrantly expressed DNA methyltransferases to histone deacetylases, methyl transferases, etc. For this reason, we will utilize a cell line-based model consisting of malignant melanoma, non-melanoma epidermoid carcinoma, normal keratinocyte cell lines and a validated 3D reconstituted human melanoma tissue model (both of which will constitute our *in vitro* model of human malignant melanoma). A wide range of methodologies will be utilized in an attempt to delineate the mechanistic basis of how these drug compounds modulate/reverse the aberrant epigenetic landscape and thus exert their therapeutic potency. In our opinion, given the unmet need for more efficient therapeutic means in malignant melanoma, such experimental approaches can form the basis of preclinical studies towards their translation to the clinical setting.

Project plan (years 2, 3 & 4):
In year 2, a detailed experimental approach will be followed including toxicokinetic analyses of various epigenetic drug compounds (in single and/or combinational treatments) in an in vitro model of cell line-based and a 3D reconstituted human malignant melanoma (stated previously) in order to develop a clinically relevant and

validated therapeutic protocol. Moreover, any observed anti-melanoma activity will be also evaluated in normal human keratinocyte cells, with the aim to optimize in vitro model of human malignant melanoma our experimental approach(es) against any potential side effects induced by the action of these compounds. In year 3, the experimental plan will aim to identify the major pathways of epigenetic drugs-induced cell death (apoptosis, autophagy, etc.,) involved as part of their anti-melanoma potency. To this end, key genes and proteins will be identified (by genomic and proteomic methodologies, respectively) as critical cell death modulators and consequently as potential biomarkers of therapeutic outcome. Moreover, we will aim to identify specific epigenetic modifications [i.e., altered DNA methylation patterns, histone lysine methylation(s), acetylation(s), etc.], with the potential to modulate the expression of genes and/or proteins towards their therapeutic action. In year 4, a more detailed approach will be employed (chromatin immunoprecipitation assays, etc.) with the scope to delineate the underlying molecular mechanism(s) by which epigenetic drug compounds act on their target molecule(s), thereby interfering with specific marks of the epigenome and thus modulating the epigenetic response in a manner compatible with their anti-melanoma potential.

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

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

T2: The potential role of DNA methylation in Thoracic Aortic Aneurysms

Hosting Department/Clinic/Group/Unit:
Cardiovascular Genetics and the Laboratory of Forensic Genetics
https://www.cing.ac.cy/easyconsole.cfm?id=310

Contact Persons:
Dr Anna Keravnou (annak@cing.ac.cy)

Abstract:
Thoracic Aortic Aneurysm and Dissection (TAA/D) represents a potentially lethal disease group characterized by an increased risk of dissection or rupture of the aorta. Although, both genetic and environmental risk factors have been implicated in TAA formation, the precise genetic markers involved and the factors influencing their expression remain an area of ongoing investigation. Epigenetics refers to modifications of the genome independent of changes in the primary DNA sequence and includes DNA methylation, histone modifications and non-coding RNAs. Several lines of evidence strongly support a causative role of epigenetic DNA methylation modifications in the pathogenesis of TAA however, there is currently very limited information regarding the role of epigenetic control in TAAs.

This study aims to enhance and further understand the role of DNA methylation and gene expression in TAAs which is crucial for understanding its pathogenesis, as the molecular mechanisms and pathways underlying TAAs remain obscured. The overall objective of this project is to compare genome-wide DNA methylation of aortic tissue from non-TAA control and TAA samples to identify new epigenetic markers and target genes associated with TAAs. The identification of epigenetic markers and several candidate genes for TAA may lead to novel tools for better prognosis, early diagnosis and treatment of patients with TAA.

