CYPRUS SOCIETY OF HUMAN GENETICS

ABSTRACT BOOKLET

March 17th & 18th, 2023

9th International Conference of the Cyprus Society of Human Genetics, Learning Resource Center "Stelios Ioannou"

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Welcome Address

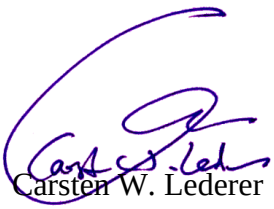
Dear CSHG members, colleagues and friends,

I am delighted to be able to welcome you to the 9th International Conference of the Cyprus Society of Human Genetics (CSHG) at the University of Cyprus New Campus in Nicosia.

As the major scientific event for human genetics in Cyprus, our conference has a wide scope and a lot to deliver this year during an exciting time of rapid progress in our field. In two full days of lectures and discussion on 17th and 18th March, the event touches on cutting-edge developments in biobanking and open-data science, advanced therapies, cancer biology, medical and reproductive genetics, diagnostics, biomarkers and disease mechanisms. Consequently, we will look at the evolving role of human genetics in medicine and society in a discussion of bioethics, patient and counselling viewpoints, and have a strong representation of young scientists and of fresh perspectives in our poster session and selected abstract presentations, and with two early-stage researchers among our international invited speakers.

Some of the current scientific, technological and conceptional advances in human genetics have already begun to shape our lives today, and we have the privilege to be an active part of that process. I hope you will find our conference with its formal lectures, networking breaks and conference dinner a perfect opportunity to forge new collaborations, to gain new insights and inspiration for your own work, and to help us take our field forward.

I thank my colleagues of the CSHG Executive Board for their hard work in making this event possible, our Scientific Committee for their diligent evaluation of abstracts and posters, our sponsors for allowing us an unprecedented number of international in-person lectures, and all of our speakers, poster presenters and participants for contributing to the diversity and excitement of the event.



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Conference Programme

CYPRUS SOCIETY OF HUMAN GENETICS

9th CSHG International Conference

17 & 18 March, 2023

Learning Resource Center "Stelios Ioannou"

University of Cyprus

Friday, March 17th

8:30		Registration & Coffee
		Opening
9:00		Welcome Address CSHG-*Carsten W. Lederer, President*
9:10		Welcome Address Ministry of Health -*Christina Yiannaki, Permanent Secretary*
9:20	**Keynote Lecture**	[art] x [science] -***Marisa Satsia***
	Session 1	**Biobanking and Open-data Science** *Chairs: Harris Stefanou & Gregory Papagregoriou*
10:00	Invited Lecture	Biobanking requirements for onco-genetics *Karine Sargsyan, Cedars Sinai Los Angeles US, Medical University of Graz*
10:30	Invited Lecture	Pan-European electronic patient registries for rare haematological diseases *Petros Kountouris, Cyprus Institute of Neurology & Genetics*
10:50	Special Announcement	Local Exhibition: Women of Mathematics from around the World: a Gallery of Portraits" *Margarita Zachariou, Cyprus Institute of Neurology & Genetics*
10:55		*Coffee Break*
11:15	Invited Lecture	Capitalizing on a National Biological Repository to Characterize the Cyprus Genome *Paul A. Costeas, Karaiskakio Foundation*
11:35	Invited Lecture	Biobanking and next generation biomedical research: Quō vādis *Constantinos Deltas, University of Cyprus, School of Medicine and Center of Excellence in Biobanking and Biomedical Research, biobank.cy*
11:55	Selected Abstract	Characterization of microRNA's differential expression during puberty in female mice *Maria Morrou, Cyprus Institute of Neurology & Genetics*
12:05	Selected Abstract	Genetic analysis of inherited cardiac diseases in Cypriot patients *Despina Vangeli, University of Cyprus*
12:20		Round-table Discussion-*All session speakers*
12:45		Lunch Break

	Session 2	**Cancer Diagnosis, Mechanisms & Therapies** *Chairs: Alexia Eliades & Kyriaki Michailidou*
14:05	Invited Lecture	Surprising targets for CAR T cell therapy *Sébastien Wälchli, Oslo University Hospital*
14:35	Invited Lecture	Exploring the heterogeneity of neuroblastoma and other childhood cancers *Kirsti Marie Gjersvoll Paulsen, Oslo University Hospital*
15:05	Invited Lecture	From CRISPR-Cas9 drop-out and enrichment screens to novel therapeutic approaches *Vassilia Tamamouna, The Center for the Study of Haematological and other Malignancies*
15:25	Selected Abstract	Large scale case-control analyses of BRCA1 and BRCA2 *Denise G. O'Mahony, Cyprus Institute of Neurology & Genetics*
15:38	Selected Abstract	Evaluation of the differences in the breast cancer polygenic risk score distribution in individuals from different European ancestry populations *Kristia Yiangou, Cyprus Institute of Neurology & Genetics*
15:50	Selected Abstract	Evaluation of the spatiotemporal intratumor molecular heterogeneity in tumor and liquid biopsies from patients with metastatic colorectal cancer *Alexia Eliades, Medicover Genetics*
16:00		Coffee Break & Posters
	Session 3	**Therapies for Inherited Disorders** *Chairs: Carsten W Lederer & Sébastien Wälchli*
17:00	Invited Lecture	Gene therapies for CMT inherited neuropathies: are we getting closer? *Kleopas Kleopa, Cyprus Institute of Neurology & Genetics*
17:20	Invited Lecture	Genome editing for founder diseases: Lesson from DADA2 *Pavel Kopcil, Centre for Molecular Medicine Norway*
17:50	Selected Abstract	Genome editing for beta-haemoglobinopathies without double-strand DNA cleavage *Nikoletta Papaioannou, Cyprus Institute of Neurology & Genetics*
18:02	Selected Abstract	Preclinical validation of HBBIVSI-110(G>A)-specific gene editing as advanced therapy for thalassemia *Petros Patsali, Cyprus Institute of Neurology & Genetics*
18:14	Selected Abstract	A novel hotspot ATP1A1 variant and its functional evaluation in a demyelinating Charcot-Marie-Tooth patient *Feride Cinarli Yuksel, Cyprus Institute of Neurology & Genetics*
20:00		Conference Dinner_Palia Ilektriki

Saturday, March 18th

8:30		Registration & Coffee
	Session 4	**Counselling & Bioethics** *Chairs: Violetta Anastasiadou & Thessalia Papasavva*
9:00	Invited Lecture	The Concept of Genetic Privacy *Georgia Charalambidou, University of Cyprus, Center of Excellence in Biobanking and Biomedical Research, biobank.cy*

9:20	Invited Lecture	Genetic counselling; A hybrid profession *Elena Spanou, Cyprus Institute of Neurology & Genetics*
9:40	Invited Lecture	The patient's perspective on a chronic, genetic condition. *Miltos Miltiadous, Cyprus Thalassaemia Association*
10:00	Invited Lecture	Is it time to revisit the written informed consent? Marios Cariolou, *Cyprus Institute of Neurology & Genetics*
10:20		Round-table Discussion
10:50		Coffee Break
	Session 5	**Medical & Reproductive Genetics** *Chairs: Panayiotis Myrianthopoulos & Elena Kypri*
11:10	Invited Lecture	Universal PGT: achievements and challenges *Eftychia Dimitriadou, KU Leuven*
11:40	Invited Lecture	Clinical application, safety, and efficacy of Day 4 biopsy for preimplantation genetic testing. A retrospective cohort analysis. *George Liperis, University of Sydney*
12:00	Invited Lecture	Targeted exome sequencing in fetuses with ultrasound findings: a powerfull tool in prenatal diagnosis *Vasiliki Chini, Intergenetics by Medicover*
12:30	Selected Abstract	A novel SMN1 splicing variant disrupts the expression of the functional SMN1 transcript and expands the spectrum of the SMN1 conventional variants *Christina Votsi, Cyprus Institute of Neurology & Genetics*
12:42	Selected Abstract	Fetal genetic factors associated with sonographic abnormalities and pregnancy loss *Andrea Hadjipanteli, Cyprus Institute of Neurology & Genetics*
12:55	Selected Abstract	Expanding the prenatal phenotype of WT1 related disorder *Sofia Ourani, Archbishop Makarios III Hospital*
13:10		Lunch Break
14:10	Platinum Sponsor Presentation	Sponsored talk -*Elias Chris Tzortzatos, Lab Supplies*
14:30	Platinum Sponsor Presentation	Implementing precision oncology through next generation sequencing within the clinical practice. *Gisncarlo Pruneri, Fondazione IRCCS Instituto Nazionale Tumori*
	Session 6	**Novel Biomarkers & Mechanistic Insights** *Chairs: George Koumbaris & Marios Phylactides*
14:50	Invited Lecture	The metastatic spread of breast cancer accelerates during sleep *Zoi Diamantopoulou, Swiss Federal Institute of Technology (ETH) Zurich*
15:20	Invited Lecture	Temporal dynamics of metastatic breast cancer lung colonization *Panos Papageorgis, European University Cyprus*
15:40	Invited Lecture	The genomics of Breast Cancer *Vessela N. Kristensen, Oslo University Hospital*

16:10	Invited Lecture	Gaining insights for candidate biomarkers, candidate repurposed drugs and disease-related mechanisms though network-based bioinformatics approaches *George Spyrou, Cyprus Institute of Neurology & Genetics*
16:30	Selected Abstract	Muscle-specific miRNAs as potential monitoring biomarkers of muscle wasting progression in DM1 *Kristia Georgiou, Cyprus Institute of Neurology & Genetics*
16:42	Selected Abstract	Investigation of the role of anoctamin 10 protein in cell division *Androniki Chrysanthou, Cyprus Institute of Neurology & Genetics*
		Closing
16:55		Announcement of Best Poster and Best Selected Abstract awards; CSHG/ESHG award
17:10		CSHG announcements (Achievements, Acknowledgements and Good-byes)

Abstracts

Invited Lectures

Biobanking requirements for onco-genetics

Author Names
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Pan-European electronic patient registries for rare haematological diseases

Author Names
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Molecular Genetics Thalassaemia Department, The Cyprus Institute of Neurology & Genetics, Nicosia, Cyprus

Abstract
Rare haematological diseases (RHDs) represent a highly heterogeneous group of oncological and non-oncological conditions, often causing chronic health problems or even death. Their diagnosis, management and treatment require multidisciplinary teams and a lot of resources, representing a public health challenge. Like other rare diseases, it is often challenging to bring together sufficient data regarding patients affected by RHDs and, therefore, a European approach is needed. However, the lack of uniform standards for data collection has led to the implementation of many patient registries through different approaches, gathering non-comparable and fragmented data, thus hampering collaborative research and generation of robust evidence for the translation of results into clinical practice.

The European Rare Blood Disorders Platform (ENROL) and the Rare Anaemia Disorders European Epidemiological Platform (RADeep) are recently stablished pan-European patient registries for RHDs and rare anaemias, respectively. Their aim is to promote basic and clinical research on RHDs at the EU level, while securing the patient's rights in agreement with EU General Data Protection Regulation (GDPR). They are built in line with the recommendations of the EU Rare Disease Platform. The registries are implemented ensuring interoperability with existing platforms, aligned with the FAIR principles, through the use of international ontologies (e.g., ORDO, HPO, ICD) and the implementation of a pseudonymisation tool.

The standardisation in data collection enable and support other research projects requiring the collection or use of clinical data, such as GenoMed4All, IMPACT-AML, and INHERENT. The goal is to promote data-driven healthcare and provide the evidence required to enhance clinical trials, guidelines and health policies for RHDs, which will allow better provision of healthcare to RHD patients across the Europe.

Capitalizing on a National Biological Repository to Characterize the Cyprus Genome

Author Names
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Karaiskakio Foundation

Abstract
High-throughput DNA sequencing has become a central feature of studies in human disease and clinical diagnostics as the information available linking diseases becomes more prevalent and precise. Although most diseases are prevalent in most populations, the variants impacting the function of the underlying molecular mechanisms of the disease may be different and driven by alternative genetic variants that are population specific. Thus, it is essential to be aware of population-specific variants and their frequency in a study population to improve the design of studies that attempt to understand the causality of genetic diseases in the population. This requires extensive databases of variants and phenotypic data to act as reference points for variant identification and research across populations. The creation of population-specific variome databases is an essential resource for the study of inherited diseases across populations and can provide key insights into disease susceptibility and responses The history of the Mediterranean island of Cyprus is complex and characterised by several periods of prolonged occupation and large-scale immigration from European and Middle-Eastern countries since the byzantine era Such a heterogenous genetic background has implications for the application of clinical research and diagnostics in the Cypriot population which are based on the currently available reference genomes. Therefore, there is a need for a genome variant database specific to the Cypriot population. Our study involves the generation of a Cypriot variant genome based on whole exome sequencing of 10,000 healthy Cypriot individuals and comparisons to the human reference genome (hg19). The cohort for this study comprised of 10,000 healthy bone marrow volunteer donors registered in the Cyprus Bone Marrow Donor registry. Exome library preparation from 10 independent pool samples was performed using Agilent's SureSelect Human All Exon V8 probe (for whole exome sequencing) and were sequenced on a NextSeq 2000 sequencing platform. The variant data from the bioinformatic analysis has been included in an IGV platform and made available for public use as a website at www.cyprusgenome.org.

Biobanking and next generation biomedical research: Quō vādis

Author Names
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Abstract
The first Biobank in Cyprus was founded in November 2011, through EU and national funding of a Strategic infrastructure project. While initial biobanking on a disease focused basis was well on its way, it was after October 2019 that general population biobanking was substantially enhanced and empowered through the CY-Biobank EU funded project (Grant Agreement No. 857122). Building on the previous infrastructure, we established high quality biobanking and upgraded the prospects for next generation biomedical research in Cyprus. The material archived is a unique treasure waiting to be mined and explored by researchers in the fields of genetics/genomics, genetic epidemiology, clinical trials, metabolomics, etc. Particularly exciting is the preparation of the 1000 Cypriot human genome, which for the first time is providing worthwhile and trusty information on the details and secrets of the Cypriot DNA at population level, associated with deep phenotyping. The findings and conclusions to radiate by diving into its secrets, will enable us to improve our procedures for the diagnosis, prognosis and prevention of diseases. This progress is pushing the frontiers of the Cyprus genetics and biomedical field beyond our expectations and is creating brand new capabilities and responsibilities to the current and the next generation of researchers.

Surprising targets for CAR T cell therapy

Author Names
Sébastien Wälchli
Author Affiliations
Oslo University Hospital

Abstract
Immunotherapy-based Adoptive Cell Transfer (ACT) relies on the transfer of immune cells (effector cells, mainly T cells or NK cells) modified to express a therapeutic receptor specific for cancer. More precisely, the receptor can recognize any molecule (protein, sugar) strictly exposed on the surface of malignant cells. This costly clinical procedure depends on the modification of effector cells ex vivo followed by their injection back to the patients. These enhanced therapeutic cells are referred to as “living drugs”. Different types of molecules have been tested in patients and, to date, the most clinically efficient is the Chimeric Antigen Receptor (CAR). CAR therapy against the common B-cell marker CD19 accounts for the majority of the trials and can be considered as an undeniable success against hard-to-beat cancers. However, recent reports highlighted a relapse level of up to 50% mainly due to antigen loss, thus there is still a need to identify novel targets. I will present several surprising targets that could well replace or be combined with the usual suspects that have mainly been exploited so far.

