ABSTRACT

BOOK

UNIVERSITEIT GENT

CCMAR
Centro de Ciências do Mar
Algarve

Programme

INTEC 2024 event will take place in the amphitheater 1.4 (meeting) of the pedagogic building or in room 102 (workshops) of the *Escola Superior de Educação e Comunicação* (ESEC), both located on the Campus da Penha of the University of Algarve

DAY 1 - September 12, 2024	
09.30	**Registration**
10.00	**Welcome -** Olivier Vanakker & Leonor Cancela
Session 1: ANIMAL MODELS AND HUMAN PATHOLOGIES Chair: Paulo Gavaia and Hervé Kempf	
10.15	**The Japanese medaka fish as model for human chondrodysplasias and heterotopic ossification disorders** Christophe Winkler – National University of Singapore
10.45	**Matrix gla protein mutations in vascular and cartilaginous tissues: new insights from genetic studies** Monzur Murshed – McGill University, Montreal, Canada
11.15	**Modeling developmental capacity and constraint in skeletal differentiation in Akt and PROS somatic mosaic activation in the zebrafish** Matthew Harris – Harvard Medical School, USA
11.45	**In memoriam: Pedro Valdivielso**
12.00	**Lunch**
	Session 2: Oral presentations early career researchers Chair: Leonor Cancela and Olivier Vanakker
13.30	**Mineralisation in the eye: a biomarker and target for treatment** Imre Lengyel - Queen's University Belfast, UK **ENNP1 is associated with ectopic calcification of the Bruch's membrane and functional changes in the mouse retina** Pietro M. Bertelli - Queen's University Belfast, UK **Anti-elastin targeted HSA-based DTPA-loaded nanoparticles resolve subretinal calcifications in a PXE mouse model** Frank Rutsch - Muenster University Children's Hospital, Germany **The role of elastin degradation in the calcification process** Daniela Quaglino - University of Modena and Reggio Emilia, Italy **Activation of the autotaxin - lysophosphatidic acid receptor pathway contributes to the pathogenesis of pseudoxanthoma elasticum** Robbe Derudder - Ghent University Hospital, Belgium

	Therapeutic applications of ENPP1-Fc prevent muscle calcification following cardiotoxin-induced-muscle damage in Abcc6$^{-/-}$ mice Hervé Husson - Inozyme Pharma **Ferroptosis of valvular interstitial cells as a result of erythrophagocytosis promotes calcific aortic valve stenosis** Sven-Christian Pawelzik - Karolinska Institutet, Sweden **The vascular calcification process in PXE** Georges Lefthériotis - University Hospital Nice, France	
15.30	**Coffee break**	
16.00	**Networking**	**WORKSHOP 1:** INTRODUCTION TO PRESENTATION SKILLS by Tom De Moor (participants must bring their computer)
18.30	**Closure of the first day**	
20.00	**Dinner & dance "Restaurant- Bar Castelo"**	
DAY 2 - September 13, 2024		
Session 3: HUMAN CASE STUDIES Chair: Olivier Vanakker and Natércia Conceição		
09.00	**Patients - partners for life** Marta Jacinto - Association of PXE Patients, Portugal	
9.30	**Calcific Aortic Valve Disease (CAVD): expression analysis of osteoblastic biomarkers and calcium content** Antonella Forlino - University of Pavia, Italy	
10.00	**Calcific tendinitis, a concise review** António Camacho - Hospital São Jose, Lisboa, Portugal	
10.30	**Coffee break**	
11.00	**Case studies on debilitating ectopic soft tissue ossifications and calcifications** Arun Kumar Kaliya Perumal - Nanyang Technological University, Singapore	
	Session 4: Oral presentation industrial partners Chair: Márcio Simão and Paulo Gavaia	
11.30	**The T50® Calciprotein Crystallization test (T50 test)** Andreas Pasch - Calciscon **Discovering FOXO3-based therapeutics to repair, restore and rejuvenate** Wolfgang Link - Refoxy Pharma **Ion Ampliseq technology for targeted resequencing** Marcos Morey Villar - Thermo Fisher Scientific	

12.15	**Closure**	
12.45	**Lunch**	
14.00	**CONSORTIUM MEETING** (only for INTEC consortium members)	**WORKSHOP 2:** TALKING AND WRITING SCIENCE by Pedro Quintino de Sousa (participants must bring their computer)

WIFI network: **ualg-conferencias** | Login: **intec2024** | Password: **95975722**

Welcome from the organizers

We thank all participants for joining us in Faro for this third INTEC meeting, making this conference a truly international event allowing networking in a beautiful environment, and aiming at providing time, space and occasions for many fruitful and relaxing discussions. INTEC meeting focusing on the molecular, physiological and clinical aspects of Ectopic Calcifications will contribute to widen our understanding of the "friend or foe" effects linked to this topic and have the chance to gain a deeper understanding of this broader field of interest. We hope you will have a great and successful INTEC conference!