Project plan (years 2, 3 & 4):

Year 2: Genome-wide DNA methylation analysis between TAA and non-TAA control tissue samples

To examine the role of DNA methylation in TAA, whole-genome bisulfite sequencing (WGBS) analysis of aortic tissues resected from patients will be carried out and compared with non-TAA control samples. Pairwise comparison of global DNA methylation will detect strong differences in methylation patterns between the two groups. The investigation of genome-wide DNA methylation patterns will provide new

epigenetic markers for TAA. WGBS approach will identify altered methylation in CpG and non-CpG sites throughout the genome. These results will reveal the diverse roles of DNA methylation at single-base resolution throughout the genome, reflecting the epigenetic variations in patients with TAA.

Validation of differentially methylated regions

To further confirm the reliability of the methylomic results, a validation step for the selected genes that are hypomethylated or hypermethylated in TAAs compared to non-TAA control will be performed in the two sample groups, using bisulfite-based quantitative Real Time PCR (qRT-PCR).

Year 3 and 4: Functional annotation and enrichment analysis

A set of genes with concordant DNA methylation pattern between the two groups (hypermethylated in TAAs and hypomethylated in non-TAA controls or vice versa) will be analysed through gene and pathway enrichment analysis in order to better understand the biological implications of alterations in DNA methylation with TAA. For this purpose, in-silico tools will be used to provide functional predictions and pathway networks for methylated or unmethylated candidate genes. To analyze the interaction between differentially methylated genes, a protein interaction network analysis of candidate genes will be performed on protein-protein interaction networks, indicating groups of genes that are interacting with each other.

Gene expression analysis of potential candidate genes

In order to determine whether the methylated/unmethylated genes, may show a specific pattern of differential expression, the expression levels of the mRNAs from these genes will be examined. The identification of genes whose expression levels may be significantly correlated, positively or negatively, with DNA methylation levels will be considered, revealing candidate genes associate with aortic aneurysm development. Measurement of candidate gene expression based on the results of gene methylation will be performed by qRT-PCR.

Protein expression and localization within aortic tissues

Immunofluorescence analysis will be performed in order to ensure that the differential gene activity obtained between TAA and non-TAA controls is applicable at the site of aneurysm in the aortic wall. Immunofluorescence analysis will be performed on formalin-fixed paraffin-embedded aortic tissue to visualize expression and localization of differentially expressed proteins via ZEISS fluorescence microscope.

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

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

T3: Classification of Variants of Uncertain clinical Significance (VUS) in BRCA1 and BRCA2 genes

Hosting Department/Clinic/Group/Unit:

Biostatistics Unit https://www.cing.ac.cy/en/about-us/biostatistics_unit

Contact Persons:

Dr Kyriaki Michailidou (kyriakimi@cing.ac.cy).

In collaboration with Prof. Amanda Spurdle (QIMR Berghofer Medical Research Institute) and Prof. Paul James (Melbourne University)

Abstract:

Clinical genetic testing for hereditary breast and ovarian cancers often result to the identification of variants of uncertain clinical significance (VUS). Individuals carrying VUS cannot benefit from cancer risk reducing interventions or targeted therapies and thus constitute a significant burden to patients and their families. For the classification of variants in the *BRCA1* and *BRCA2* genes, a multifactorial model has been developed and used, primarily by the ENIGMA consortium (https://enigmaconsortium.org/). More recently the standards and guidelines, proposed by the American College for Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology (AMP) groups have been implemented and are widely used to aid the classification of variants identified. Strength levels (very strong, strong, moderate and supporting) are assigned to independent lines of evidence for or against variant pathogenicity. These strengths are then used in a scoring system to provide a clinical class, expressed as pathogenic, likely pathogenic, likely benign, benign, or VUS. These guidelines integrate various sources of information including variant's nature, variant's prevalence in affected individuals and controls, data from functional studies, disease co-segregation within families, family history, co-inheritance of the VUS with a known pathogenic variant, bioinformatics prediction, pathology and other data. With this project we aim to evaluate the applicability of different sources of evidence for the classification of variants using the multifactorial likelihood model and also the point system by the ACMG/AMP for variants identified in the *BRCA1* and *BRCA2* genes.