Exploring the heterogeneity of neuroblastoma and other childhood cancers

Author Names
Kirsti Marie Gjersvoll Paulsen
Author Affiliations
Oslo University Hospital

Abstract
The outcome of a childhood cancer diagnosis varies greatly. It ranges from spontaneous regression without intervention to aggressive and therapy resistant disease that inevitably causes death. Neuroblastoma (NBL) is the most common extracranial solid tumour in children. It is an embryonal tumour, starting its development in immature sympathetic nerve cells. NBL is an extremely heterogeneous cancer: Patients with tumours classified as 'low risk' have a survival rate of about 90%, but for patients with 'high risk' tumours, event-free survival is reduced to 40-50%. Further, 'high risk' patients with relapse have a survival rate of only 8%. The varied outcome is reflected by the diverse biology and genetic heterogeneity within and between childhood cancers. Clearly we still do not know enough about the origin, progression, therapy resistance and relapse triggers of these diseases. In our research group, we focus on various sequencing technologies to explore the genetic heterogeneity, to better understand the different characteristics and phases of neuroblastoma and other childhood cancers.

From CRISPR-Cas9 drop-out and enrichment screens to novel therapeutic approaches

Author Names
Vassilia Tamamouna
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The Center for the Study of Haematological and other Malignancies

Abstract
The study of gene function is essentially the business of deciphering the relationship between genotype and phenotype. In experimental studies, one way to understand this relationship is to introduce a genetic mutation and study its consequences in cells, organs or whole organisms. This is reverse genetics, and it can be applied very widely, because you can introduce whichever mutation you like in whichever context you like and then describe its consequences. Sometimes, however, you are interested in phenotypes and have a burning desire to find out what if their genetic basis and this is forward genetics. Usually, forward genetics is more like a search, and an excellent way to search for the genetic basis of a phenotype is to perform a genetic screen. In a genetic screen, you introduce mutations and look for the phenotype. The new kid on the block is CRISPR-Cas9, which has changed the way we approach gene editing, providing precision that was never seen before. In our laboratory, we have established CRISPR-Cas9 technology, and we are using it on a daily basis to study different types of cancer. For example, an important hurdle to identifying genes that represent good therapeutic targets is the lack of knowledge about possible clinical toxicities associated with their inhibition. One way to reduce the likelihood of toxicities is to target genetic vulnerabilities that are specifically associated with particular cancer driver mutations. Whilst it is theoretically possible to gain such insights by comparing the vulnerabilities of cancer cell lines with and without certain mutations, the large numbers of additional mutations in such lines are prohibitive of this approach, as is the fact that some important mutations are rarely seen in cell lines. We utilize our powerful genome-editing CRISPR-Cas9 platform in a systematic manner to create an inventory of novel TP53-mutant AML-specific genetic dependencies that can serve as targets for innovative new therapies. In our laboratory, we are also

interested in studying the different hallmarks of cancer. For example, we are studying the ability of the Basal-Like Breast Cancer (BLBC) tumor cells to escape from the steady state of tumor dormancy and become metastatic. Investigation of breast cancer cell migration and lung infiltrating cancer cell escape from dormancy will elucidate the regulatory mechanisms of numerous pathways and genes that are associated with cancer. Using a combination of genetic and genomic methodologies, the key signalling events that induce breast to lung metastasis will be identified.

Gene therapies for CMT inherited neuropathies: are we getting closer?

Author Names

Kleopas Kleopa

Author Affiliations

Department of Neuroscience and Center for Neuromuscular Disorders, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Abstract

Charcot-Marie-Tooth (CMT) inherited neuropathies are genetically heterogeneous disorders caused by variable molecular-genetic alterations in neurons and their axons, or in Schwann cells and the myelin sheath they form. CMT-associated mutations lead to either loss of function or toxic gain of function cellular mechanisms. In order to treat the most common demyelinating CMT neuropathies we have developed gene replacement or gene silencing approaches targeted to myelinating Schwann cells. Lumbar intrathecal injection of AAV vectors in CMT neuropathy models resulted in widespread biodistribution and therapeutic gene expression throughout the PNS. To treat CMT1A, the commonest CMT type, caused by PMP22 gene duplication and gene-dosage effect, we developed a microRNA-based gene silencing approach. Delivery of microRNA by AAV9 in a mouse model overexpressing the human PMP22 gene both at early as well as late stages of the disease efficiently silenced PMP22 expression in PNS tissues, leading to functional and morphological phenotypic improvements. For the second most common CMT type, X-linked CMT1 resulting from Schwann cell-autonomous loss of function of the gap junction protein Cx32, we delivered the GJB1 gene in different knockout and transgenic mouse models of the disease resulting in restoration of Cx32 expression in Schwann cells. Pre- and post-onset gene replacement therapy led to improved motor function, nerve conduction velocities, and nerve pathology. Likewise, replacement of the SH3TC2 gene associated with CMT4C resulted in functional and morphological improvement in a model of CMT4C neuropathy. Clinically relevant, treatment-responsive blood biomarkers have been validated across different neuropathy models. Our studies provide proof of concept for the therapeutic potential of gene replacement or gene silencing therapies to treat inherited demyelinating neuropathies.

Genome editing for founder diseases: Lesson from DADA2

Author Names
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Centre for Molecular Medicine Norway

Abstract
Primary Immune Deficiency Diseases (PIDDs) are represented by various genotypes and phenotypes complicating diagnostic and therapeutic intervention. Although very rare on its own, combined prevalence are putting them on a spotlight as a suitable candidate for a promising novel gene therapy employing CRISPR/Cas9-based techniques. Here, we present our editing pipeline established recently to tackle patient specific mutation correction and to model and to improve the precise editing in wild type peripheral and mononuclear cells (PBMCs) and in cord blood derived CD34+ hematopoietic stem and progenitor cells (HSPCs).

Deficiency of adenosine deaminase 2 (DADA2) is a PIDD caused by numerous mutations spanning across ADA2 gene region. In our presented work, we focused on one most prevalent mutation in Scandinavian countries, R169Q. We showed our pipeline is functional in the context of healthy donor and patient derived PBMCs in which we can achieve over 50% HDR without any detectable off-targets and reaching up to 80% HDR after improved editing pipeline using NHEJ inhibitor. Similarly, we employed our pipeline to healthy donor HSPCs, where we subjected edited HSPCs to colony forming unit assay (CFU) and performed transplantation experiments with NSG mouse model (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ). We ruled out negative effect of editing on HSPCs differentiation potential and behaviour in vivo. To conclude, our editing pipeline is a universal and applicable for both PBMCs and HSPCs precise correction of several amendable PIDD-causing mutations.

The Concept of Genetic Privacy

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Abstract
The focus of the presentation is primarily the concept of genetic privacy within the contexts of healthcare, biobanking and research. Firstly, the concept of privacy is analysed in the wider sense to enable understanding of genetic privacy. The presentation then proceeds to consider whose genetic rights are affected along two axes: the transactional axis—genetic privacy rights held by patients and research participants; and the relational axis—genetic privacy right held by genetic relatives and genetic groups. Subsequently, genetic rights held by patients and research participants, genetic relatives and genetic groups will be considered. Lastly, brief discussion as to the numerous complex issues emerging due to sharing of genetic data in different contexts will be held.

Genetic counselling; A hybrid profession

Author Names
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Abstract
Genetic counselling is a profession that has been around for the last fifty or so years. It is a profession that involves both science and art. The art of communication. Genetic counselling is coming further to the forefront with the easier accessibility to genetic testing. It is important to clarify through this presentation the role of a genetic counsellor in all aspects of healthcare. So, a review of the development of this profession and a look into the future will hopefully answer questions as to who this breed of health professionals is.

The patient's perspective on a chronic, genetic condition

Author Names
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Abstract
A chronic, genetic condition never comes alone. It brings with it a multitude of challenges and problems that need careful and well-planned handling. Being a person with a genetic condition, which indeed requires constant monitoring and therapeutic interventions, requires one to plan life with the condition in mind but without allowing it to define or limit one.
It must be realized, by all social and governmental bodies, that people with genetic conditions need to be considered and treated as equal members of the community and for this to happen, a series of measures and policies need to be in place and applied to a state and a society.
It is also important to ensure that people with genetic conditions should have access to a full range of services in a well-designed health system, with high quality services and safety levels, but also support, in the form of services, to address the phenomena of social exclusion, due to stigmatization or consequent disabilities.

Is it time to revisit the written informed consent?

Author Names
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Abstract
The signing of a written informed consent is requested by all individuals who take part in research projects. The informed consent is the "contract" between the researchers and research participants. Recent developments in the analysis of human DNA such as Next Generation Sequencing (NGS) or Massively Parallel Sequencing (MPS), data mining and access to big data pose a question as to whether the informed consent we have been using up-to this point in time is adequate or whether it requires a revisit and revision. The presentation will consider the various issues involved and whether a revision is warranted in the near future for the written informed consent.

Universal PGT: achievements and challenges

Author Names
Eftychia Dimitriadou
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Abstract
Preimplantation genetic testing for monogenic disorders has been evolving remarkably the past years. The field moved from targeted to generic and genome-wide approaches. These provide not only information about the inheritance of Mendelian disease alleles but also about numerical and structural chromosome anomalies and haplotypes genome-wide. Importantly, meiotic segregation errors can be distinguished from mitotic ones and genome-wide ploidy violations can be detected, one the one hand allowing the transfer of embryos that would otherwise be discarded and on the other hand avoiding the transfer of embryos that could lead to the birth of children carrying clinically relevant aneuploidies.

At the same time, technological progress has been taken along. Haplotyping strategies now often rely on SNP array technology, whole-genome sequencing (WGS) or a combination. As sample throughput and cost-efficiency can be possibly hindered, reduced representation sequencing provides a promising alternative. Finally, long read sequencing approaches are being introduced at the preclinical PGT work-up, particularly for couples carrying de novo pathogenic variants, but also for direct mutation detection in embryos.

Clinical application, safety, and efficacy of Day 4 biopsy for preimplantation genetic testing. A retrospective cohort analysis

Author Names
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University of Sydney

Abstract

Study question: To evaluate the safety and efficacy of Day 4 embryo biopsy

Summary answers: The developmental potential of embryos and cycle outcomes suggest that day 4 biopsy is a safe and effective stage at which to perform embryo biopsy.

What is known already: Embryo biopsy routinely takes place on day 3 (cleavage stage) or day 5-6 (blastocyst stage) of embryonic development; with the latter preferred due to the ability to biopsy more cells, providing increased genetic material for analysis. A limitation of blastocyst biopsy is that several embryos capable of generating a pregnancy may not be biopsied if they fail to reach the blastocyst stage. Transfer of euploid embryos following morula biopsy on day 6 has been shown to result in significantly lower implantation rate and birth rate compared to transfer of euploid blastocysts.

Study design, size, duration: A retrospective analysis was undertaken of patients having day 4 embryo biopsy for PGT at a single Centre between February 2014 and June 2017. The reasons for PGT-A included implantation failure, history of recurrent miscarriages and advanced maternal age, whilst PGT-M was employed for specific gene defects and PGT-SR for structural rearrangements. Average patient age was 36.4 ± 4.8 years. A total of 152 cycles of PGT from 148 couples were biopsied on day 4 (1890 embryos).

Participants/materials. Setting, methods: Participants were consented patients undergoing IVF/ICSI cycles combined with PGT. Following fertilisation, fresh or frozen-thawed embryos, cryo-preserved at the 2PN stage for banking purposes, were cultured to day 4. On day 4, all embryos suitable for biopsy were de-compacted in Ca2+/Mg2+ free medium and 2-6 cells were removed for analysis. The genetic analysis was performed on the same day and euploid/non affected embryo(s) were either transferred on day 5 or cryopreserved.

Main results and the role of chance: Couples had an average of 12.4 ± 4.1 embryos biopsied with an average of 2.7 ± 1.3 cells per embryo aspirated. Following genetic analysis, 2.5 ± 1.9 euploid/normal embryos available per couple (22.6% per couple, 451/1890 total embryos). Cycle outcomes were compared for embryo transfers taking place 24 hours following the day 4 biopsy (119, 1.1 embryos per ET). No differences were observed between groups with an overall clinical pregnancy of 42.9% and 66 live births recorded. Neonatal outcomes included five preterm deliveries (5/63, 7.9%) with zero babies born with small for gestational age weight and zero days in NICU for deliveries ≥37 weeks of gestation (0.03 ± 0.2 days). A high proportion of biopsied embryos per patient with no genetic abnormalities reached the blastocyst stage (79.2%) when assessed on day 5. However, a large proportion of utilised embryos per patient did not reach the expanded blastocyst stage on day 5 (45.4%). A total of 11 additional live births resulted from day 4 biopsy method from embryos that would otherwise not have met the requirements for undergoing a day 5 blastocyst biopsy.

Limitations, reasons for caution: The results represent the experience gained from current practice and not of a prospective controlled study. No embryos were cultured to day 6, therefore the developmental

potential of certain embryos classified as not expanded blastocysts at day 5 of embryo development is unknown.

Wider implications of the findings: Day 4 embryo biopsy can be a safe and effective stage for obtaining genetic material for PGT. Therefore, this method can be used as an alternative approach to current embryo biopsy practices or used in combination with blastocyst biopsy when there is a small cohort of available embryos for biopsy

Targeted exome sequencing in fetuses with ultrasound findings: a powerfull tool in prenatal diagnosis

Author Names
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Medicover Genetics, Greece

Abstract
One of the biggest challenges in prenatal diagnosis are the limitations of existing ultrasonographic tools regarding the true fetal phenotype. Even at an advanced gestation age, ultrasound findings may not be clear in many cases.
Thus, prenatal genetic testing of syndromes in utero is a one-way approach.
To assess the potential diagnostic yield of prenatal targeted exome sequencing, a retrospective analysis of a cohort of fetuses with ultrasound anomalies and normal microarray results was performed and the results are presented here.
In the period 2016-2022, 113 pregnancies with fetal ultrasound anomalies with normal prenatal microarray results were referred for additional molecular testing, after genetic counseling. Only cases that underwent invasive prenatal sampling (chorionic villi sampling or amniocentesis) were included in this cohort. Fetal anomalies from our cohort were suspected by routine ultrasound scanning, mostly in the setting of second-trimester ultrasound screening. Twin pregnancies, where both fetuses had different anomalies and were both sampled, were counted as two separate cases. Targeted analysis through next-generation sequencing (NGS) of 874 genes associated to syndromes with fetal ultrasound findings was performed. Reporting and evaluating of findings was according to the guidelines of ACMG (Monaghan et al, 2020).
In 34 out of 113 cases (30%), pathogenic and likely pathogenic variants, clinically relevant to the referred ultrasound findings, were detected. This agrees with the pooled incremental yield of exome sequencing (31%) reported by Mellis (Mellis et al, 2022), while it seems to be 4-fold higher than the diagnostic yield of microarray analysis alone in fetuses with abnormal ultrasound scans (7%) (Callaway et al, 2013).
Prenatal targeted exome sequencing, following a normal chromosomal microarray, would notably increase the diagnostic yield in fetuses with ultrasound anomalies by ~30% and would allow early diagnosis of a genetic disorder, irrespective of the limited fetal phenotype.