And while you are here, we hope you take some time to visit Faro. The Algarve's capital is a small town but with a lot to see...its attractive marina, parks and plazas, pedestrian streets and an old town ringed by medieval walls. You can also enjoy museums, churches, a bone chapel and many small restaurants and cafes. There is also an extended beach that you can reach easily by boat...as well as different boat trips to visit a number of islands where you can enjoy walking, swimming and relaxing. Enjoy the visit and the food, it is well worthwhile...

Leonor Cancela and Olivier Vanakker

Organizing committee

Karolien Aelbrecht (Univ. Ghent)

Leonor Cancela (CCMAR)

Márcio Simão (CCMAR)

Natércia Conceição (CCMAR)

Olivier Vanakker (Univ. Ghent)

Paulo J. Gavaia (CCMAR)

Vincent Laizé (CCMAR)

Invited talks

The Japanese medaka fish as model for human chondrodysplasias and heterotopic ossification disorders

Christoph Winkler

National University of Singapore

Small laboratory fish, such as zebrafish and medaka, have become popular models in bone research and to model human skeletal disorders. We have established protocols that allow the highly efficient knock-in of human disease mutations into the medaka genome by using CRISPR-mediated homology directed repair (HDR). Using this method, we have introduced a disease mutation in collagen type X into medaka that is found in human patients suffering from metaphyseal chondrodysplasia type Schmid (MCDS). We show that the resultant medaka mutants faithfully recapitulate human disease phenotypes and discovered that impaired cell polarity of skeletal cells results in irregular bone matrix deposition and ectopic bone formation. In a second project, we have introduced a R206H mutation into the medaka locus encoding activin receptor type 1 (Acvr1). This mutation is reported to lead to heterotopic ossification in soft tissues in patients suffering from Fibrodysplasia ossificans progressive (FOP), a rare disorder with unknown mechanism. In my talk, I will focus on our methodological approaches that are similarly useful to study ectopic calcification in fish models.

Christoph Winkler has been using zebrafish and medaka models for the past 30 years to study developmental, degenerative and repair processes in the nervous and skeletal systems. His lab has generated fish models that helped to understand the mechanisms underlying selective motor neuron degeneration in Spinal Muscular Atrophy patients and impaired bone homeostasis in osteoporosis patients. Christoph Winkler studied Biology in Munich, obtained his PhD in Wuerzburg, Germany and did a postdoc at the University of Washington in Seattle. He was an independent group leader at University of Wuerzburg before being appointed as Associate Professor at the National University of Singapore (NUS), where he has served the Department of Biological Sciences as Deputy Head, Assistant Head and Chair of the PhD program. He currently is the Scientific Director of the AquaPolis aquaculture program at NUS.

Matrix gla protein mutations in vascular and cartilaginous tissues: new insights from genetic studies

Monzur Murshed

McGill University, Montreal, Canada

Matrix Gla protein (MGP) is a key inhibitor of soft tissue calcification, and its deficiency leads to Keutel syndrome—a rare disorder characterized by widespread cartilage and vascular abnormalities, including abnormal mineral deposition (calcification). We used a genetic approach to understand how MGP's conserved residues—the N-terminal serine residues and γ-carboxylated glutamic acid (Gla) residues—contribute to its anti-mineralization function. Our findings indicate that the serine residues are essential for preventing mineral deposition in both vascular and cartilaginous tissues, while the Gla residues are primarily necessary for inhibiting cartilage calcification. We also identified FAM20C as a kinase responsible for phosphorylating MGP's serine residues.

More recently, we examined a dominant mutation in MGP's signal sequence, which impacts both cartilage and vascular tissues and presents with traits overlapping with Keutel syndrome, such as shortening of long bones, facial anomalies, and short distal phalanges. This mutation also causes spondyloepiphyseal dysplasia, which is not seen in Keutel syndrome. Mouse models reveal that this mutation impairs MGP secretion, leading to ER stress, abnormal chondrocyte death, and premature growth plate cartilage calcification, but does not induce vascular calcification. Instead, vascular smooth muscle cells transdifferentiate into chondrocytes, resulting in arterial wall thickening. These results advance our understanding of MGP's role in vascular and cartilage tissues and offer insights into potential therapeutic approaches for diseases linked to MGP mutations.