Project plan (years 2, 3 & 4):

We are going to evaluate the different ACMG/AMP criteria and compare them with the multifactorial probability model results, both by simulated datasets and real data from collaborators of the ENIGMA consortium. In the first instance the different rules will be explored and simulated data will be created using statistical and other software, to follow different real datasets and specific scenarios. The successful candidate will work in an international team and collaborative projects. We are going to evaluate the ACMG rules and by using simulated data and real data and identify where these rules are not applicable for the specific genes of interest.

Furthermore, we are going to evaluate these rules in comparison to the so-called "moderate risk" or "hypomorphic" variants so that we can provide recommendations

for evaluation of these variants and their use in clinical practise. These variants although they are risk associated they do not confer risk to the carriers in the same levels as the classical pathogenic variants and thus are more difficult to identify and a lot of times are classified as VUS. The different rules will be explored in order to identify their behaviour when such a variant is identified.

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

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

T4: Metabolites and the role of the Mediterranean Diet in Cypriot FAP patients

Hosting Department/Clinic/Group/Unit:
Neuroepidemiology Department
https://www.cing.ac.cy/en/about-us/clinical-sciences/ncd

Contact Persons:
Dr. Eleni Zamba Papanicolaou ezamba@cing.ac.cy
Dr Christiana Christodoulou christianachr@cing.ac.cy

Abstract:
Familial amyloidosis polyneuropathy (FAP) is an autosomal dominant inherited disease, there are more than 120 mutations in the Transthyretin (TTR) gene. Common manifestations of the disease include peripheral axonal neuropathy and/or cardiomyopathy. Untargeted metabolomics analysis allows for detection of various metabolite classes within a biological fluid. Metabolomics is being utilized to identify metabolites or novel metabolites in different diseases. Additionally, the Mediterranean Diet (MD) is well-characterized for its anti-oxidant, neuro-protective and anti-inflammatory properties in providing protection to neurons from inflammation and reactive oxygen species (ROS).
The aim of this PhD research study is to obtain blood samples from 60 FAP carriers and patients and 60 gender-age matched controls. Serum will then be collected and stored for untargeted metabolomics analysis, which will be performed using Liquid-Chromatography-Mass Spectrometry (LC-MS). Furthermore, Food Frequency Questionnaires will be obtained from all study participants to evaluated dietary intake and the effect of the MD in delaying disease onset and possibly improving symptoms. Statistical Analysis and Bioinformatics tools and databases will be utilized to identify the differentially abundant metabolites between FAP carriers and controls, FAP patients and controls and between FAP carriers and patients.

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

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

T5: Computational investigation of cell-to-cell communication networks and signalling mechanisms inferred by single cell omics

Hosting Department/Clinic/Group/Unit:

Bioinformatics Department
https://www.cing.ac.cy/easyconsole.cfm?id=1358

Contact Persons:

Prof George Spyrou (georges@cing.ac.cy)
Dr Anastasis Oulas (anastasioso@cing.ac.cy)
Dr Margarita Zachariou (margaritaz@cing.ac.cy)
Dr George Minadakis (georgem@cing.ac.cy)
Prof Mihalis Panayiotides (mihalisp@cing.ac.cy)

Abstract:

Cell-to-cell communication networks have critical roles in coordinating diverse processes, such as tissue development, immune cell response, disease initiation and/or progression. However, these communication networks are not so well understood since there is already a great complexity in the intracellular signaling networks that defines a great part of the intercellular communication. In parallel, the development of high throughput single-cell sequencing technologies has made it cost-effective to profile thousands of cells from diverse samples containing multiple cell types. We need to be able to study how these different cell types work and communicate together, to predict and visualize cell-to-cell communication networks from single-cell omics data. Through this project we will work on publicly available omics data to reconstruct cell-to-cell communication networks, analyze them in terms of finding the most influential nodes as well as studying the information flow between them, highlighting differences in intercellular communication when profiling given cell types under different conditions. We have strong interest on cell-to-cell communication in conditions like neurodegeneration, infectious diseases and cancer as well as the interplay between some of them. The project includes also a small experimental part for the validation of some of our findings and verification of the computational methods that will be developed.