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The metastatic spread of breast cancer accelerates during sleep

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Abstract
The metastatic spread of cancer is achieved by the haematogenous dissemination of circulating tumour cells (CTCs). However, the temporal dynamics that dictate the generation of metastasis-competent CTCs are largely uncharacterized and it is often assumed that CTCs are constantly shed from growing tumours. Here I will present data showing a striking and unexpected pattern of CTC generation dynamics in both patients with breast cancer and mouse models, highlighting that most spontaneous CTC intravasation events occur during sleep. Further, I will show that rest-phase CTCs are highly prone to metastasize, whereas CTCs generated during the active phase are devoid of metastatic ability. Finally, I will present single cell RNA sequencing data of CTCs revealing a marked upregulation of mitotic genes exclusively during the rest phase in both patients and mouse models, and how key circadian rhythm hormones such as melatonin, testosterone, glucocorticoids and insulin dictate CTC generation dynamics. Taken together, the results that I will present demonstrate that the spontaneous generation of CTCs with a high propensity to metastasize does not occur continuously, but it is concentrated within the rest phase of the affected individual, providing a new rationale for time-controlled interrogation and treatment of metastasis-prone cancers.

Temporal dynamics of metastatic breast cancer lung colonization

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Abstract
Introduction and aims: Metastasis, a multistep process during which cancer cells disseminate to secondary organs, represents the main cause of death for breast cancer patients. Following successful treatment, breast cancer patients considered survivors often develop macrometastases after years of latency. This phenomenon represents a major clinical obstacle in cancer patient care. We hypothesize

that the escape of metastatic breast cancer cells from a dormant state is mediated via the accumulation of yet unidentified molecular alterations that allow metastatic cells to colonize. Our main objective is to elucidate the molecular mechanisms underlying the escape from dormancy of metastatic breast cancer cells in the lungs. Our specific aims are: 1. To characterize an in vivo dormancy model for metastatic breast cancer. 2. To assess the temporal transcriptional landscape associated with the transition from dormancy to metastatic colonization and 3. To functionally evaluate the contribution of differentially expressed genes in controlling dormancy and lung colonization.

Materials and methods: Lentiviral gene transduction was used to engineer metastatic MIV-Luc-GFP cells. In vivo model for characterization of metastatic breast cancer lung colonization was established by intravenous injection of MIV-Luc-GFP cells in NOD/SCID mice coupled with whole-body bioluminescence imaging and flow cytometry for dormancy-related markers. Whole-exome RNA sequencing was performed in GFP+ cells sorted from murine lungs at distinct time points during metastatic colonization. Functional validation of the role of candidate genes in regulating lung colonization was performed using in vitro and in vivo approaches.

Results: Differential gene expression analysis accompanied by leveraging publicly available Kaplan-Meier patient survival datasets revealed a transcriptional signature of 10 candidate genes as potential regulators of the dormant state and metastatic colonization in the lungs. Stable lentiviral-mediated delivery of inducible shRNA for silencing each of these genes was performed in MIV-Luc cells. Doxycycline-mediated knockdown of P4HA1 gene decreased cell viability in MTT assays, increased sub-G1 fraction in cell cycle analysis and was associated with cleavage of PARP, caspase-3 and caspace-8 in western blotting. Most importantly, inducible in vivo silencing of P4HA1 delayed primary tumor formation and dramatically suppressed formation of lung metastasis. RNA sequencing in P4HA1-depleted versus control cells revealed deregulation of key genes involved in actin cytoskeleton remodeling, ECM-receptor interaction and PI3K/Akt signaling pathway.

Discussion and conclusions: Our findings could provide the basis for designing novel targeted therapies to improve the clinical management of breast cancer patients and as well as development of diagnostic tools for closer monitoring of patients with increased probability of metastatic disease relapse.

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The genomics of breast cancer

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Abstract
The lecture will address BC pathophysiology integrating multi-omics data from different clinical trials, also through novel single-cells technologies to identify biomolecular signatures of responders vs. non responders and to dissect tumor heterogeneity and individual response to treatment. We will describe systems medicine approaches to integrate multiple levels of omics data on the example of clinical trials with hormonal therapy, chemotherapy and targeted treatment. Through our Horison2020 project RESCUER we may explore also organoid and primary cultures of breast tumor cells for ex vivo drugs

screening of cells with certain copy number alterations and will apply mechanistic modelling to simulate in silico the treatment effects on cancer cells. This will allow to propose alternative treatment combinations, individually adjusted doses, and administration frequencies, based on in silico statistical models instructed by and validated in animal models and organoid structures. It will also suggest new biomarkers to be routinely examined in the clinic.

Gaining insights for candidate biomarkers, candidate repurposed drugs and disease-related mechanisms though network-based bioinformatics approaches

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Abstract
Network-based methods and tools for mechanism understanding, drug repurposing and biomarker discovery, developed by the Bioinformatics Department at CING will be presented. Network science, together with machine learning and computational modeling, lays out a roadmap for the further development of bioinformatics towards a more efficient exploitation of single-level as well as multi-omics. Network-based inference and integration provide great opportunities to develop innovative methodologies that lead to new insights into candidate biomarkers, repurposed drugs, and disease-related mechanisms.

Selected Abstracts

Characterization of microRNA's differential expression during puberty in female mice

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Abstract

Introduction: Puberty is defined as the transition period from childhood to sexual maturation, displaying both physiological and psychological changes. It is mainly controlled by the hypothalamic-pituitary-gonadal axis, which is activated by the pulsatile release of gonadotropin-releasing hormone, marking the onset of puberty. Pubertal disorders like precocious puberty, with disrupted pubertal timing, are often related to changes in the activation of GnRH. The GnRH expression and release is highly regulated by a complex network of both intracellular and extracellular factors in the hypothalamus, including microRNAs (miRNAs). MiRNAs are short noncoding RNAs of approximately 22 nucleotides, with a post-transcriptional activity that causes the silencing of gene expression, mostly by binding to the 3'-untranslated region of mRNAs. In this study, we demonstrate the miRNA profiling of female mice during puberty.

Material & Methods: C57BL/6 mice in three developmental stages, pre-pubertal (postnatal day 14, PND14), pubertal (postnatal day 35, PND35) and post-pubertal (postnatal day 56, PND56) were sacrificed for collection of the whole hypothalamus tissue, serum and ovaries. Small RNA isolation was performed and expression level of miRNAs was acquired through small RNA sequencing by next generation sequencing.

Results: Bioinformatic analysis showed the higher amount of differentially expressed miRNAs between PND14 and PND56 stages, with 146 transcripts in hypothalamus tissue, 105 transcripts in ovaries and 45 transcripts in serum. Comparing PND14 and PND35 stages, a total of 76 transcripts in hypothalamus tissue, 55 transcripts in ovaries and 4 transcripts in serum were observed. In addition, a comparison between PND35 and PND56 stages showed only 6 transcripts in hypothalamus, 31 transcripts in ovaries and 3 transcripts in serum differentially expressed (p-value < 0.05, fold change > 2 or fold change < -2). A pathway analysis of the validated gene targets of all miRNAs was performed, classifying the genes based on their molecular function, biological process or cellular component.

Discussion & Conclusions: The extended amount of differentially expressed miRNAs between pre-pubertal and post-pubertal stages compare to the findings between pubertal and post-pubertal stages in every tissue, strongly indicates the existence of a post-transcriptional regulatory network related to the switch from juvenile to puberty. Validated gene targets of these miRNAs can reveal the important signalling pathways that are involved in every single stage during puberty. Disruptions in these pathways may have an effect on the timing of the pubertal activation process, as it is observed in many pubertal disorders.

Genetic Analysis of Inherited Cardiac diseases in Cypriot patients

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Abstract

Introduction: Cardiomyopathies are defined as disorders of the cardiac muscle and considered as the leading cause of sudden death in young athletes worldwide. The following study emphasizes on inherited cardiac diseases which are classified into cardiomyopathies and channelopathies, and there are several sub-categories based on the clinical and genetic phenotype. Inherited cardiomyopathies are divided into 4 sub-groups: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) and restrictive cardiomyopathy (RCM). The most common inherited channelopathies are divided into long QT syndrome (LQTS), short QT syndrome (SQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and Brugada syndrome.

Objective: The main objective of the project was the genetic analysis of the DNA of probands with a suspicion of an inherited form of a cardiac disease, to identify genetic alterations that could be related to the probands' clinical phenotype and identify the family members at risk.

Material & Methods: The genetic variations in probands' DNA samples were identified through Next Generation Sequencing (NGS) using a panel of 72 genes related to cardiac diseases. The NGS results were validated through Sanger Sequencing. Once the proband was validated positive for a genetic variation, the testing was repeated for every family member with an available DNA sample. Once the validation of the results was completed, the pathogenicity of the genetic variants under study was evaluated in different databases (HGMD, ClinVar, Franklin, Varsome). Furthermore, bioinformatic algorithms were used to predict the pathogenicity at the cDNA level and protein level (Mutation taster, Polyphen-2, SIFT).

Results: In this study, 11 families were examined with the panel of the 72 genes, and 25 genetic variations were identified, 20 of them novel. Most of the variants are responsible for probands clinicals phenotype although patterns of incomplete penetrance and co-inheritance of multiple genetic variants have been observed.

Discussion & Conclusions: Inherited cardiac diseases are characterized by genetic and phenotypic heterogeneity. The results indicate that in some cases these disorders may have oligogenic inheritance pattern, however further studies are required to verify it. Finally, To our knowledge, this is the first systematic study attempted in Cyprus for inherited cardiac conditions, enabling us to develop information about the genetic basis of these diseases and guidelines for new therapeutic approaches.

Large scale case-control analyses of *BRCA1* and *BRCA2*

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Abstract

Introduction: Clinical genetic testing in high risk-genes can lead to the identification of variants of uncertain clinical significance (VUS), that complicate management of carriers and their families. Variant frequency in affected vs unaffected individuals for a disease is commonly considered as evidence in the interpretation of variants identified by genetic testing. We have developed a novel rare variant case-control analysis method that incorporates gene- and age-specific penetrance, which has shown to outperform existing methodologies. We applied our method, on a large case-control dataset sequenced using the Breast Cancer Risk after Diagnostic Gene Sequencing (BRIDGES) panel, with the aim of improving VUS interpretation.

Materials & Methods: Our case-control likelihood ratio (ccLR) method was used for the analysis of 1,555 rare BRCA1 and BRCA2 variants (with at least two carriers and MAF<0.0010) from 35,674 breast cancer cases and 32,532 controls from the Breast Cancer Association Consortium (BCAC). Resulting LRs were categorised as weights for or against pathogenicity following recommendations arising from Bayesian modelling of the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) variant classification framework. The analyses were also conducted for European vs Asian ancestry and population- vs familial-based studies. Missense variants were also analysed separately.

Results: Of the analysed variants, we provide evidence in favour of pathogenicity for 60 variants and against pathogenicity for 975 variants. For the variants having a definitive ClinVar class the overall consistency in classification was 90%. Additionally, informative evidence was estimated for 246 variants, for which no ClinVar class is available. Overall, our ccLR analysis provided evidence relevant for variant classification for 70% of BRCA1 and BRCA2 rare variants analysed.

Discussion & Conclusions: Identified LR estimates will be integrated with other evidence for the derivation of a final variant class in BRCA1 and BRCA2. Overall, the ccLR method can be applied in the analysis of case-control data for rare variants identified by genetic testing and provides more informative results compared to other methods used in clinical practice. Our method offers the potential to inform patient testing and disease management.

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Evaluation of the differences in the breast cancer polygenic risk score distribution in individuals from different European ancestry populations

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Abstract

Introduction: Polygenic risk scores (PRS) combine the effects of multiple common-low risk variants identified via genome-wide association studies (GWAS), and could be potentially used for individualized breast cancer risk assessment and inform targeted screening and preventative strategies. A 313-SNP PRS (PRS313) has been constructed for the classification of women of European ancestry based on their breast cancer risk. However, specific evaluation of the distribution of the mean PRS313 across the different European populations has not been explored. Potential differences in this distribution could influence the classification of some individuals, and thus have important implications in their future clinical management.

Material & Methods: Here, we aimed to explore the distribution of the mean PRS313 across 21 different European ancestry populations, using data from 111,814 female breast cancer cases and 94,718 female controls from 84 studies participating in the Breast Cancer Association Consortium (BCAC). All samples were previously genotyped using either the iCOGS or the OncoArray platforms. Initially, the PRS313 was calculated in each participant and mean and standard error of PRS313 by country were calculated separately in cases and controls. In order to explore potential sources of variability in the mean PRS; we used ancestry informative Principal Components, removed variants with high frequency variability across countries and Empirical Bayes approaches.

Results: The mean PRS313 differed significantly across the different European populations in the control dataset with heterogeneity I2 = 80% (p-value < 0.01), being highest in the Republic of North Macedonia, Greece and Italy, and lowest in Ireland. Excluding the variants with the most variable frequency across the countries, did not change the variation in the mean PRS (I2 = 80%, p-value < 0.01). In contrast, when the PRS was adjusted by the first 6 ancestry informative principal components the heterogeneity was almost eliminated (I2 = 1% and p-value > 0.05). When an Empirical Bayes approach was used, as an alternative method to estimate the country specific means, estimates for countries with small available sample size had greater shrinkage towards overall estimates.

Discussion & Conclusions: These results indicated that the genetic background influences the PRS313 distribution even within Europe leading to an observed overestimation in south-east Europe and underestimation in west Europe if not appropriately accounted for. Thus, country-specific calibration of PRS313 will be required before its widespread utility in risk-stratified prevention and implementation in risk prediction models in European countries. Principal Components seem to

correct the distribution differences; although are not always available since array genotyping data are needed to generate them. The Empirical Bayes approach provided an alternative method to derive country-specific means, which considers both the true variation in the mean PRS across the countries as well as the uncertainty due to small available sample size.

Evaluation of the spatiotemporal intratumor molecular heterogeneity in tumor and liquid biopsies from patients with metastatic colorectal cancer

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Abstract

Introduction: Despite the widespread establishment of primary prevention, colorectal cancer (CRC) remains the second leading cause of cancer-related death in industrialized western countries. Dynamic molecular evolution of cancer cell sub-populations and intratumor heterogeneity (ITH), as well as metastasis and therapeutic resistance, represent the major causes of relapse and cancer-related death. The mechanisms underlying high drug resistance and relapse rates after multi-modal treatment in patients with colorectal cancer (CRC) and liver metastasis (LM) remain poorly understood.

Materials & Methods: To assess ITH and serial ctDNA molecular heterogeneity in the perioperative setting, we designed a prospective protocol encompassing multiple intra-lesional and matched plasma samples for each individual patient. A total of 28 patients diagnosed with metastatic colorectal adenocarcinoma were recruited in the study. In total, 94 FFPE samples were collected from both primary tumors (PTs) and LMs. For 16 out of 18 patients, plasma was collected at multiple time points during treatment, before and after surgery, to assess the molecular dynamics of the disease using liquid biopsies.