Prof. Murshed earned his doctoral degree from the University of Cologne, Germany, and completed his postdoctoral training at Baylor College of Medicine, USA, specializing in mineralized tissue biology. His research utilizes genetically modified mouse models to explore the complex pathways regulating skeletal development, bone mineralization, and remodeling. For the past 22 years, Dr. Murshed has focused on the mineralization inhibitor matrix Gla protein (MGP), a critical regulator of soft tissue calcification and skeletal development. His work has been supported by CIHR, NSERC, FRQS, and other national and international funding agencies.

Modeling developmental capacity and constraint in skeletal differentiation in Akt and PROS somatic mosaic activation in the zebrafish

Matthew Harris

Harvard Medical School, USA

The Harris lab is focused on the genetic and developmental basis of skeletal form and differentiation. We use the zebrafish and other small laboratory fish as windows in which to identify the instructions of skeletal development, maintenance with age and how these signals are integrated in cases of skeletal disorders. One key insight arise from studies of how skeletal progenitor cells, carrying a somatic mutation, behave when out of context with their surroundings, as the conflict in signals can illuminate mechanisms of their control. Through modeling of somatic mosaic overgrowth disorders in zebrafish, we can show distinct tissue, temporal and environmental constraints and capacities on activation and maintenance of growth programs determining susceptibility to bone overgrowth. Through these models we define molecular contexts that can break growth constraints. Lastly, we have developed a new model for damage-induced heterotopic ossification that shows distinct areas for study of this disorder in light of our identified regulators. These findings in the zebrafish model open up areas for therapeutic intervention.

Dr. Harris specializes in comparative genetics and genomics focusing on the regulation of skeletogenesis and form. His work uses experimental models of vertebrate development, centering on the zebrafish as a platform for discovery of novel gene functions in development. Similarly, through comparative genomic approaches, his group identifies genetic networks regulating unique properties of development exposed through natural selection. Dr. Harris trained in the lab of Dr. John Fallon at the University of Wisconsin-Madison and with Dr. Christiane Nuesslein-Volhard at the Max-Planck Institute for Developmental Biology in Tuebingen, Germany.

http://www.fishbonelab.org
Twitter: @fishyskeleton
Blsky@fish4walking.bsky.social

Patients - partners for life

Marta Jacinto

Association of PXE Patients, Portugal

Patients do all sorts of things for a living. They may be nurses, teachers, electricians, IT personnel, lawyers, doctors, caregivers, ..., you name it. When getting the label 'patient', everything else seems to vanish when it comes to the ones following the former as such. Nevertheless, the knowledge patients had beforehand never vanishes and their expertise can be put to a good use and this should be kept in mind. Moreover, some patients go beyond the diagnosis and gather with others to build Patient Associations in order to help other patients, their families, science, health professionals, which will then help the patients back with their effort and findings. Some of the goals are to raise awareness, improve empowerment and give hope. This voyage is an amazing one. I for one can testify for it in what comes to ectopic calcifying diseases, Pseudoxanthoma Elasticum (PXE) in particular, but also beyond a particular group of diseases, with the National RD Patients' Organizations in Portugal.

What is the reality concerning PXE in Portugal? Which projects did the Portuguese Patient Association develop? How can the patients be of help to health care, research and academic professionals? How do the several associations relate in Portugal, in Europe, globally? Which projects could be replicated elsewhere? These are some of the questions to be addressed.".

With a master's degree in Computer Science and working in IT since 1999, Marta Jacinto is the President of the Portuguese Association for Pseudoxantoma Elasticum, member of the board of RD-Portugal, member of the EB of ISSEC and leader of RD-Portugal's project Informar sem Dramatizar (Informing without Dramas). She gave several interviews for newspapers and radio and TV programs, wrote multiple opinion articles for national, regional and local newspapers, and spoke about RD in several conferences. All she does for these institutions is pro bono.

Calcific Aortic Valve Disease (CAVD): expression analysis of osteoblastic biomarkers and calcium content

Antonella Forlino

University of Pavia, Italy

Calcific aortic valve disease (CAVD) represents a frequent valvular heart diseases for which, beside surgery, no definitive treatment is available. The investigation of alternative strategies for early diagnosis, for better understanding CAVD progression and to find new therapies is a need for the patients. The expression of osteoblastic markers in CAVD human samples, the calcium content and a complete microbiology analysis have been evaluated in aortic valve leaflets from 37 CAVD patients (stage III and IV) and 9 controls (affected by nonvalvular cardiac disease). Aortic valve leaflets were classified as right (R), left (L) and non-coronary (NC). Total valve RNA was extracted from samples to determine the tissue expression of early (RUNX2, OSX) and late (ALP, COL1A1, BGLAP) osteogenic markers by RT-qPCR. Multivariable analysis evaluated the role of risk factors (age, gender, chronic kidney disease and dyslipidemia) on osteogenic markers expression. The expression of ALP, OSX and COL1A1 was significantly increased in patients compared to controls; for BGLAP an increasing trend was present. Calcium quantitation of the samples was also performed by mass spectrometry revealing significant increase amount of calcium in all patients. Of note, isolation of cells from cardiac leaflets demonstrated for the patinets an intrinsic ability to mineralized in vitro compared to control isolated cells. Bacterial DNA was detectable in 38% of the patients' samples at least in one leaflet and in 22% of the controls. Correlation studies of molecular data with clinical features are undergoing and will provide new insight into the disease likely paving the way for novel target and therapies.