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

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

T6: Identification and investigation of neurodegenerative disease pathogenic variants through NGS approaches

Hosting Department/Clinic/Group/Unit:
Neurogenetics Department

https://www.cing.ac.cy/easyconsole.cfm?id=370

Contact Persons:
Dr Christina Votsi (votsi@cing.ac.cy)
Prof Kyproula Christodoulou (roula@cing.ac.cy)

Abstract:
Hereditary cerebellar ataxias (HCA) and hereditary spastic paraplegias (HSP) comprise two groups of genetically and clinically heterogeneous neurodegenerative disorders with more than 70 genes and loci reported for each group. Considerable phenotypic overlap between them has also been described, thus defining a new concept known as spastic ataxia (SA) and making their genetic diagnosis more challenging. Several families and sporadic patients with ataxia, spastic paraplegia and spastic ataxia exist in the Cypriot population and have been studied for many years. Recent advancements in genetic testing such as next generation sequencing (NGS), enabled the genetic diagnosis for some of them. However, many cases have been excluded for known mutations and repeat expansions and remain undiagnosed. Moreover, novel pathogenic variants that have been recently identified and are pending functional investigation also exist. The project will focus on identifying and characterizing novel and/or known genes/mutations. Molecularly undiagnosed families and sporadic patients will be analyzed using NGS methods and maybe an additional technique such as array genotyping for the needs of linkage analysis. Bioinformatics tools will be employed for the analysis of NGS derived data. Novel and/or known genes/variants are expected to be identified through this analysis, and molecular genetic studies will enable their verification. Further functional studies using transcriptomics, proteomics techniques and/or in vitro modeling, will enable the characterization of one or more of the verified variants and lead to new knowledge on the disease pathogenetic mechanism.

Project Plan for years 2, 3 and 4

Year 2: The project will begin with the NGS analysis [either whole-genome sequencing (WGS) or whole-exome sequencing (WES)] of selected families and/or family trios of sporadic patients. More specifically, families who have been already analyzed by WES and pathogenic variants in coding regions have not been detected, should be investigated by WGS and linkage analysis towards the identification of a possible deep intronic variant or a novel repeat expansion. Family trios of sporadic cases and/or

families not investigated by WES before should be initially analyzed by WES. In parallel, other published disease repeat expansions which have not yet been tested, should be tested. In addition, according to the clinical features of the patients, gene-targeted exon deletion/duplication testing by the use of the MLPA technique could be performed if there is an available assay.

Year 3: NGS derived data will be analyzed to prioritize candidate variants followed by segregation analysis with Sanger sequencing.

Year 4: Functional studies on one or more verified variants will be performed. If no such variants are identified through the project, alternatively, analysis on one already identified variant pending further functional characterization will be performed.

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

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

T7: Redefining balanced structural rearrangement breakpoints using long-read sequencing

Hosting Department/Clinic/Group/Unit:

Cytogenetics and Genomics Department
https://www.cing.ac.cy/easyconsole.cfm?id=322

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

Dr Athina Theodosiou (athinat@cing.ac.cy)