Results: The proportion of patients with ITH were 53% and 56% in primary CRC and LM respectively, while 35% of patients harbored de novo mutations in LM indicating spatiotemporal tumor evolution and the necessity of multi-regional analysis. Among the 56% of patients with alterations in liquid biopsies, de novo mutations in cfDNA were identified in 25% of patients, which were undetectable in both CRC and LM. All 17 patients with driver alterations, harbored mutations targetable by targeted drugs, either approved or currently under evaluation.

Discussion & Conclusions: Our prospective study provides initial evidence on potential clinical superiority of IPH and warrants the conduction of precision oncology trials to evaluate the clinical utility of ITH-driven matched therapy."

Genome editing for beta-haemoglobinopathies without double-strand DNA cleavage

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Abstract

Introduction: Haemoglobinopathies, such as sickle-cell disease and β-thalassaemia, are the commonest monogenic diseases. Of these, β-thalassaemia has high prevalence in Cyprus and is marked by low adult haemoglobin (α2β2, HbA), owing to defective β-globin (HBB) expression. Increased levels of foetal haemoglobin (α2γ2, HbF) can ameliorate the severity of the disorder and may be achieved by erythroid reduction of γ-globin repressors, such as the transcription factor BCL11A. While corresponding clinical trials are currently based on double-strand-break (DSB)-dependent CRISPR/Cas editors, DSB-independent base editors (BEs) may instead be employed as safer and likely more efficient tools for curative γ-globin induction. Mindful of the high clinical potential of BE technology, this study aims to adopt the newest generation of BEs for application to targets of relevance for β-haemoglobinopathies.

Materials & Methods: Based on in silico design of target- and platform-specific guide RNAs (gRNAs) and on mRNA/gRNA delivery of BE technology in HUDEP-2 and patient-derived CD34+ cells by nucleofection, this study modified therapeutic targets for β-haemoglobinopathies. In the process, editing efficiencies and functional parameters at the DNA, RNA and protein level were measured in comparisons of different BEs against one another and against ribonucleoprotein (RNP)-based delivery of CRISPR/Cas DSB-based technology targeting the well-known BCL11A erythroid enhancer. To achieve higher HbF levels, a duplex base editing strategy was established targeting both and trans-acting factors and cis-acting elements.

Results: Establishment of in vitro mRNA synthesis for mRNA/gRNA-based delivery of BEs allowed efficient, non-toxic BE delivery. Our data indicate differential same-target efficiency of different BEs for the clinically relevant BCL11A target, with peak precision editing of 86% bulk efficiency for the BE4-PpAPOBEC1 BE in HUDEP-2 cells. Duplex base editing in patient-derived CD34+ cells of both, trans-acting factors (BCL11A) and corresponding cis-regulatory elements (HBG), resulted in 1.8-fold elevated HbF induction compared to simplex edits and in up to 60% increase of HbF levels compared to baseline. Moreover, duplex BE application resulted in 70% contribution of HbF to total hemoglobin compared to 60% for DSB-based editing technology, at vastly decreased risk of genome recombination events.

Discussion & Conclusions: The present study demonstrates high efficiency, low toxicity and superior editing outcomes of RNA-based delivery for base editing technology compared to the clinically applied RNP-based DSB-mediated editing standard. In particular duplex editing of BCL11A as therapeutic target resulted in superior editing outcomes compared to simplex BE and DSB-based editing application, and to superior, therapeutically relevant HbF induction."

Preclinical validation of HBBIVSI-110(G>A)-specific gene editing as advanced therapy for thalassemia

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Abstract

Introduction: β-Thalassemia is among the commonest single-gene disorders worldwide, caused by deficient production of β-globin. For one of the commonest β-thalassemia mutations, HBBIVSI-110(G>A), which creates an aberrant intronic splice site in β-globin and has a relative carrier frequency of 76% in Cyprus and above 20% in many EU countries, we established proof of concept for an efficient mutation-specific therapy, based on designer nucleases (CRISPR/Cas9 RNA-guided nuclease (RGN) and TALENs) and specific DNA cleavage of the intronic mutation to allow the prevailing non-homologous end joining mechanism to destroy the aberrant splice site thus restoring normal splicing and HBB expression at clinically relevant efficiencies on patient-derived primary cells. With this project we want to take our approach forward for preclinical assessment of edited cells in vitro and in vivo in chimeric NOD,B6,SCID Il2rγ-/-Kit(W41/W41) (NBSGW) mice, developed to support engraftment of human hematopoietic stem cells (HSCs) without irradiation, and for validation of suitability for clinical trials regarding efficacy, safety and long-term repopulation (LTR) potential of modified cells. In the process we will compare the mutation-specific approach with a universal therapy targeting the BCL11A enhancer element for the induction of HbF, currently in clinical trials for β-thalassemia and sickle cell disease.

Material & Methods: RGN and TALENs were delivered via nucleofection to mobilized HBBIVSI-110 patient-derived HSCs as ribonucleoprotein complexes (RNPs) and in vitro transcribed mRNAs, respectively, both targeting the HBBIVSI-110 mutation and as RNPs targeting the BCL11A enhancer element (sg1617 RGN). The therapeutic potentials of the genome editing tools were assessed in vitro with induced erythroid differentiation (ED) cultures, in which correction was evaluated at the DNA (on- and off-targeting, Sanger sequencing), protein (HPLC and RP-HPLC) and late-stage ED levels (flow cytometry), in clonogenic assays for erythroid and myeloid lineage potential and in vivo with xenotransplantation of the treated HSCs in NBSGW mice for the assessment of the LTR potential of the edited cells in the bone marrow of the recipients 16-weeks post-transplantation (flow cytometry).

Results: Overall, both mutation-specific designer nucleases let to high on-targeting (IVSI-110 RGN 89%, TALENs: 68%) with undetected off-targeting, whereas BCL11A enhancer targeted disruption was low (16%). RP-HPLC analysis of the in-vitro ED cultures, showed functional correction of HBB-like globin proportion in the mutation-specific edited populations (HBB; IVSI-110 RGN: 69%; TALENs: 54% vs UT: 21%) and a minor increase of HBG in the sg1617 RGN edited population relative to the untreated (UT) (HBG; sg1617: 78% vs Untreated: 72%). There was a clear correction of late-stage erythroid differentiation in the mutation-specific edited populations and genome editing did not affect the erythroid and myeloid lineage potential of the edited HSCs. Importantly, analysis of BM chimerism in xenotransplanted NBSGW mice showed high engraftment for all samples (hCD45+: 75% and hCD34+ves: 10%).

Conclusion & Discussion: Even though, analysis on the biosafety of the RGN- and TALEN-based mutation-specific approach is still in progress, the current data indicates IVSI-110 RGN as the most promising approach for clinical application, since therapeutic levels were achieved while the multi-lineage and long-term repopulating capacity of the edited HSC population was maintained.

A novel hotspot ATP1A1 variant and its functional evaluation in a demyelinating Charcot-Marie-Tooth patient

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Abstract

Introduction: Charcot-Marie-Tooth (CMT) represents motor and sensory hereditary neuropathies characterized by high genetic and clinical heterogeneity. CMT is traditionally classified as demyelinating (CMT1), axonal (CMT2) and intermediate (I-CMT) based on neurophysiological findings. ATP1A1 is one of the rare CMT genes associated with autosomal dominant CMT2 and I-CMT to date. ATP1A1 encodes for the catalytical α1 subunit of Na+/K+ ATPase, an essential plasma membrane protein expressed in all mammalian cells. The Na+/K+ ATPase is responsible for regulating sodium and potassium levels in the cytosol hence, maintaining the electrochemical gradient across the plasma membrane. The α subunit is required to assemble with an auxiliary β subunit expressed by an ATP1B gene (ATP1B1-ATP1B3) to form a functional pump protein. In addition to neuropathy, ATP1A1-associated phenotypes include spastic paraplegia, renal hypomagnesemia and intellectual disability. We report the first demyelinating CMT case associated with a novel ATP1A1 variant within the hotspot region of previously identified CMT2 variants.

Material & Methods: Patient genomic DNA subjected to the whole exome sequencing to identify the candidate pathogenic variant. Sanger sequencing was performed to validate the candidate variant, c.1799 C>G (p.P600R) in ATP1A1 and confirm its segregation. For functional analysis, blood-derived mRNA and protein levels of the ATP1A1 and ATP1B1 were investigated by qPCR and western blotting in the patient versus controls. The p.P600R was overexpressed in the human neuroblastoma SH-SY5Y cells to evaluate any functional effects the variant could exert on this cell line.

Results: The p.P600R in ATP1A1 is a novel variant absent in the local and global datasets. The wild type proline residue is highly conserved and highly constrained for missense variations. The mRNA and the protein expression levels of ATP1A1 and ATP1B1 were significantly reduced by ~50% in the patient compared to controls. Genetic analysis confirmed that the reduced ATP1A1 levels are not due to a splicing or a biosynthesis defect caused by the c.1799 C>G variant. Finally, overexpression of the p.P600R variant in the SH-SY5Y cells did not seem to cause a significant change in cell morphology.

Discussion & Conclusions: We present a Cypriot CMT1 patient with a negative family history of any neuromuscular disease suggesting that she might have acquired the novel ATP1A1 p.P600R variant de novo. Reduced ATP1A1 and β1-encoding ATP1B1 expression suggests that reduced Na+/K+ ATPase activity impaired the peripheral nerve homeostasis in this case. Previously described CMT2 variants p.P600A and p.P600T were reported to cause pathogenicity through different mechanisms such as reduced Na+ currents. Such variation in phenotype due to same amino-acid substitution is likely due to nature of the mutation and the effects it exerts on the tertiary structure of the protein. This phenomenon is already documented in other CMT genes such as MPZ. To conclude, our results broaden the genetic and phenotypic spectrum of ATP1A1-related CMT variants and confirms that the p.P600 is a hotspot CMT locus in ATP1A1.

A novel SMN1 splicing variant disrupts the expression of the functional SMN1 transcript and expands the spectrum of the SMN1 conventional variants

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Abstract

Introduction: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular genetic disorder caused by pathogenic variants in the SMN1 gene. The most common pathogenic variant is the homozygous deletion of exon 7 (95%), while compound heterozygous patients with a conventional variant and an exon 7 deletion are less frequent (5%). Diagnosis of patients with conventional variants is challenging due to the highly homologous SMN2 gene, which mainly produces a truncated non-functional protein (SMN-d7) instead of the full-length functional (SMN-FL). In cases of a conventional variant, verifying its occurrence in the SMN1 rather than the SMN2 gene is necessary; hence, the sequencing procedure becomes more complex and challenging. The current study aimed to investigate an SMA patient harbouring a heterozygote SMN1 exons 7/8 deletion.

Material and Methods: The proband's and her parents' DNA samples were initially subjected to SMN1/2 genes exon dosage analysis using the MLPA method. Sanger sequencing was then performed to investigate the presence of an SMN1-specific pathogenic variant in the family. Additional RNA studies, including a) qualitative transcript analysis of all the SMN1/2 transcripts by conventional PCR, restriction enzyme digestion, agarose gel electrophoresis, gel excision-extraction and Sanger sequencing, and b) quantitative transcript analysis by Real-Time PCR, were employed to delineate the

effect of the identified variant. An improved allele-specific Real-Time PCR assay was designed by us and has been used for this purpose.
Results: MLPA analysis revealed a heterozygous deletion of SMN1 exons 7 and 8 in the proband and her father. Sanger sequencing of SMN1/2 exons and intronic flanking regions, followed by sequencing of an SMN1-specific long-range PCR-derived product, revealed a novel heterozygous SMN1 gene splice-site variant in the proband and her mother. RNA studies confirmed a disruptive effect of this variant on SMN1 splicing, clearly showing the absence of exon 7 in the proband. RNA expression analysis aiming at the determination of the four transcript levels (SMN1/2-FL, SMN1/2-d7) demonstrated the lack of the functional SMN1-FL transcript in the proband, a remarkable expression of the SMN1-d7 transcript and increased levels of the SMN2-FL/SMN2-d7 transcripts.
Discussion: The current findings successfully verified the occurrence of a non-deletion variant in the SMN1 gene. The pathogenic role of the novel variant is also supported, thus expanding the SMN1 variants spectrum and increasing the percentage of intragenic variants identified in our population, which is higher than in other populations. Moreover, the early diagnosis of the affected child was achieved, thus facilitating an early treatment initiation.

Fetal genetic factors associated with sonographic abnormalities and pregnancy loss

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

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

Results: Out of eighteen tested trios, causative/potentially causative variants were uncovered in six cases. A known de novo heterozygous missense variant within SCN2A was found in a fetus presenting Developmental and Epileptic Encephalopathy 11 phenotypes. Two inherited novel missense variants

in SCN4A were found in a compound heterozygous fetus resulting in severe SCN4A-related congenital myopathy. A known homozygous nonsense variant in KLHL40 was found in a fetus with Nemaline Myopathy 8. Potentially causative heterozygous variants were identified in three cases, in genes USP18, CC2D2A and CPLANE1 with autosomal recessive inheritance.

Discussion & Conclusions: We identified causative variants in 3/18 cases as well the possible involvement of heterozygous variants in genes USP18, CC2D2A and CPLANE1 in fetal development. Further investigation is required to assess the clinical significance of the latter findings with the use of techniques offering a higher resolution than array-CGH for the detection of small copy number variants. Expression studies using RT-PCR will also be carried-out where applicable. Accurate identification of variants in such genes creates new genotype-in utero phenotype associations, leading to the prospect of new additions in preconception and prenatal diagnostic panels.

Expanding the prenatal phenotype of WT1 related disorder

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Abstract

Introduction: Frasier syndrome, Denys-Drash syndrome, and Meacham syndrome were originally described as distinct disorders on the basis of clinical findings but they now represent a continuum of features caused by WT1 heterozygous pathogenic variants. Given the extensive clinical as well as the genetic overlap between these disorders they are all currently described in the literature as WT1 disorder characterized by congenital/infantile or childhood onset of a progressive glomerulopathy that does not respond to standard steroid therapy. Additional common findings include disorders of testicular development (with or without abnormalities of the external genitalia and/or mÃ¼llerian structures) and Wilms tumor. Less common findings are congenital anomalies of the kidney and urinary tract (CAKUT) and gonadoblastoma.