Dr. Antonella Forlino obtained her Degree in Biological Science, her Doctoral Degree in Biochemistry and her Speciality Degree in Genetic at the University of Pavia, Pavia, Italy. She spent 5 years as post-doctoral fellow at the National Institute of Health, Bethesda, USA, and became later on researcher and Associate professor at the Dept of Molecular Medicine, Section of Biochemistry, at the University of Pavia where she is now working as Full professor. Since October 2019 she is Vice-Rector for the Internationalization and President of the Center for Global Strategic Engagement. Her research activity has been and is focused on the molecular, biochemical, and functional study of genetic diseases of the connective tissue, in particularly the brittle bone disease Osteogenesis Imperfecta (OI). She developed and characterized the first non-lethal knock in murine model for OI and used this model for investigating OI phenotypic variability using proteomic and transcriptomic approaches and to develop novel cell/gene therapy and repositioning drug strategy. She generated and characterized other murine models and more recently she activated a D. rerio facility at the University of Pavia to create by CRISPR/Cas gene editing, to characterize and to use for drug screening several zebrafish models of skeletal dysplasias. She recently started a collaboration with the IRCCS San Matteo Hospital, Pavia, Italy, to investigate the molecular basis of the ectopic valve calcification using human samples.

Calcific tendinitis, a concise review

António Camacho

Hospital São José, Lisboa, Portugal

Calcific tendinitis is a painful tendon disorder characterised by either single or multiple calcium hydroxyapatite crystals deposits within the tendon. The process is unique and distinct from degenerative joint disease. It's a dynamic process that evolves through several stages. Successive stages have been characterized as having distinct radiographic and pathologic features, which often correlate with clinical symptoms. The exact mechanism of calcification, and the process of spontaneous resorption of the calcific deposits, are yet not clearly understood.

Consultant orthopedic surgeon with an interest in traumatology and physiology of tendon and bone healing.

Case studies on debilitating ectopic soft tissue ossifications and calcifications

Arun Kumar Kaliya Perumal

Nanyang Technological University, Singapore

Ectopic soft tissue ossifications and calcifications often result in significant morbidity. Within the musculoskeletal system, Heterotopic Ossification (HO) disorders are particularly notable for causing such morbidity. While some rare forms of this disorder are driven by genetic mutations, others occur in response to triggering events, such as injury, and are relatively common. Among the genetic varieties, Fibrodysplasia Ossificans Progressiva (FOP) is the most extensively studied and debilitating form. All reported FOP patients carry a heterozygous gain-of-function mutation in the gene encoding activin A receptor type I (ACVR1). This mutation leads to dysregulated bone morphogenetic protein (BMP) signaling, resulting in extensive HO at extra-skeletal sites, including muscles, ligaments, tendons, and fascia. In contrast, acquired heterotopic ossification, which occurs in response to trauma, is more localized to the site of injury and can be classified into two varieties: Myositis Ossificans Traumatica (MOT) and Neurogenic Heterotopic Ossification (NHO). MOT is relatively common and is triggered by either a single episode or repeated instances of injury to the same area, leading to localized ossification within the soft tissues at the injury site. NHO, while also localized, typically occurs alongside central nervous system injuries, such as spinal cord or traumatic brain injury, and is often more extensive and severe than MOT. This presentation will focus on these debilitating conditions, showcasing clinical examples and discussing disease progression, as well as the diagnostic and therapeutic challenges they present.

Dr. Arun Kumar Kaliya Perumal is an Indian Orthopaedic Surgeon with advanced International Fellowship Training in Spine Surgery. Following his Orthopaedic Residency in India, he developed a strong interest in spine surgery and completed clinical fellowships at Chang Gung Memorial Hospital in Taiwan and Tan Tock Seng Hospital in Singapore. He obtained a PhD in Molecular Genetics from Nanyang Technological University in Singapore, focusing on the mechanisms underlying injury-induced heterotopic ossification and the development of novel therapeutic strategies for the disorder. Affiliated with the Rehabilitation Research Institute of Singapore, he continues to pursue his interest in translational research within Orthopaedics, with a focus on enhancing the quality of healthcare delivery for the elderly.