Abstract:
Apparently - balanced chromosomal rearrangements (ABCRs) present great challenges in terms of detection and interpretation. Each case is unique and accurate mapping of breakpoints can offer additional insights into the molecular mechanisms underlying phenotype presentation in ABCR carriers as well as the generation of these structural variants (SVs). Clinical consequences depend on the type and nature of each SV, while different rearrangements occur by different mechanisms. The major challenge is resolving complex ABCRs that can lead to severe phenotypic consequences. The breakpoints of ABCRs are traditionally defined by conventional methodologies such as chromosomal analysis or Fluorescense *In Situ* Hybdidization (FISH). However, the resolution of these technologies does not allow for accurate breakpoint predictions and may fail to decipher the underlying molecular mechanisms. In the last years, next-generation sequencing (NGS) has enabled researchers to detect and resolve simple and complex chromosomal rearrangements at the base-pair level. Specifically, whole-genome sequencing (WGS) data can universally detect all types of SVs including deletions, insertions, tandem duplications, inversions, and translocations. The Department of Cytogenetics and Genomics has previously applied low-coverage whole-genome mate-pair sequencing (WG-MPS) and identified additional complexity, cryptic imbalances as well as other molecular mechanisms underlying phenotype presentation in ABCR carriers.
The main aim of this PhD project is to delineate ABCR breakpoints at the base-pair level mainly in affected and non-affected carriers of complex chromosomal rearrangements. Since the previously used WG-MPS library preparation kit from Illumina has been discontinued, alternative long-read WGS library preparation

approaches will be initially sought that are suitable for simple and complex ABCR detection and characterization. WGS data from samples carrying simple or complex ABCRs will be analyzed, validated and further interpreted in order to associate specific ABCRs with the clinical phenotypes. Ultimately, the data produced during this project will help decipher the mechanisms and consequences of rare and poorly understood chromosomally determined disorders. Comparison of data derived from testing affected and non-affected individuals will provide valuable information regarding the impact of the precise genomic location of each breakpoint. The research outcome will be exploited after the completion of the project, in order to unravel the full potential of the newly produced data.

Project Plan for years 2, 3 and 4

Year 2

During year 2, the possibility of applying alternative methodologies to resolve ABCRs will be explored. For example, there is an emerging Transposase Enzyme Linked Long-read Sequencing (TELL-Seq™) technology, recently developed by Universal Sequencing Technology (UST), which enables short-read sequencers to produce super long-read results (average 20kb to 100kb). Alternatively, third generation long-read sequencing technologies, such as Nanopore sequencing by Oxford Nanopore Technologies, will be considered as these offer new opportunities for in-depth investigation of SV breakpoints and overcome most of the sequencing challenges currently faced by short-read sequencing approaches. While the TELL-Seq approach could be implemented on site using high-throughput sequencing platforms available locally, the Nanopore sequencing (or other third generation NGS) approach would have to be investigated through a collaboration with a suitable sequencing provider abroad.

Year 3

WGS data produced during year 2 from samples carrying simple or complex ABCRs will be analysed, with the help of a bioinformatician, and further interpreted by the PhD student. Rearrangement breakpoints as well as potential single nucleotide variants identified in candidate disease genes will be validated using conventional PCR and Sanger sequencing. A thorough investigation of all possible disease mechanisms as well as mechanisms underlying the generation of simple and complex ABCRs will be performed.

Year 4

Genotype-phenotype associations will be performed by functional studies and the results will be gathered, published in peer-reviewed scientific journals and presented in scientific conferences. As the PhD student maybe on a part-time basis, the research activities planned for years 2-4 may be extended to year 5.

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

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

T8: Identification of Extracellular Vesicles as biomarkers for abnormal puberty

Hosting Department/Clinic/Group/Unit:
Molecular Genetics, Function and Therapy
https://www.cing.ac.cy/easyconsole.cfm?id=346

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

Abstract:

Puberty is a period of notable physical and psychological development that results in sexual maturation. These changes are under the control of the hypothalamic-pituitary-gonadal (HPG) axis, which controls puberty period and reproduction and is highly regulated by both excitatory and inhibitory hormonal factors. When the secretion of these hormonal factors exceeds the levels that are normally expected during the pubertal period this leads to the pathological state of Precocious Puberty. On the contrary, when these hormonal levels fall beyond the expected levels the pathological condition of Delayed Puberty is observed. Precocious puberty refers to the pubertal onset before the age of 8 in girls and the age of 9 in boys, while delayed puberty is defined by the absence or incomplete pubertal onset by the age of 13 in girls and the age of 14 in boys. The timing of puberty is associated with significant biological, psychosocial and long-term health implications, thus making a pubertal disorder due to abnormal onset a severe condition. Despite the strong heritability of pubertal timing, our understanding of the underlying molecular and genetic mechanisms is limited. Last decade, a major discovery was that secreted extracellular vesicles (EVs) communicate between mammalian cells or tissues by carrying molecules, such as proteins and small noncoding regulatory RNAs (sncRNAs). In vitro experiments showed that sncRNAs were found in EVs in a stable form as a cargo and that the EVs function as mediators of exchanging molecular information between different tissues.