Material and methods: Newborn infant was admitted to neonatal intensive care unit because of respiratory failure at six hours of life During the 2nd trimester of pregnancy oligohydramnios was noted and monitored with ultrasound. No genetic test, either invasive or noninvasive, was performed prenatally. Fetal MRI at 29+4weeks confirmed oligohydramnios, without signs of renal or other congenital abnormalities. The baby was born by caesarean section at 36+1/40weeks with apparent normal first hour of life. A few hours later, the baby presented with breathing difficulty and was transferred to the NICU, where right pneumothorax was diagnosed, and appropriate management was provided. On 6th day of life, high levels of plasma creatine and urea were noted leading to the

diagnosis of congenital nephrotic syndrome. Thorough investigation according to standard guidelines was initiated, including genetic testing.
Results: Clinical examination showed facial edema, hypotelorism, very wide anterior fontanelle with diastasis of all cranial sutures, feminine external genitalia with pronounced labia majora and minora. Chromosomal analysis revealed a normal however male chromosome constitution in all cells examined consistent with a disorder of sex development explaining part of the clinical phenotype. Chromosomal microarray did not reveal any clearly pathogenic copy number variants. Whole exome sequencing showed a de novo heterozygous c.1316G>A variant at exon 8 of the WT1 gene (NM_024426.6). This variant is a missense substitution changing the amino acid arginine to histidine at position 439, found in a mutational hot-spot of the protein. ClinVar database classifies this variant as pathogenic associated with previously reported as Denys-Drash Syndrome and Frasier Syndrome.
Discussion and Conclusion: We report the first case of WT1 related disorder with prenatal findings of oligohydramnios, expanding the currently described phenotype during pregnancy. Multidisciplinary work led to the quick precise diagnosis and guided the clinical management of the patient including the approach to gonadal dysgenesis, renal failure, and appropriate surveillance for the appearance of Wilms tumor. Furthermore, genetic counseling of the family can be accurately offered. Importantly, the expansion of prenatal phenotypes will support phenotype-driven prenatal exome and genome sequencing for precise genetic diagnostics of rare diseases.

Muscle-specific miRNAs as potential monitoring biomarkers of muscle wasting progression in DM1.

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Abstract
Introduction: Myotonic dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy primarily affecting the skeletal muscle tissue. DM1 is a multisystemic disorder characterized by progressive muscle weakness, wasting and myotonia. Muscle wasting progression in DM1 is highly variable, exposing a pressing need to develop reliable non-invasive biomarkers for its characterization. Although many biomarkers have been established for various diseases, limited work has been performed regarding the rare muscular dystrophies including DM1. We previously showed that the levels of four muscle-specific miRNAs, miR-1, miR-133a, miR-133b and miR-206, correlate with muscle

wasting progression in DM1 suggesting that they have the potential to be used as biomarkers in clinical practice. Having associated miRNA levels in DM1 patients who are degenerating, the aim of this study was to associate the four muscle-specific miRNAs with muscle wasting during the course of the disease.

Material & Methods: To achieve our aim, we analyzed the levels of the four muscle-specific miRNAs circulating in the serum of DM1 patients at different time points of the disease course. In this study, serum samples from DM1 patients participated in 'PhenoDM1' study (Newcastle, UCLH, UK) were used. Specifically, the participants provided serum samples yearly for a period of three years. DM1 patients were categorized as progressive or non-progressive (stable) at the time of blood collection based on the outcome measures of the patients suggested by the Outcome Measures in Myotonic Dystrophy type 1 (OMMYD) consortium. Total RNA, including miRNA, was extracted from the serum samples of DM1 patients followed by Real-Time PCR analysis specific for the four muscle-specific miRNAs.

Results: Our results show that the levels of miR-1, miR-133a, miR-133b and miR-206 remain stable or even decrease in DM1 patients that were categorized as stable at their follow-up visit. Interestingly, we show that the levels of the four muscle-specific miRNAs follow an increasing trend in DM1 patients classified as progressive. Moreover, we show that the levels of the four muscle-specific miRNAs do not correlate to the age, gender or CTG repeats size.

Discussion & Conclusions: Based on our results we suggest that the levels of the four muscle-specific miRNAs reflect the progression status of the patients and these molecules have the potential to be used as monitoring biomarkers in DM1.

Investigation of the role of anoctamin 10 protein in cell division

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Abstract

Introduction: Anoctamin 10 (ANO10), also known as TMEM16K, is a member of a broader family of dual function proteins exhibiting phospholipid scrambling and ion transport activity. Endosomal sorting, spindle assembly, calcium signalling, cell volume regulation, and apoptosis are other biological processes that have been associated with ANO10. The primary localization of ANO10 is in the endoplasmic reticulum (ER); however, a recent study also showed an association with acetylated tubulin of spindles in mouse macrophages. Moreover, the ANO10 ortholog in Drosophila, Axs, has been associated with microtubules during the meiotic spindle formation, while protein defects were found to cause abnormal spindle assembly and chromosome segregation. These findings may indicate the involvement of ANO10 in spindle formation and cell cycle progression. Variants in the ANO10 gene are known to cause a rare type of autosomal recessive spinocerebellar ataxia named SCAR10. Degeneration of Purkinje cells in the cerebellum, mediated by a defective ANO10 protein, is the proposed mechanism of SCAR10 pathogenesis. In specific, it is suggested that ANO10 defects deregulate calcium signalling in Purkinje cells; however, the exact role of ANO10 in SCAR10

pathomechanism remains unclear. The aim of this study is to examine the biological role of human ANO10, and especially, to investigate the impact that ANO10 depletion has at the cell division level.
Materials and Methods: Immunofluorescence microscopy was performed to assess ANO10 localization in the human SH-SY5Y and U2OS cell lines, using two different antibodies. In addition, we employed ANO10 silencing using RNA interference technology to resemble and study the effects of a pathogenic variant (c.289del [p.Thr96_Met97ins*]) that creates a truncated protein identified in three individuals of a Cypriot family with SCAR10 phenotype. Validation of gene knockdown was carried out by qPCR and Western-blot analysis.
Results: ANO10 was found to localize at the centrosomes of mitotic U2OS and SH-SY5Y cells, and the ER in agreement with previous studies. Transfection of cells with siRNA targeting ANO10 mRNA resulted in a significant reduction in both gene and protein expression levels.
Discussion and Conclusion: Centrosomic localization of ANO10 suggests a potential role of the protein in cell division. The effects of ANO10 silencing are currently examined to further characterize the protein and delineate its role in the cell cycle, cell growth and ciliogenesis. Future investigations include co-immunoprecipitation experiments to reveal possible interactors of ANO10.

Poster Abstracts

HBBIVSI-110(G>A)-Specific gene editing as advanced therapy for β-thalassemia

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Abstract

Introduction & Aims: β-Thalassemia is a disease affecting millions of patients worldwide, with a particularly high carrier rate in Cyprus. The disease is brought about by defective β-globin (HBB) formation, with patients suffering from many symptoms, in some cases even depending on regular blood transfusions and iron chelating agents for survival. Current cures are limited to allogeneic hematopoietic stem cell (HSC) transplantation from compatible donors or transplantation of autologous HSCs after gene addition, both of which inaccessible to many and harbour high risks and cost. Sequence-specific genome editors, utilized for the correction of genetic defects, have revolutionized the therapy of monogenic diseases. Especially attractive for clinical translation are base editors (BEs), which catalyse base transitions at a targeted base without relying on potentially mutagenic double-strand breaks (DSBs) typical of first-generation DNA editors. Cytosine BEs enable C>T transitions, whereas adenine BEs enable A>G transitions. Here I am evaluating gRNA designs for four recently published BEs with relaxed protospacer adjacent motif (PAM) requirements, for their ability to correct functionally the common Cypriot HBB[IVSI-110(G>A)] splice mutation.

Materials & Methods: GFP reporter plasmids encoding ABEs and CBEs were obtained from Addgene, and the T7 promoter was added to CBE plasmids, to allow in vitro mRNA transcription for BEs. Editors were delivered into primary hematopoietic cells by nucleofection, of mRNA/gRNA mixtures for BEs and of Cas9/gRNA/donor mixtures for DSB-based editing. Clonal cell models based on HUDEP-2 cells were plate-sorted on a BD FACS Aria III after DSB-based editing. Editing efficiency was assessed at the DNA level and, after erythroid differentiation, at the protein level. DecodeR and EditR were used to assess DSB-based and base editing, respectively.

Results: The strategies considered aim to prevent aberrant splicing by (i) precise correction of the mutated A of the aberrant AG splice motif, (ii) mutation of the G of the same motif, and (iii) alteration of upstream sequence elements critical for aberrant splicing. This work has established efficiency of two nearly PAM-less ABEs for HBB[IVSI-110(G>A)] target sites and has demonstrated their ability to correct β-globin expression. Furthermore, removal of the GFP reporter from ABEs doubled on-target efficiency for the SPRY editor to 65%. We additionally applied DSB-based precision editing to create an HBB[IVSI-110(G>A)]-homozygous cell model as tools for further analyses, based on HUDEP-2 cells. Of facility for the study of on-target and bystander editing events, also at the clonal level, of alternative HBB[IVSI-110(G>A)]-proximal targets and of sequences affecting splicing, a total of 16 clones were characterised, with HBB[IVSI-110(G>A)]-homozygotes displaying characteristically decreased β-globin levels, as confirmed by HPLC analysis.

Discussion and Conclusions: This work has established efficiency of two novel nearly PAM-less ABEs and has demonstrated the impact of their editing power on improving the HBB/HBA ratio following differentiation. Editing efficiency was observed in both the HUDEP-2 cell line as well as primary CD34+ patient cells. Moreover, editing capacity was nearly doubled following excision of the GFP reporter from original plasmids. Finally, HBBIVS-110(G>A)-homozygous clones were produced through HDR-disruption, which has facilitated for correctional work to be done on these cells.

Genomic landscape of the immunogenicity regulation in skin melanomas with diverse tumor mutation burden

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Abstract

Introduction: Skin melanoma cells are tightly interconnected with their tumor microenvironment (TME), which influences their initiation, progression, and sensitivity/resistance to therapeutic interventions. An immune-active TME favors patient response to immune checkpoint inhibition (ICI), but not all patients respond to therapy.

Material & Methods: Here, we assessed differential gene expression in primary and metastatic tumors from the TCGA-SKCM dataset, compared to normal skin samples from the GTEx project, and validated key findings across 4 independent GEO datasets, and using immunohistochemistry data from an independent patient cohort. We focused our attention on examining the expression of various immune receptors, immune-cell fractions, immune-related signatures, and mutational signatures across cutaneous melanomas with diverse tumor mutation burdens (TMB).

Results: Globally, most immunoreceptor expression correlated with patient survival but did not differ between TMBhigh and TMBlow tumors. Melanomas were enriched in "naive T-cellâ€•, "effector memory T-cell"•, "exhausted T-cell", "resting Treg T-cell" and "Th1-like"• signatures, irrespective of their BRAF, NF1 or RAS mutational status. Somatic mutations in IDO1 and HLA-DRA were frequent and could be involved in hindering patient response to ICI therapies. We finally analyzed transcriptome profiles of ICI-treated patients and associated their response with high levels of IFNγ, Merck18, CD274, CD8, and low levels of myeloid-derived suppressor cells (MDSCs), cancer-associated fibroblasts (CAFs) and M2 macrophages, irrespective of their TMB status.

Discussion & Conclusions: Overall, our findings highlight the importance of pre-existing T-cell immunity in ICI therapeutic outcomes in skin melanoma and suggest that TMBlow patients could also benefit from such therapies, depending on the patient's TME

Whole-exome sequencing of 736 Cypriot female breast cancer cases and controls

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Abstract

Introduction: Germline pathogenic variants (PVs) in BRCA1, BRCA2 and other genes, have been associated with an elevated risk of breast cancer. Individuals carrying PVs can benefit from risk management strategies including closer surveillance at an earlier age, prophylactic surgery and targeted therapies. It is estimated that around 7 to 10% of women with breast cancer carry a PV in an established breast cancer susceptibility gene. However, the prevalence of PVs and the associated risk estimates of breast cancer among the Cypriot population are currently unknown. Herein, we aimed to investigate the prevalence of protein-truncating variants (PTVs) and the associated risk estimates of breast cancer in a large series of breast cancer cases and controls in Cyprus.

Material & Methods: We used whole-exome sequencing data from the MASTOS population-based case-control study of breast cancer in Cyprus. The dataset comprised 339 breast cancer cases and 397 healthy controls, selected for early age of onset (<50) or family history of breast cancer. Library preparation was conducted using the Agilent SureSelect Human all exon V7 kit and libraries were sequenced on a NovaSeq 6000 system. Variant calling followed the Genome Analysis ToolKit (GATK) best practices.

Results: The prevalence of PTVs in established breast cancer risk genes (ATM, BRCA1, BRCA2, CHEK2, PALB2, BARD1, RAD51C, RAD51D, TP53) was 4.13% among cases and 1.48% among controls. Among the patients, the highest prevalence of PTVs was observed for BRCA2 (n = 6, 1.77%), ATM (n = 4, 1.18%).

Discussion & Conclusions: Here, using large-scale whole-exome sequencing data, we report the prevalence of PTVs and the associated estimates of breast cancer risk among 736 Cypriot breast cancer cases and controls. We further aim to explore the prevalence of variants with unknown effect on protein function, including rare missense variants, in-frame indels and variants within intronic and untranslated regions.

Extracts derived from Sideritis scardica cultivated in Greece demonstrate neuroprotective activity against Aβ25-35 toxicity

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Abstract

Introduction: Alzheimer's disease (AD) is the commonest neurodegenerative disease affecting primarily the elderly. Despite the considerable time and resources invested in the last decades, no therapy has been developed yet. In recent years, research focused on ameliorating the cytotoxic amyloid beta (Aβ) peptide aggregates and the increased elevated oxidative stress, two interconnected main AD hallmarks. Medicinal plants constitute a large pool for identifying bioactive compounds or mixtures with a therapeutic effect. Sideritis scardica (SS) has been previously characterized as neuroprotective toward AD, and the aim of this work is to reconfirm this finding using specific SS plant material cultivated in Greece.

Material & Methods: We investigated the neuroprotective ability of SS by generating eight distinct solvent fractions and assessing their antioxidant potential and ability to reverse the cytotoxicity caused by the highly neurotoxic Aβ25-35. Furthermore, we chemically characterized the SS fractions, both qualitatively and quantitatively, by ultra-performance liquid chromatography tandem mass spectrometer with electron spray ionization in either positive or negative mode.

Results: The SS extracts showed a significant antioxidant activity that correlated positively with their corresponding flavonoid and phenolic contents, except for petroleum ether extract. In addition, four fractions partly rescued the cell viability in Aβ25-35 treated SH-SY5Y human neuroblastoma cells, the most potent being the initial aqueous fraction that demonstrated similar activity in retinoic acid-differentiated SH-SY5Y cells as well. These extracts were rich in specific substances of known neuroprotective properties, such as apigenin, myricetin-3-galactoside, and ellagic acid.

Discussion & Conclusions

Our results suggest that particular SS mixtures from plant material cultivated in Greece possess properties that can be useful to the pharmaceutical industry for developing herbal drugs and functional food products that may alleviate AD. These abilities can be attributed to the presence of specific bioactive compounds.

Systemic Sclerosis: from proteomic biomarkers to essential signalling pathways and biological processes

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Abstract

Introduction: Systemic sclerosis (SSc) is a complex rheumatic disease characterised by inflammatory, vascular and fibrotic processes. To date, the aetiopathogenesis of the disease is still unknown, and the implicated pathways/mechanisms are not clarified. In this work, we gathered candidate SSc proteomic biomarkers discovered through our previous study and other proteomic studies and then further analysed them to extract possible pathways and mechanisms involved in the development of the disease.