Oral presentations

Mineralisation in the eye: a biomarker and target for treatment

Lengyel I

Queen's University Belfast, Belfast, UK

Introduction: Calcification is required to form and maintain skeletal and dental tissues in health. However, dysregulation of the mineralisation process can lead to ectopic calcium phosphate mineral deposition. In our work, we explored how ectopic calcification contributes to age-related macular degeneration (AMD) and Alzheimer's disease (AD).

Materials and Methods: Our study involved the ethical acquisition of post-mortem eyes and brains from patients diagnosed with AMD or AD, as well as from healthy controls. We employed various techniques, such as flat-mounting whole eyes onto glass slides and embedding eye and brain tissues in paraffin for sectioning. To visualise calcification, we used OsteoSense680EX, a fluorescent dye specific for the calcium phosphate hydroxyapatite (HAP). We also utilised advanced analytical tools like energy dispersive x-ray spectroscopy (EDX) and time of flight-secondary ion mass spectrometry (TOF-SIMS) to confirm the elemental composition of the structures stained with OsteoSense680EX.

Results: We found that AMD and AD are associated with increased deposition of mineralised spherules in sub-RPE deposits and Bruch's membrane. In AMD, large, calcified nodules heralded the rapid progression to end-stage disease. We also found that ectopic calcification of neurons and their nuclei is associated with p-Tau in the brains of AD patients.

Conclusions: Our findings suggest that ectopic calcification could be a promising target for interventions in AMD and AD. Alterations in calcium and phosphate levels, either through dietary changes or supplementation, as well as targeted interventions involving proteins and lipids associated with mineralisation, are potential strategies for further research and development.

ENNP1 is associated with ectopic calcification of the Bruch's membrane and functional changes in the mouse retina

Bertelli PM[1], Pilgrim MG[1], Brown C[1], Augustine J[1], Friedel T[1], Cunningham F[1], MacRae V[2], Kortvely R[3], Lengyel I[1]

[1] *Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, Belfast, UK*
[2] *The Roslin Institute, The University of Edinburgh, Midlothian, Scotland, UK*
[3] *Roche Pharma Research and Early Development, Immunology, Infectious Diseases and Ophthalmology (I2O) Discovery and Translational Area, Roche Innovation Centre Basel, Switzerland*

Introduction: Under physiological conditions, calcium and phosphate concentrations are tightly regulated, ensuring that calcification is restricted to bones and teeth. However, ectopic calcification in the extracellular space of soft tissues has been associated with ageing disorders including age-related macular degeneration (AMD), pseudoxanthoma elasticum (PXE), and generalized calcification in infancy (GACI). Ectopic calcification is associated with mutations on ectonucleotide pyrophosphatase/ phosphodiesterase 1 (ENPP1). Here, we characterized the effects of exon 9 deletion in ENPP1 on ocular calcification and retinal structure and function.

Materials and Methods: ENPP1 transgenic mice were generated by deletion of the functional exon 9 and maintained for up to 6 months. Retinal function was assessed by colour fundus photography (CFP), optical coherence tomography (OCT) and electroretinography (ERG). In addition, murine eyes were assessed for calcification using hydroxyapatite-specific fluorescent staining (Osteosense-680RD) and confocal microscopy. ENPP1$^{-/-}$ animals were compared to ENPP1$^{+/-}$ and ENPP1$^{+/+}$ controls.

Results: Visible lesions were identified in the ENPP1$^{-/-}$ animals using CFP. However, OCT retinal segmentation showed no structural differences among the groups. When using ERG, no changes were seen in scotopic a-wave and b-wave. ENPP1$^{-/-}$ showed significant decreased c-wave amplitude, used to assess the functional integrity of the photoreceptors, the pigment epithelium and the interactions between them. Osteosense staining showed ectopic calcification in the Bruch's membrane and vasculature ENPP1$^{-/-}$ retinas but not in ENPP1$^{+/-}$<ENPP1$^{-/-}$ control animals.

Conclusions: Here we show that deletion of exon 9 in the ENPP1 results in changes to retinal structure and function. We show the functional changes in the retina to be associated with Bruch's membrane calcification. Our results suggest ENPP1 transgenic animals as a valuable pre-clinical model to study the effects of calcification in ocular diseases, such as AMD, PXE and GACI, for the development of novel intervention strategies.