Since puberty is a paracrine process, the aim of this project is to identify EVs and characterize their content in patients with pubertal disorders. Initially, EVs will be isolated from patient serum samples and their sncRNA content will be analysed by Next Generation Sequencing. Using bioinformatic analyses a pubertal disease sncRNA profile will be generated. Emerging key factors of puberty development will be further evaluated by immunostaining, real time PCR and immunoblot analyses. In addition,

isolated EVs will be analysed for their type by state of the art methods such as size exclusion chromatography, Tunable Resistive Pulse Sensing (TRPS) and Electron microscopy followed by analysis of their content.

Extracellular vesicle profiles will provide the first evidence concerning the extracellular communication in the pathophysiology of puberty. Moreover, for the first time, an attempt will be made to identify pubertal biomarkers in the blood serum that would hold prognostic/diagnostic potential for puberty and pubertal disorders. A successful completion of this project will be beneficial for patients with pubertal disorders.

Related publications from our department

1. Neocleous V, Fanis P, Toumba M, Gorka B, Kousiappa I, Tanteles GA, et al. Pathogenic and Low-Frequency Variants in Children With Central Precocious Puberty. Frontiers in endocrinology (2021) 12:745048. Epub 2021/10/12. doi: 10.3389/fendo.2021.745048. PubMed PMID: 34630334; PubMed Central PMCID: PMC8498594.

2. Neocleous V, Fanis P, Toumba M, Tanteles GA, Schiza M, Cinarli F, et al. GnRH Deficient Patients With Congenital Hypogonadotropic Hypogonadism: Novel Genetic Findings in ANOS1, RNF216, WDR11, FGFR1, CHD7, and POLR3A Genes in a Case Series and Review of the Literature. Frontiers in endocrinology (2020) 11:626. Epub 2020/09/29. doi: 10.3389/fendo.2020.00626. PubMed PMID: 32982993; PubMed Central PMCID: PMC7485345.

3. Koutsoulidou A, Phylactou LA. Circulating Biomarkers in Muscular Dystrophies: Disease and Therapy Monitoring. Molecular therapy Methods & clinical development (2020) 18:230-9. Epub 2020/07/09. doi: 10.1016/j.omtm.2020.05.017. PubMed PMID: 32637452; PubMed Central PMCID: PMC7327849.

4. Mytidou C, Koutsoulidou A, Katsioloudi A, Prokopi M, Kapnisis K, Michailidou K, et al. Muscle-derived exosomes encapsulate myomiRs and are involved in local skeletal muscle tissue communication. FASEB journal : official publication of the Federation of American Societies for Experimental Biology (2021) 35(2):e21279. Epub 2021/01/24. doi: 10.1096/fj.201902468RR. PubMed PMID: 33484211.

Project Plan for years 2, 3 and 4:

<u>2nd year</u>

- Collection and isolation of extracellular vesicles from patient serum samples.
- Isolation of small noncoding RNAs from isolated EVs
- Transcriptomic analysis by NGS followed by bioinformatic analyses.

<u>3rd year</u>

- Validation of initial results by real-time PCR and immunoblot.
- Isolated EVs will be analysed for their type by size exclusion chromatography, TRPS and Electron microscopy.

<u>4th year</u>

- Analysis of the content of EVs by real-time PCR and immunoblot.
- Writing and submission of manuscript.

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