Material & Methods: All candidate Mass-Spectrometry (MS) based biomarkers for SSc were recorded. A number of candidate biomarkers were selected for in-silico analysis using specific criteria. Enrichr Web Server was used to classify the proteins/genes based on pathways and cellular components. PANTHER and STRING tools were used to assess the biological processes and interactions of the recorded proteins, respectively.

Results: Pathway analysis extracted several pathways associated with the three basic stages of SSc pathogenesis; inflammation, vasculopathy and fibrosis. Some pathways related to other diseases, such as autoimmune diseases, were also extracted. It was observed that these biomarkers are located in several cellular components and implicated in many biological processes. STRING analysis showed that some proteins interact with each other and create significant clusters.

Discussion & Conclusions: For the first time, we performed integrated pathway analyses of all MS-based SSc proteomic biomarkers. They are essential for producing new data associated with the disease pathogenesis and identifying the biological mechanisms and molecular interactions that lead to the disease. They also highlight the complexity of SSc and indicate that further investigation of the extracted pathways/biological processes and interactions may enable study of the disease from a different perspective.

Evaluation of mono- and bi-functional GLOBE-based vectors for therapy by HBBβAS3 gene addition and mutation-specific RNA interference

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Abstract

Introduction: Therapy by gene addition of the anti-sickling β-globin transgene βAS3 is potentially curative for all β-hemoglobinopathies and therefore of particular clinical and commercial interest. **Material & Methods**: This study investigates GLOBE-based lentiviral vectors (LVs) for βAS3 addition and evaluates strategies for increased β-like globin expression without vector dose escalation. **Results:** First, we report the development of a GLOBE-derived LV, GLV2-βAS3, which compared to the parental vector adds anti-sickling action and a transcription-enhancing 850-bp terminator element, retains high vector titers, and allows superior β-like globin expression in primary patient-derived hematopoietic stem cells (HSCs). Second, prompted by our previous correction of HBB^{IVSI-110(G>A)} thalassemia based on RNApol(III)-driven shRNAs in mono- and combination therapy, we analyzed a series of novel LVs for RNApol(II)-driven constitutive or late-erythroid expression of HBB^{IVSI-110(G>A)}-specific miR-NA30-embedded shRNAs (miR30-shRNAs), including bifunctional LVs allowing concurrent βAS3-globin expression. LVs were thus initially compared for their ability to achieve high β-like globin expression in HBB^{IVSI-110(G>A)}-transgenic cells, before evaluation of shortlisted candidate LVs in HBB^{IVSI-110(G>A)}-homozygous HSCs. The latter revealed that β-globin promoter-driven designs for monotherapy with HBB^{IVSI-110(G>A)}-specific miR30-shRNAs only marginally increased β-globin levels compared to untransduced cells, whereas bifunctional LVs combining miR30-shRNA with βAS3-globin expression showed disease correction similar to that achieved by the parental GLV2-βAS3 vector. **Discussion & Conclusions**: Our results establish the feasibility of high titres for LVs containing the full HBB terminator, emphasize the importance of the HBB terminator for high-level expression of HBB-like transgenes, qualify the therapeutic utility of late-erythroid HBB^{IVSI-110(G>A)}-specific miR30-shRNA expression, and highlight the exceptional potential of GLV2-βAS3 for the treatment of severe β-hemoglobinopathies.

The Complexity of RCCX Locus in the Diagnosis of Congenital Adrenal Hyperplasia

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Abstract

Introduction: Genetic abnormalities in the CYP21A2 gene are associated with 95% of cases causing Congenital Adrenal Hyperplasia (CAH). The gene is part of the RCCX locus, which is located in the major histocompatibility complex (MHC) class III region on chromosome 6p21.3. RCCX structure consists of four genes - RP, C4, CYP21 and TNX - and the number of RCCX segments ranges between one (monomodular) and three (trimodular) on a chromosome. The functional genes RP2, C4B, CYP21A2 and TNXB reside in tandem with their highly homologous pseudogenes RP1, C4A, CYP21A1P and TNXA, leading to unequal crossover during meiosis. Such events between CYP21A2 and CYP21A1P or between the TNXB and TNXA produce a non-functional chimeric gene. To date, nine types of CYP21A1P/CYP21A2 chimeric genes and three types of TNXA/TNXB chimeric genes have been identified. In this study, we present an efficient approach to identify and characterize the chimeric genes in patients with CAH.

Material & Methods: Forty-four genomic DNA samples from patients previously identified with various genetic defects in the CYP21A2 gene were re-analyzed for chimeric genes using multiplex ligation-dependent probe amplification (MLPA) and long-range PCR, followed by Sanger sequencing and restriction enzyme digestion. In addition, 46 genomic DNA samples previously screened by sequencing and identified as carriers for the pathogenic p.Gln319Ter, were tested by MLPA and a real-time PCR Copy number Variation (CNV) assay.

Results: Eight patients were found to carry in heterozygosity a CYP21A1P/CYP21A2 chimeric gene, two to carry in heterozygosity a TNXA/TNXB chimeric gene and one carried in compound heterozygosity two CYP21A1P/CYP21A2 chimeric genes. Furthermore, both MLPA and CNV analyses by real-time PCR confirmed the RCCX bimodular haplotype in 19/46 (41.30%) p.Gln319Ter carriers and the RCCX trimodular haplotype the remaining 27/46 (58.70%) p.Gln319Ter carriers.

Discussion & Conclusions: Using the applied methodologies, we were able to identify and characterize the types of chimeric genes in cases that underwent unequal crossover during meiosis. Furthermore, by analyzing the genetic architecture of the RCCX locus, we were able to distinguish p.Gln319Ter carriers into those with the bimodular and those with the trimodular RCCX haplotype. Therefore, it is extremely important the detection these haplotypes for prenatal diagnosis, treatment and genetic counseling in patients with CAH.

Bespoke PGT-M: from in silico to implementation

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Abstract

Introduction: Preimplantation genetic testing for monogenic disorders (PGT-M) is a molecular prevention test provided to couples with high reproductive risks for a single-gene genetic disorder. PGT-M combines molecular genetic testing with assisted reproductive technology (ART) to ensure the transfer of unaffected embryos after IVF.

Genetic testing is performed on single blastomere or 4-8 cell trophectoderm biopsies from cleavage-stage or blastocyst-stage embryos, respectively. Every PGT-M assay is designed or tailored to meet the unique demands of each family.

Here we present the current strategy and methodology followed by our laboratory for designing and implementing bespoke PGT-M assays.

Materials and Methods: Couples with family history and high genetic risks for a specific diagnosed disorder. Feasibility study for evaluating the gene and the pathogenic variation(s) responsible for the specific genetic disorder, and the initial assessment of the genomic and flanking sequences for the presence of potentially usable polymorphic sites for linkage analysis.

In-silico design of a combined multiplex PCR assay for direct mutation detection and linkage analysis. For linkage analysis, potential short tandem repeat (STR) polymorphisms are identified which should be both proximal and distal to the gene. Selection is based on a number of criteria to ensure that the assay will be robust.

Optimization entails the assessment of the suitability of the designed STR markers and primers on DNA samples from the family, followed by a series of trials to establish optimal sensitivity and specificity of the test.

Validation by application of the optimized assay on 40 single-cell samples in at least 5-6 separate experiments.

Results: PGT-M methodology was introduced in 2004; since then, more than 500 cases have been successfully examined with no report of misdiagnosis. We have successfully developed, validated, and implemented PGT-M assays for more than 35 monogenic disorders, such as β-thalassaemia (autosomal recessive), Huntington's Disease (autosomal dominant), Duchene Muscular Dystrophy (X-linked).

Conclusion: Our data demonstrates that PGT is an efficient and reliable tool in reproductive care and that the Bespoke PGT-M assay design allows couples with reproductive risks to achieve a healthy pregnancy without the stress and risks of invasive prenatal testing.

A significant increase in the PGT-M uptake has been observed, especially with expanding gene panels for carrier screening. This demonstrates the utility of offering PGT-M prospectively to couples at risk for various heritable disorders.

The investigation of genetic and environmental risk factors of ALS in Cyprus

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Abstract

Introduction: Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly lethal degenerative disease of motor neurons, presenting with relentlessly progressive muscle atrophy and weakness. Since the identification of the first causative gene SOD1 in the 1990s and with recent advances in genetics, more than 50 potential causative or disease-modifying genes have been identified, with SOD1, TARDBP, FUS and C9orf72 being the most common. However, the etiology of ALS remains unexplained for over 85% of all cases, suggesting that various environmental risk factors are implicated in the pathogenesis of the disease. This study aimed to conduct a detailed genetic epidemiological investigation and to detect potential exogenous risk factors of ALS in the Cypriot population.

Material & Methods: A total of 82 ALS patients including 21 fALS (26%) and 61 sALS (74%), provided the cohort for the variant screening in the most common causative genes of ALS including C9of72, SOD1, TARDBP, FUS, ATXN2, and SMN1. In addition, a case-control study was conducted with a total of 56 ALS patients and 56 healthy controls of Greek-Cypriot ethnicity. Demographic, lifestyle characteristics and environmental risk factors were collected through the use of a detailed questionnaire.

Results: One patient with the pathogenic c.800A>G (p.Asn267Ser) genetic variant in the TARDBP (1.25%) and 16 additional patients with a pathogenic hexanucleotide repeat expansion in C9orf72 (20%) have been identified. No pathogenic variants have been identified in the remaining genes. Furthermore, statistical analysis of the case-control study revealed no significant difference in the demographic characteristics between the two groups. Logistic regression analysis will be performed for the exogenous risk factors including exposures to chemicals, head trauma and electric injury.

Discussion & Conclusions: Collectively, findings indicate that C9orf72 repeat expansions are indeed causative for ALS in the Cypriot population, and agree with findings from other European countries. However, genetic clusters of the pathogenic variants in the remaining genes are not present in the Cypriot population, as seen in other neighborhood countries. Finally, the case-control investigation will shed some light on the nature of ALS epidemiology in Cyprus, by demonstrating a number of environmental determinants of ALS in the Cypriot population.

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

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Abstract

Introduction: Endoplasmic reticulum mitochondria contact sites (ERMCs) are a highly dynamic platform of communication between the endoplasmic reticulum (ER) and the mitochondrial network implicated in several biological processes. Distortions in ER-mitochondria communication have been linked to various pathological conditions including metabolic disorders. ERMCs have been recently identified as important hubs for insulin signaling. In addition, perturbations in ERMCs integrity have been associated with insulin resistance. Nevertheless, because of contradicting reported findings, the precise role of ERMCs in insulin resistance remains elusive. Starch binding domain-containing protein 1 (Stbd1) is an ER-resident, glycogen-binding protein of poorly characterized function which also localizes at ERMCs. As previously reported, Stbd1 was demonstrated to undergo co-translational N-myristoylation which determines its subcellular targeting. The non-N-myristoylated form of the protein Stbd1(G2A) was shown to be preferentially targeted to the ERMCs and enhance ER-mitochondria association. The non-N-myristoylated Stbd1(G2A) variant can therefore serve as a useful tool to address the effects of enhanced ER-mitochondria communication in both physiological and pathological processes. The current study aims to address the consequences and implications of increased ER-mitochondria association in mitochondrial function, glycogen metabolism and insulin resistance in vitro.

Material & Methods: To this end, hepatocyte AML12 cell lines stably overexpressing either Stbd1(G2A) or wild-type Stbd1 were generated. AML12 cells overexpressing the unrelated GFP protein served as controls. The above cell lines were used to study glycogen metabolism as well as several biological processes related to ERMCs such as calcium transfer, ER-mitochondria interactions, mitochondrial dynamics, morphology and respiratory function.

Results: As revealed by proximity ligation assay, the overexpression of Stbd1(G2A) resulted in increased ER-mitochondria interactions. In addition, immunofluorescence staining displayed considerable changes in mitochondrial morphology in cells overexpressing Stbd1(G2A). At the ultrastructural level, mitochondria appeared arranged in prominent clusters consisting of small fragmented mitochondria. Furthermore, cells overexpressing Stbd1(G2A) were found to exhibit significantly reduced glycogen content as compared to cells overexpressing the wild-type form of the protein. Despite the prominent alterations in mitochondrial morphology induced by Stbd1(G2A) overexpression, mitochondrial dynamics were not affected. As ERMCs serve as platforms for calcium transfer between the ER and mitochondria, calcium influx into mitochondria in addition to the levels of calcium in the ER,

mitochondria and cytoplasm were determined. Cells overexpressing Stbd1(G2A) displayed significantly reduced mitochondrial calcium uptake and increased mitochondrial calcium content. Consistent with the alterations in mitochondrial calcium dynamics, Stbd1(G2A) cells displayed impaired mitochondrial respiratory function. Interestingly, overexpression of wild-type Stbd1 was found to significantly improve insulin resistance induced by insulin treatment in AML12 hepatocytes, more efficiently than Stbd1(G2A).

Discussion & Conclusion: Our findings indicate that enhancement of ER-mitochondria association induced by Stbd1(G2A) overexpression results in disturbed mitochondrial morphology and respiratory function. The above appears to impact on glycogen metabolism and the response to insulin and may indicate broader changes in cell metabolism.

Alterations in Circulating miRNA Levels after Infection with SARS-CoV-2 Could Contribute to the Development of Cardiovascular Diseases

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Abstract

Introduction

The novel coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and poses significant complications for cardiovascular disease (CVD) patients. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and influence several physiological and pathological processes, including CVD.

Material & Methods

This critical review aims to expand upon the current literature concerning miRNA deregulation during the SARS-CoV-2 infection, focusing on cardio specific miRNAs and their association with various CVDs, including cardiac remodeling, arrhythmias, and atherosclerosis after SARS-CoV-2 infection.

Results

Despite the scarcity of research in this area, our findings suggest that changes in the expression levels of particular COVID-19-related miRNAs, including miR-146a, miR-27/miR-27a-5p, miR-451, miR-486-5p, miR-21, miR-155, and miR-133a, may be linked to CVDs.

Discussion & Conclusions

While our analysis did not conclusively determine the impact of SARS-CoV-2 infection on the profile and/or expression levels of cardiac-specific miRNAs, we propose a potential mechanism by which the miRNAs mentioned above may contribute to the development of these two pathologies. Further research on the relationship between SARS-CoV-2, CVDs, and microRNAs will significantly enhance our understanding of this connection and may lead to the use of these miRNAs as biomarkers or therapeutic targets for both pathologies.

CHD2 pathogenic nonsense variant in a three-generation family with variable phenotype and a paracentric inversion 16

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Abstract

Introduction

Chromosomal inversions are usually balanced, with no impact on the carrier's phenotype. Their main clinical consequence is the risk for unbalanced gametes and affected offspring. Rarely though, inversions are associated with a clinical phenotype, mainly due to sub-microscopic Copy Number Variants (CNVs) or disruption at the breakpoints of a functionally important gene and/or genomic elements. Here we report a female paediatric patient with a familial paracentric inversion on chromosome 16 segregating in affected and unaffected family members with discordant phenotypes.

Material & Methods

Karyotype and Fluorescence in situ hybridization (FISH) were carried-out using standard procedures and a chromosome-specific multicolor probe set. For array-based Comparative Genomic Hybridization (array-CGH), SurePrint ISCA array (Agilent) was used. Clinical Exome Sequencing (CES) applied TruSight One panel on NextSeq500 (Illumina). Data was processed with an in-house bioinformatics pipeline.