Anti-elastin targeted HSA-based DTPA-loaded nanoparticles resolve subretinal calcifications in a PXE mouse model

Rutsch F[1], Nitschke Y[1], Begasse T[2], Heiduschka P[3], Eter N[3], Hansen U[4], Deering J[5], McKee MD[5], Langer K[2]

[1] *Department of General Pediatrics, Muenster University Children's Hospital, Muenster, Germany*
[2] *Institute of Pharmaceutical Technology and Biopharmacy, University of Muenster, Muenster, Germany*
[3] *Department of Ophthalmology, Muenster University Hospital, Muenster, Germany*
[4] *Institute of Musculoskeletal Medicine, Muenster University Hospital, Muenster, Germany*
[5] *Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Canada*

Introduction: Our aim was to develop a PXE mouse model with robust subretinal calcifications and a targeted treatment to resolve these calcifications.

Materials and Methods: Ttw/ttw mice received a high-phosphate diet, which promotes calcification. Eyes were studied at 9 weeks of age by optical coherence tomography (OCT) and fluorescein angiography, and retinal function by electroretinography (ERG). Eye calcification at 12 weeks was examined by X-ray microscopy, Alizarin red and Osteosense staining, and by electron microscopy. Calcium content in the eyes was quantified by the O-cresolphthalein-complexon method at 9 and 12 weeks. For treatment, the chelating agent DTPA was covalently bound to human serum albumin (HSA)-based nanoparticles (NPs). NP surfaces were modified by an anti-elastin antibody to target Bruch's membrane. NPs were injected twice per week at a concentration of 20 µg/g body weight starting from 10 weeks of age (4 injections in total) into the lateral tail vein of ttw/ttw mice.

Results: Calcified subretinal deposits were evident at 9 weeks of age in ttw/ttw mice, and calcifications were more pronounced at 12 weeks of age. Subretinal calcifications were associated with decreased ERG a- and b-wave amplitudes. Systemic administration of NPs starting at 10 weeks of age led to dissolution of retinal calcifications in ttw/ttw mice, which was highly significant ($p \leq 0.0005$).

Conclusions: Ttw/ttw mice subjected to a high-phosphate diet show robust subretinal calcifications recapitulating the ocular PXE phenotype. Systemic delivery of DTPA-HSA-NPs containing anti-elastin targeting antibodies is a promising approach to treat PXE manifestations in the eye.

The role of elastin degradation in the calcification process

Quaglino D, Lofaro FD, Mazzilli A, Bonacorsi S, Boraldi F

Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Elastic fibres (EF) are mainly composed by elastin and microfibrillar molecules that assemble during development and have a very low turnover rate throughout life. This makes EF more prone to enzymatic proteolysis, oxidation, calcium accumulation and carbamylation. Calcification of EF has been linked to progressive degradation of elastin, that exposes sites promoting mineralization, as observed in several pathologic conditions, either acquired (e.g., vascular calcification in atherosclerosis) or inherited (e.g., PXE, pseudoxanthoma elasticum). Indeed, PXE patients exhibit elevated levels of matrix metalloproteinases, and PXE fibroblasts are characterized by raised degradative potential. To demonstrate that elastin degradation precedes and favours the mineralization process, an in vitro cell-free model was used to explore if: i) conditioned media from human dermal fibroblasts isolated from calcified or from healthy tissues could differently degrade elastin fibrils modulating their calcification; ii) the addition of glycosaminoglycans (i.e., HS, heparan sulphate or CS, chondroitin sulphate) during coacervation phase of elastin fibrils could counteract the mineralization. Results indicate that: i) conditioned media from fibroblasts from calcified areas have an increased proteolytic potential compared to healthy cells; ii) calcification is increased on fibrils degraded by pathologic conditioned media; iii) the presence of HS significantly reduces calcification in a dose-dependent manner, whereas CS has no effect. These data, further supported by the analysis of the fibroblasts' secretome, underline: i) the correlation between elastin degradation and mineralization, ii) the importance of glycosaminoglycans in modulating the resistance of elastin to degradation. Moreover, fibroblasts play an active role in this process having a different capacity to secrete proteases and their inhibitors, and this can explain why not all elastic fibres are calcified even in the same tissue of the same subject.

Work supported by PXE Italia Odv

Activation of the autotaxin - lysophosphatidic acid receptor pathway contributes to the pathogenesis of pseudoxanthoma elasticum

Derudder R[1,2,3], Dangreau L[1,2,3], Coucke P[1], De Kesel P[4], Nollet L[1,2,3], Vanakker OM[1,2,3]

[1] *Department of Biomolecular Medicine, Ghent University, Ghent, Belgium*
[2] *Ectopic Mineralization Research Group Ghent, Ghent University, Belgium*
[3] *Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium*
[4] *Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium*

Introduction: Pseudoxanthoma elasticum (PXE) is a rare hereditary disorder caused by bi-allelic ABCC6 mutations, leading to calcification of the skin, arteries, and eyes. Autotaxin (ATX/ENPP2), a lysophospholipase D enzyme, produces lysophosphatidic acid (LPA) from lipoprotein(a) phospholipids. LPA signaling, via LPA receptors, promotes IL-6 and BMP-2 secretion, stimulating osteochondrogenic transdifferentiation and calcification. This study evaluated the involvement of ATX-LPAR signaling in PXE-related ectopic calcification.

Materials and Methods: Skin tissue and dermal fibroblasts (n=5) were obtained from PXE patients and age- and sex-matched controls (n=5). Immunohistochemical staining for ATX, LPAR, and downstream targets was performed, and calcium crystals were visualized using Von Kossa staining. Differential gene expression analysis of ATX/LPAR pathways was conducted in PXE and control fibroblasts using RT-qPCR. In an in vitro calcification assay, mRNA levels of ATX/LPAR targets were measured in PXE fibroblasts at 0, 10, and 20 days. PXE fibroblasts were treated with the LPAR inhibitor Ki16425 (10 µM), and calcification and soluble TNAP enzyme activity were assessed. In PXE (n=26) and control (n=25) serum samples, ATX levels were measured using ELISA, and lipoprotein(a) concentrations were determined by immunoassay.

Results: Immunohistochemical staining revealed high expression of ATX, LPAR3 and downstream targets in PXE skin, co-localizing with osteogenic markers and calcium deposits. Increased mRNA levels of ATX, LPAR1, PIK3CA, ERK1, and IL-6 were found in PXE fibroblasts compared to controls. In a 20-day in vitro calcification assay, ATX, LPAR1, ERK1, and IL-6 mRNA levels increased with calcification. Treatment with Ki16425 significantly reduced calcium deposits and soluble TNAP activity. In serum, ATX levels were 2-fold higher in PXE patients compared to controls, while lipoprotein(a) levels were normal.

Conclusion: Increased ATX/LPAR signaling may be involved in PXE-related calcification. LPAR inhibition reduced matrix mineralization and TNAP activity in vitro. Currently, experiments evaluating ATX/LPAR inhibitor HA130 are underway.

Therapeutic applications of ENPP1-Fc prevent muscle calcification following cardiotoxin-induced-muscle damage in Abcc6$^{-/-}$ mice

Price T[1], O'Brien K[2], Howe J[2], Flaman L[2], Lynch A[2], Moran M[3], Li J[4], Husson H[2], Plaas A[4], Sabbagh Y[2]

[1] *Departments of Surgery, Rush University Medical Center W Harrison Street Chicago*
[2] *Inozyme Pharmaceuticals, 321 Summer Street, Boston MA*
[3] *Anatomy & Cell Biology, Rush University Medical Center W Harrison Street Chicago*
[4] *Internal Medicine, Rush University Medical Center W Harrison Street Chicago*

Ectopic mineralization (EM) from burns, blast injury, spinal cord injury, or surgical trauma range from dystrophic calcifications to heterotopic ossification. No effective therapeutic intervention exists, apart from surgical intervention which can prolong or exacerbate recovery from the initial injury. Specific mutations in genes in the Pi/PPi metabolic pathway such as ABCC6, ENPP1, ANKH and FETUIN A are known to cause hereditary disease of EM in humans and knock-out mouse models. ABCC6 is highly expressed in liver and proximal collecting kidney tubule cells. It is presumed to transport ATP from inside to outside of the cell, where ENPP1 hydrolyzes ATP into AMP and pyrophosphate (PPi), the latter of which is a potent inhibitor of mineralization. The Abcc6$^{-/-}$ mouse strain has 50% lower circulating PPi, leading to spontaneous mineralization of soft tissues. 12 week male Abcc6-/- mice were injected with Cardiotoxin, Naja pallida (CDTX) in the left quadriceps muscle. 10 mg/kg ENPP1-Fc was administered weekly subcutaneously for 2 weeks starting 1 day after CDTX injection. Muscle tissue was assessed via μCT and gene expression changes were assessed using qPCR. Terminal plasma was collected to measure circulating PPi levels. Mineralization begins in damaged muscle starting 7 days after CDTX injection and increases through day 14, preceded by transient increases in macrophage/inflammation genes (Itgam,Siglec1,Emr1,Il6,Tnf), myogenic genes (Myf5,Myog,Myod1), and followed at 7-14d by induction of chondrogenic/hypertrophic genes (Vcan,Cd44,Has2,Acan,Col10,Mmp3,9&13). Dosing of ENPP1-Fc prevents >90% of muscle mineralization. Experiments are ongoing to confirm the effect of ENPP1-Fc on circulating PPi, and RNASeq analyses of muscle to elucidate the effects of ENPP1/PPi on inflammation, chondrogenesis, mineralization and resorption pathways during the regenerative responses of the injured muscle tissue. The data support our previous publications to highlight therapeutic potential of ENPP1-Fc in mitigating and possibly resolving post-traumatic or genetically induced soft tissue mineralization in a variety of clinical settings.