Results

A paracentric inversion [inv(16)(q22.3q24.1)] was detected and confirmed by FISH, in a three-generation family with discordant phenotypes with/without epilepsy and/or intellectual impairment, as well as with an unaffected carrier. Array-CGH revealed no CNVs. CES detected a pathogenic nonsense variant in CHD2 gene [NM_001271.4:c.5035C>T p.(Arg1679Ter)], associated with Developmental and Epileptic Encephalopathy 94. Family testing showed that the variant segregated with phenotypic heterogeneity in the affected individuals and is probably causative.

Discussion & Conclusions

To the best of our knowledge, this is the first CHD2 pathogenic variant segregating in a three-generation family and the fourth familial case reported. These results further support our previous findings that the majority of familial, balanced rearrangements with discordant phenotypes in the same family are coincidental.

Case report demonstrating certain pitfalls and challenges in NGS data interpretation.

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Abstract

Introduction

Menke-Hennekam syndrome-1 (MKHK1) is a congenital disorder characterized by developmental delay, intellectual disability, variable facial characteristics, as well as, feeding difficulties, autistic behaviour, recurrent upper airway infections, hearing impairment, short stature, and microcephaly. It is caused by heterozygous mutations in exon 30 or 31 of the CREB Binding Protein (CREBBP) gene, whereas mutations elsewhere in the CREBBP gene result in Rubinstein-Taybi syndrome-1 (RSTS1), which is phenotypically distinct.

Material & Methods

The patient along with his parents was referred for trio-based clinical exome sequencing (CES). The patient is a 21-month-old boy with severe global developmental delay, failure to thrive, arched eyebrows, long lashes and prominent forehead.

CES was performed on Illumina NextSeq 2000 platform using TruSight One sequencing panel. Bioinformatic analysis, annotation and interpretation were performed with the VarSome Clinical platform (version 11.3, hg19).

Sanger sequencing confirmed the CES findings.

Results

A de novo, missense variant was identified and confirmed by Sanger Sequencing, at exon 31 of NM_004380.3 transcript in CREBBP gene (NC_000016.9:3779680A>G), resulting in a cys-to-arg substitution at codon 1790 (Cys1790Arg).

Discussion & Conclusions

Based on the clinical data, the detected variant was linked to the patient's phenotype. Even though the same gene is responsible for another phenotypically distinct syndrome (RSTS1), the specific exon where the variant is located has differentiated the final diagnosis to MKHK1. This finding highlights the pitfalls and challenges in NGS data interpretation; the importance of a detailed phenotypic description in combination with an in-depth review of all gene-related data available through literature and databases, is determinant to reach an accurate genetic diagnosis.

LIN28B controlled expression system for controlled expression of the transcriptional repressor BCL11A and foetal haemoglobin

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Abstract

Introduction

Sickle cell disease (SCD) is a recessively inherited monogenic disorder caused by a single nucleotide substitution in the open reading frame of the β-globin gene, leading to the production of sickle-haemoglobin (HbS). Hereditary Persistence of Foetal Haemoglobin (HPFH) ameliorates the SCD phenotype, thus many research efforts are focused on reactivation of foetal haemoglobin (HbF) expression, including identifying new potential pharmacological targets. Such a target is LIN28B which was found to reduce the translation of BCL11A, a transcription factor directly repressing the foetal haemoglobin genes HBG1 and HBG2 in adult human erythroid cells. In order to investigate the kinetics of LIN28B-mediated HbF regulation, an expression system that can be switched on and off in a controlled manner would pose an ideal way to carry this out.

Material & Methods

HUDEP2 cells, a model for human adult erythropoiesis, normally do not express LIN28B and have completely silenced HBG1/2 genes. A LIN28B-HiBiT/HaloTag construct (LIN28B-HH) was prepared and transduced in HUDEP2 cells. Expression of LIN28B-HH protein can be conveniently monitored with the Nano-Glo lytic detection system which sensitively quantifies HiBiT-tagged proteins in cell lysates. Upon addition of HaloPROTAC3, the expression of LIN28B-HH can be decreased through targeted protein degradation via the VHL E3 ligase. Thus, the expression of LIN28B-HH can be monitored and controlled quickly and efficiently, allowing subsequent investigation of the effects on BCL11A and HBG1/2 expression.

Results

We have found that overexpression of LIN28B in HUDEP2 cells leads to a decrease in BCL11A protein levels and reactivation of HBG1/2 expression. Expression of LIN28B-HH was monitored before and during the treatment with HaloPROTAC3, in both a low-expressing clone and the pool, to assess the kinetics of HaloPROTAC3-mediated LIN28B degradation. In both cases, the signal loss was similarly fast, as well as signal recovery after HaloPROTAC3 withdrawal.

Discussion & Conclusions

The expression of LIN28B-HH was successfully controlled with the use of HaloPROTAC3 over a period of time, which allows investigation of the kinetics of LIN28B in association with BCL11A and HbF levels. HaloPROTAC3 can be used as a toggle for LIN28B-HH expression in HUDEP2 cells. The detailed knowledge of the LIN28B pathway that was previously described combined with the investigation of the protein's kinetics may be translated into clinical applications aimed at increasing HbF levels in SCD patients.

Investigation of a novel muscle communication pathway

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Abstract

Background

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

Methods

To achieve our aim we designed oligonucleotides and administered them intramuscularly in mice. An antagomiR against miR-133b was initially used to assess the downregulation of miR-133b in distant tissues. Three different versions of the antagomiR were designed, a 3â€² cholesterol conjugation, a 5â€² cholesterol conjugation and one without a cholesterol conjugation. Differences of the conjugate effect on the communication pathway were assessed by examining the efficacy and biodistribution of each antagomiR. Intramuscular and intravenous administration methods were also compared. Moreover, fluorescently labelled miR-133b mimic with similar chemistry to the antagomiR was designed in order to assess its biodistribution.

Results

Intramuscular injections in the tibialis anterior (TA) of mice showed remarkable systemic delivery, reaching a reduction of miR-133b levels of ~40-50% in the heart in all versions of the inhibitor at a dose of only 3mg/kg. Interestingly, the 5â€² modification showed enhanced downregulation of miR-133b in various skeletal muscles, over the 3â€² cholesterol, reaching 40-50% downregulation. Intramuscular administration of 5â€²-antagomiR showed enhanced inhibition of target miRNAs in skeletal muscles compared to intravenous. The miR-133b mimic showed similar biodistribution to the distant tissues where the antagomiR showed miR-133b downregulation.

Conclusion

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

Molecular characterization of patients with gliomas using multi-gene next-generation sequencing panel

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Abstract

Gliomas are the most common primary intracranial tumour, characterised by poor prognosis and high mortality in adults. Prognosis and therapy are driven partly by molecular characterisation of the disease. The present study provides a comprehensive understanding of the molecular profile and clinical features of 32 patients diagnosed with glioma using an 80-gene targeted NGS panel. Clinicopathological characteristics and outcomes were associated with molecular findings. In total, 129 genetic alterations including 33 structural variants were identified in 38 distinct genes. Among 96 variants (single nucleotide variants and insertions and deletions), 38 were pathogenic and 58 variants of unknown clinical significance. TP53 was the most frequently mutated gene, followed by PTEN and IDH1 genes. Glioma patients with IDH1 mutant tumours were younger and had significantly longer overall survival compared to patients with wild-type IDH1 tumours. Subsequently, a comparison of mutational profiles of samples analysed by 2 independent assays was also performed. The comprehensive 80-gene pan-cancer panel identified 24 additional variants, 22 of which were in regions that were not targeted by the 55-gene panel. Overall, the present study demonstrated that using an extended tumor profile assay instead of a glioma-specific tumor profile panel identified additional genetic changes that may be taken into consideration as potential therapeutic targets for glioma diagnosis and molecular classification.utility of these protein biomarkers in routine clinical practice.

Prevalence of the p.Cys1400Ter pathogenic CFTR mutation in Cyprus: Evidence of a founder effect

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Abstract

Introduction

Cystic Fibrosis (CF) and CFTR-related disorders (CFTR-RD), can be largely attributed to the high variety of mutations found in the CFTR gene. In the island of Cyprus, several rare CFTR mutations have been identified, among them the rare p.Cys1400Ter.

Material and Methods

Genotypic analysis of the CFTR gene was performed using the Devyser CFTR NGS kit on an Illumina MiSeq platform using a 150 bp paired-end reads with a minimum coverage of 150X. Read alignment and variant calling were done with the Amplicon Suite software. Haplotype analysis was also performed by multiplex PCR using five STR markers flanking the CFTR gene and analysed by capillary electrophoresis on an ABI 3500xl genetic analyser.

Results

The p.Cys1400Ter mutation has so far been identified in 6 probands from 6 non-related Cypriot families and in a total of 17 subjects. Three of these are diagnosed with CF, presenting with persistent respiratory symptoms, pancreatic insufficiency and a second CF-causing mutation. Two are diagnosed with CFTR-RD, presenting with bronchiectasis, intermediate sweat test and a second mutation. Also, three carriers have a high suspicion of CFTR-RD, with bronchiectasis and intermediate sweat test, although due to the lack of another CFTR mutation and a second functional test, definite diagnosis has not been made. In all carriers, p.Cys1400Ter mutation was found to co-segregate with the intronic variant c.489+3A>G in the same allele. Interestingly, the results of haplotype analysis provided evidence of a common haplotype in all individuals with p.Cys1400Ter.

Discussion & Conclusions

The provided molecular evidence speculates that p.Cys1400Ter co-segregated with c.489+3A>G is an ancestral variant combination that has spread in Cyprus due to a possible founder effect. Due to its common prevalence, it has been added to the regular testing panel of CFTR mutations in the island of Cyprus.

Apparently benign cryptic complexity in an affected carrier of a de novo translocation

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Abstract

Introduction

The majority of apparently balanced translocation (ABT) carriers are phenotypically normal; however, reproductive complications are often reported. Furthermore, phenotype association has been estimated in ~27% of de novo cases due to direct dosage-sensitive gene disruption, cryptic complexity, and long-range position effect. Therefore, detailed breakpoint mapping and derivative chromosome delineation are essential to identify causal genes, underlying molecular mechanisms and genotype-phenotype correlations, as well as to accurately estimate reproductive risks.

Materials & Methods

We report a follow-up investigation of a prenatal case with right-sided aortic arch and a de novo ABT [46,XX,t(6;19)(q13;p13.3)dn]. Postnatally, whole-genome mate-pair sequencing (WG-MPS), followed by Sanger sequencing, were performed in the affected child to further characterize this rearrangement.

Results

WG-MPS mapped one breakpoint on der(19) and at least five on der(6). The resulting fragments were repositioned in a random order and orientation, while a ~6kb chr19 segment was apparently deleted. Microhomology and small indels were identified at the breakpoints, while no causal genes were found within the disrupted topologically-associated domains.

Discussion & Conclusion

Our study revealed cryptic complexity in a two-way reciprocal ABT. The breakpoint junction signatures suggested non-homologous end joining as the mechanism underlying this chromothripsis event. Preliminary WG-MPS results did not provide an obvious cause for the patient's phenotypes. Further investigation is needed, including whole-exome sequencing, to decipher the aetiology of right-sided aortic arch. The identified cryptic complexity provides useful insights into the meiotic segregation of derivative chromosomes and the associated increased reproductive risk, therefore appropriate genetic counselling is strongly recommended. In conclusion, WG-MPS successfully delineates the complexity of de novo ABTs, which may or may not be causative for the patient's phenotype.

GenoMed4All: Artificial intelligence-based deep learning algorithms for patients with sickle cell disease

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Abstract

Aim

Improve prediction of SCD disease severity by developing AI-based deep learning algorithms.

Patients & methods

1000 SCD patients >1 year of age from ten European hospitals, with HbSS, HbSβo, HbSC and HbSβ+ genotypes will be enrolled. Standardized collection of data will be performed including: GWAS, metabolomics, radiomics, Lorrca oxygenscan analysis. Based on predefined and newly identified SNPs, patient clustering will be performed. Integration of collected covariates will facilitate deep learning AI-based algorithms.

Results

Study protocol has been validated and approved. Enrollment of patients has just started in two hospitals; all 1000 patients will be enrolled by the end of 2023. Subsequently, deep learning algorithms will be developed and validated to predict SCD severity (e.g. microalbuminuria, silence cerebral infarctions, stroke, vaso-occlusive crisis etc.). Following a stepwise-approach, we will first predict single clinical outcomes and radiomics patterns, then develop predictions of complete clinical phenotypes.

Conclusion

Widespread use of novel technologies including AI, has already modified diagnostic research. Combining data of 1000 SCD patients in GenoMed4All is a necessary step to improve robustness of study results. Concomitantly, standardization and linkage of SCD data repositories is promoted through this ERN-EuroBloodNet collaboration. Of course, full compliance with data protection legislation and ethical principles is safeguarded. Implementation of AI algorithms and linking of SCD health repositories promises to enhance diagnostics, to predict disease outcomes and to individualize treatment options, so urgently needed for SCD.

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Evaluation of induction of fetal hemoglobin synthesis by genome editing of cis- and trans-acting components of the β-globin locus

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Abstract

The reactivation of γ-globin can ameliorate the clinical phenotype of β-hemoglobinopathies by functional compensation of β-globin deficiency and anti-sickling action of fetal hemoglobin (HbF). Most importantly, it constitutes a universal therapy approach that can potentially be applied to all β-hemoglobinopathy patients, irrespective of genotype. This work focuses on genetic modifica-tion of globin expression regulators and the β-globin locus as potential therapeutic approaches for β-hemoglobinopathies by reactivation of γ-globin. To this end, we employed the clustered reg-ularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system to abolish expression of two known γ-globin gene repressors, BCL11A and ZBTB7A (trans editing), and a dual-targeting single RNA-guided CRISPR/Cas12a system to create a large (7.4-kb) β-δ in-tergenic deletion at the β-globin locus (cis editing). Tools were delivered as ribonucleoprotein (RNP) complexes by nucleofection of human umbilical cord blood-derived erythroid progenitor-2 (HUDEP-2) cells and primary thalassemic CD34+ cells. Edited cells were assessed for on-target editing efficiency by Inference of CRISPR Edits (ICE) and digital polymerase chain reaction (dPCR), and analysed for globin and hemoglobin expression after induction of erythroid differentiation, by high-performance liquid chromatography (HPLC). HbF expression was also analyzed by flow cytometry and subpopulations of high and low HbF-expressing cells within each bulk population of edited cells were isolated by fluorescence-activated cell sorting (FACS) and analyzed for dif-ferential on-target editing levels. The study suggests that generation of the 7.4-kb cis deletion at the β-locus, relying on the highly efficient non-homologous end-joining (NHEJ) repair mechanism of double-strand breaks (DSBs), may lead to higher HbF levels than the disruption of trans-acting components also involved in other essential functions of hematopoiesis, as are the γ-globin re-pressors BCL11A and ZBTB7A..

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

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Abstract

Introduction

Plasma membranes contain specific microdomains, the so-called lipids rafts. In neurons, lipid rafts are present in the axonal plasma membrane and are important for neuronal function. These domains are rich in sphingolipids, especially sphingomyelin, ceramide (Cer) and cholesterol and act as a platform for signal transduction molecules. Many of these molecules, such as the Src-family kinases and Rho GTPases are involved in regulation of the actin cytoskeleton, endocytosis, vesicular trafficking and axon guidance. Additionally, the formation of exosomes has been associated with lipid rafts.

Acid ceramidase (AC), a key regulatory enzyme of Cer metabolism, catalyses the conversion of Cer into a fatty acid and sphingosine inside the lysosomes and synthesises Cer at a more alkaline pH. AC deficiency due to mutations in the ASAH1 gene, leads to the early onset of the Farber disease, an autosomal recessive fatal lysosomal storage disorder. Mutations in the same gene have also been associated with Spinal Muscular Atrophy (SMA) or with SMA with Progressive Myoclonic Epilepsy. SMA is characterized by the degeneration of motor neurons in the spinal cord leading to symmetric muscle weakness and atrophy and is mostly caused by a homozygous deletion of the SMN1 gene.

In our previous work, a stable knockdown SH-SY5Y cell line was established and used to determine the functional significance of AC in neurons. Phenotypic alterations that are commonly observed in neurodegenerative diseases, were observed in AC-depleted cells including; distribution of lysosomes towards the cell periphery and significantly shortened and less branched neurites upon differentiation. Lipidomic analysis showed alteration in various lipids combined with altered of gene transcription of enzymes that are involved in the metabolism of ceramide, suggesting dysregulation of lipid rafts. The aim of this work was to investigate whether AC plays a role in particular pathways, maintaining the normal phenotype and function of neurons.

Materials & Methods

Two stable neuroblastoma SH-SY5Y cell lines have been used; ASAH1 knockdown (ASAH1KD) and ShScramble (control), to evaluate the mRNA and protein levels of particular selected targets by performing real-time PCR and WB analysis. G-Lisa assays of Rho GTPase family members were also performed.

Results

The mRNA and protein levels of various targets were significantly altered in ASAH1KD cells compared to shScramble cells. These proteins are involved into specific pathways related to lysosome positioning/function, autophagy, multivesicular bodies–exosome formation and signal transduction pathways, all related to lipid rafts. Additionally, survival motor neuron protein (SMN) and many components of the spliceosome were found to be altered in ASAH1KD cells, indicating a functional link

between AC and SMN. Lastly, dysregulation of the expression levels of specific Src-Tyrosine kinases and activity of Rho GTPases was present in ASAH1KD cells, underlining the critical role of AC in signal transduction pathways.

Discussion & Conclusion

The results indicate that AC-depletion may lead to disorganization of lipid rafts and thus dysregulation of pathways related to them, highlighting the significance role of AC in neurons and in SMA related pathogenic pathways. Further studies could lead to identification of novel molecules for the development of new SMA therapies.

MYH9: a promising partner of filtrin, a protein member of the kidney slit diaphragm

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Abstract

Introduction

Filtrin (Neph3) is a transmembrane-type I protein and a member of the immunoglobulin superfamily of the slit diaphragm (SD) multiprotein complex. Currently, there are no reports on NEPH3 mutations in humans associated with kidney diseases. Findings of our group have suggested that NEPH3 could be acting as a modifier gene by predisposing to a more severe phenotype of patients with documented thin basement membrane nephropathy (TBMN) (Voskarides et al, 2017). More specifically, in a set of 96 patients with TBMN categorized as severe or mild according to the presence or not of proteinuria, our group detected a deleterious SNP, causing a substitution of the highly evolutionarily conserved valine in position 353 by methionine (NEPH3-p.V353M). Additionally, coimmunoprecipitation experiments showed an increase in the interaction of NEPH3-p.V353M with NPHS1-wt and with NEPH3-wt or NEPH3-p.V353M itself (Voskarides et al, 2017).

Materials, methods and results

The study aims to find potential filtrin interacting partners. To achieve this, we performed immunoprecipitation experiments using NEPH3-FLAG in transiently transfected undifferentiated podocytes. SDS/PAGE was employed to separate the precipitates, and mass spectrometry was utilized to determine any potential partners. The discovery of Myh9, an actin-associated motor protein, was an intriguing finding. This interaction was confirmed through additional immunoprecipitation experiments. Moreover, reciprocal experiments were performed, this time precipitating the endogenously expressed Myh9 in human undifferentiated podocytes or podocytes transiently transfected with NEPH3-wt-FLAG. Following immunoprecipitation results, immunofluorescence

experiments were contacted to investigate Neph3 and Myh9 co-localization in human differentiated podocytes. Myh9 distribution was observed in a filamentous pattern extending to the cell surface, analogous to the pattern of Neph3 expression. The co-distribution of Neph3 and Myh9 to the foot process layer, which lines the capillary loops of the podocytes, was then shown in immunofluorescence experiments on mouse kidneys. Lastly, double labelling on normal human kidneys, using immune-gold staining demonstrated that both Neph3 and Myh9 are localized in the immediate proximity of slit diaphragms.

Discussion and conclusions

To detect new interacting partners of Neph3 other than Nphs1, Neph1 and Nphs2, an experimental approach using IP, mass spectrometry and immunostaining was employed. This process provided for the first time evidence of direct or indirect interaction of Neph3 with Myh9; an important cytoskeletal protein primarily expressed in glomerular podocytes, implicated in the development of FSGS and other rare diseases known as MYH9-related diseases (Sekine et al., 2010, Alhindawi and Al-Jbour, 2009, Epstein et al., 1972, Ghiggeri et al., 2003, Moxey-Mims et al., 1999, Naito et al., 1997, Peterson et al., 1985, Turi et al., 1992, Yap et al., 2009, Clare et al., 1979).

A THRA pathogenic variant in twin sisters with congenital hypothyroidism

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Abstract

Introduction

Congenital non-goitrous Hypothyroidism, 6 (CHNG6) is an autosomal dominant condition characterised by growth and developmental retardation, relatively low serum T4 and high serum T3 levels and delayed bone development including skeletal dysplasia reminiscent of congenital hypothyroidism. Thyroid hormone receptor (TRs) THRA is one of the several nuclear receptors for thyroid hormone that when mutated confers a resistance to thyroid hormone. The first THRA germline mutations were reported in 2013.

Materials & Methods

Twin sisters presenting with ataxic gait, poor fine motor coordination, dysarthria, truncal obesity, dysmorphic features and mild intellectual disability. Personal history was significant for severe generalized hypotonia, feeding difficulties, global developmental delay and learning difficulties. They are treated for hypothyroidism since early childhood. Previous extended investigations including karyotype, metabolic screening and molecular karyotype were non conclusive. Family history was non-contributory. Exome sequencing was performed with Agilent's Exome V8 NGS panel and data were analysed using Franklin. Sanger sequencing confirmed findings. Parents and the rest of the family were tested by Sanger sequencing.

Results

The THRA c.1187dupT p.(Phe397LeufsTer10) truncating pathogenic variant, was found in heterozygous state in the two probands, and was absent from their parents. The variant was the first reported mutation

in THRA and since then has been reported in literature in at least three affected individuals, and although it lies at the last exon of THRA, it results to pathogenicity by acting as a dominant-negative mutation.

Conclusion

We present the case of Congenital non-goitrous Hypothyroidism, 6 (CHNG6) in two monozygotic sisters harbouring a de novo pathogenic variant in THRA gene. To our knowledge about 10 individuals from 8 families were found to harbour a THRA pathogenic variant and this study can be incorporated to the limited reported cases published. In addition this study highlights the importance of the pathogenicity of truncating mutations even if these are located at the C-terminal of a protein as the mechanism of pathogenicity might be a dominant negative one.

The finding of a pathogenic variant in PBX1 as a confirmation of the diagnosis of CAKUTHED Syndrome

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Abstract

Introduction

CAKUTHED is a recently described (2017) autosomal dominant rare syndrome which compiles of Congenital anomalies of kidney and urinary tract syndrome with or without hearing loss, abnormal ears, developmental delay and other. CAKUTHED is a highly pleiotropic developmental condition (PMID: 28566479, PMID: 29036646).

PBX1 is an essential developmental transcription factor. Transcriptional misregulation in cancer and Preimplantation Embryo are two of its associated mechanisms. Annotations in the Gene Ontology (GO) for this gene include DNA-binding transcription factor activity and protein heterodimerization activity. Recently it has been connected with congenital heart anomalies as well as acute lymphoblastic leukaemia**.**

Materials and Methods

We studied a male patient, significantly short statured with macrocephaly, dysmorphic facial features and large malformed ears. Global developmental delay in childhood, moderate to severe mental retardation, extremely poor speech and autistic features are noted.. He was diagnosed in early childhood with kidney and ureteral dysplasia which led gradually to renal failure and kidney transplantation. Previous investigations including molecular and metabolic studies were non-conclusive. Exome sequencing was performed in peripheral blood DNA, with Agilent's Exome V8 NGS panel and data were analysed using Franklin software. Sanger sequencing confirmed the findings.

Results

Patient was heterozygous for c.142C>T (p.Gln48Ter) variant in the PBX1 gene. Although, to our knowledge, this nonsense mutation has not been reported earlier, this variant is absent from our population and is expected to result in a truncated PBX1 protein.

Conclusion

CAKUTHED syndrome is a recently described rare condition (2017) attributed to PBX1 (Pre B-cell leukemia Homeobox) gene haploinsufficiency. Its incidence is currently unknown. We suggest that PBX1 gene should be considered as a candidate gene in the investigation of patients with kidney –ureteral anomalies, congenital heart malformations as well as hearing deficit.

Genetic Study of Early Onset Parkinson's Disease in Cyprus

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Abstract

Introduction:

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

Material & Methods:

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

Results:

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

Discussion & Conclusions:

The EOPD-associated genes that were under investigation in this study seem to interact with each other on a structural and functional level. The function of the EOPD-associated genes has been linked to protection against mitochondrial dysfunction, the mediation of mitophagy, and to roles in synaptic transmission and phospholipid remodeling. Hence, variants in either one of the aforementioned genes have the potential to alter cellular processes and potentially increase PD susceptibility. Currently, the diagnosis of PD is clinical and based on the presence of motor features. Early onset patients have a challenging journey towards a PD diagnosis as their initial symptoms may vary and their young age of onset may lead to differential diagnoses. To date, the pathogenic variants identified in this study have explained the PD phenotype for 18.8% of the EOPD cases. Almost 1 in every 5 patients in our cohort has been identified as a carrier of either a PINK1 or PRKN pathogenic variant. Hence, the results of this study may contribute to the genetic screening of EOPD in Cyprus.

CRISPR knock out screening of potential γ globin gene expression regulators and validation studies

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Abstract

Introduction:

Reactivation of γ-globin for the production of HbF can ameliorate β-thalassemia and sickle cell disease. Advanced therapeutic strategies involving addition of a functional β-globin gene or genome editing for γ-globin reactivation are promising, and in particular knockout of γ-globin repressors BCL11A and ZBTB7A, results in high-level γ-globin expression. However, the latter are multifunctional and have wider effects in hematopoiesis, while advanced strategies altogether have high cost and limited availability, which combined make the one-off approaches inaccessible for the majority of patients. By contrast, chronic application of small-molecule drugs, such as hydroxyurea or Luspatercept, is affordable but is toxic and depending on the patient shows variable efficacy. Hence, reactivation of γ-globin with pharmacological means is still a useful and much needed avenue to explore. Hence, we want to identify and validate novel γ-globin repressors as potential druggable targets for new treatments of β-hemoglobinopathies.

Material & Methods:

A custom lentiviral library of gRNAs targeting 293 candidate genes was generated and transduced into HUDEP-2 cells, followed by FACS-based enrichment of high γ-globin expressors and identification of corresponding candidate gene knockouts. Promising candidate repressor genes are being validated individually by CRISPR/Cas-mediated knock outs based on lentiviral delivery and ribonucleoprotein nucleofection in HUDEP-2 cells. Editing efficiency is tested using ICE (Inference of CRISPR Edits), while

loss of candidate gene expression is tested using immunoblotting. HPLC and immunoblotting are used to investigate the effect of the knock-outs on γ-globin expression levels.

Results:

The CRISPR knock out screen indicated seven candidate genes encoded potential repressors of γ-globin expression. Two of the seven genes are in the process of being validated for repressor activity, one encoding a protein involved in ion transport and iron homeostasis, the other a transcriptional regulator. Initially based on four gRNAs per candidate, consistent high-efficiency editing was achieved for both genes for shortlisted gRNAs. There is no indication that the knocked-out cells are at a selective disadvantage. Surprisingly, for the first two candidates, editing efficiency observed at the DNA level does not translate to reduced levels of the target protein, even for same-day analyses. This has prompted the investigation of molecular causes and of additional candidates.

Discussion & Conclusions:

The CRISPR knock out screen performed has identified seven potential γ-globin repressor genes, which scored as highly during the screening process as some of the well known γ-globin regulators, raising hopes for the identification of novel therapeutic targets for hemoglobinopathies. While multiple repetitions of the screening protocol and validation experiments point away from experimental error, analyses in HUDEP-2 cells have not yet validated these results. Possible causes are being considered, including exon skipping, read-through translation, alternative splicing of the targeted mRNA. Finally, validation of results in CD34+ cells might allow more definite conclusions.

Evaluation of the spatiotemporal intratumor molecular heterogeneity in tumor and liquid biopsies from patients with metastatic colorectal cancer

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Abstract

Introduction:

Despite the widespread establishment of primary prevention, colorectal cancer (CRC) remains the second leading cause of cancer-related death in industrialized western countries. Dynamic molecular evolution of cancer cell sub-populations and intratumor heterogeneity (ITH), as well as metastasis and therapeutic resistance, represent the major causes of relapse and cancer-related death. The mechanisms underlying high drug resistance and relapse rates after multi-modal treatment in patients with colorectal cancer (CRC) and liver metastasis (LM) remain poorly understood.

Materials & Methods:

To assess ITH and serial ctDNA molecular heterogeneity in the perioperative setting, we designed a prospective protocol encompassing multiple intra-lesional and matched plasma samples for each

individual patient. A total of 28 patients diagnosed with metastatic colorectal adenocarcinoma were recruited in the study. In total, 94 FFPE samples were collected from both primary tumors (PTs) and LMs. For 16 out of 18 patients, plasma was collected at multiple time points during treatment, before and after surgery, to assess the molecular dynamics of the disease using liquid biopsies.

Results:

The proportion of patients with ITH were 53% and 56% in primary CRC and LM respectively, while 35% of patients harbored de novo mutations in LM indicating spatiotemporal tumor evolution and the necessity of multi-regional analysis. Among the 56% of patients with alterations in liquid biopsies, de novo mutations in cfDNA were identified in 25% of patients, which were undetectable in both CRC and LM. All 17 patients with driver alterations, harbored mutations targetable by targeted drugs, either approved or currently under evaluation.

Discussion & Conclusions:

Our prospective study provides initial evidence on potential clinical superiority of IPH and warrants the conduction of precision oncology trials to evaluate the clinical utility of ITH-driven matched therapy.

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