Ferroptosis of valvular interstitial cells as a result of erythrophagocytosis promotes calcific aortic valve stenosis

Qin Z[1], Bäck M[1,2], Franco-Cereceda A[2,3], Pawelzik S-C[1,2]

[1] *Translational Cardiology, Department of Medicine Solna, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden*

[2] *Heart and Vascular Center, Karolinska University Hospital, Stockholm, Sweden*

[3] *Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden*

Calcific aortic valve disease (CAVD) progresses to severe Aortic Stenosis and heart failure. Recent evidence indicates that intra leaflet hemorrhage (ILH) promotes valve calcification; however, how ILH contributes mechanistically to CAVD remains poorly understood. Additionally, it has been shown that dysregulated iron metabolism correlates positively with osteoblastic differentiation of valvular interstitial cells (VIC). Ferroptosis, a regulated form of cell death characterized by with lipid peroxidation, has been implicated in CAVD, but the detailed mechanisms remain unclear. Therefore, we aim to determine the role of ferroptosis in CAVD progression in the context of ILH.

VIC directly erythrophagocytosed red blood cells (RBC), which led to an excess intracellular iron overload and significantly exacerbated VIC calcification. Suppression of VIC calcification by ferroptosis inhibitors indicated a link between ILH and valvular calcification through ferroptosis. Using transcriptome data from our human aortic valve biobank, we identified ferroptosis-related genes that were upregulated in calcified valve tissue, e.g. 5-lipoxygenase (5-LO) and acyl-CoA synthetase long-chain family member 4 (ACSL4). To verify the role of ferroptosis in VIC, we cultured VIC in the presence of the ferroptosis inducer RSL3 and observed an increased vulnerability to oxidative damage and cell death, which was reversed by inhibition of 5-LO and ACSL4. Finally, we demonstrated that oxidative stress triggered by osteogenic medium was inhibited by both ferroptosis inhibitors and 5-LO inhibitors.

Our findings connect ILH- and erythrophagocytosis-induced valve calcification with oxidative stress and ferroptosis. Additionally, we identified 5-LO and ACSL4 as crucial components for the interplay between ferroptosis and oxidative stress in VIC. By connecting these pathways, we suggest that 5-LO and ACSL4 process polyunsaturated fatty acids as part of the pathophysiology of CAVD. Inhibition of ferroptosis not only reduces oxidative damage but also attenuates VIC calcification, underscoring its potential as a therapeutic target in the treatment of CAVD.

The vascular calcification process in PXE

Alouane A[1], Stefano J[1], Clotaire L[2], Martin L[3], Padovani B[1], Duranton C[2], Rubera I[2], Lefthériotis G[1,2]

[1] *University Hospital Nice, Nice, France*
[2] *Laboratory of Molecular PhysioMedicine (LP2M), Nice, France*
[3] *University Hospital Angers, Angers, France*

Introduction: PXE is a monogenic disease characterized by a progressive calcification of the peripheral arteries of the lower limbs. The progression and natural process of development of calcifications in the peripheral vasculature is not well known in this disease. As part of the PROPHECI trial (NCT0486857), we are studying the segmental progression of peripheral arterial calcification.

Methods: The analysis of the peripheral arterial calcification process is extracted from CT scans of the leg arteries, without contrast injection, using an automated segmentation method developed in our laboratory. For each CT section (n = 1,000), calcification density (determined by a threshold >132 HU), surface area and relative calcification were determined. Quantification and mapping of calcification topology before and after 12 months of treatment was determined in PXE patients enrolled in the PROPHECI trials.

Results: The first results reveal that the development of calcifications in PXE is singular, with different patterns depending on the patients. In particular, the popliteal artery remains preserved in almost all patients, which suggests a specific response of this arterial segment compared to the supra- and infra-popliteal segments to the pro-calcific context.

Conclusions: These first results will allow us to better analyse the progression/regression of calcifications in the natural history of the PXE disease and the clinical trials.

Poster presentations

UCMA loss of function contributed to accelerate growth and bone mineralization and appears to increase susceptibility for osteoarthritis progression in association with age

Simão M[2,3], Fazenda C[1,2], Vitorino M[2], Yu I-S[4], Diogo GS[5,6], Pirraco RP[5,6], Moreira-Silva J[5,6], Reis RL[5,6], Gavaia PJ[2,3], Lin S-W[7], Kempf H[7], Conceição N[2,3], Cancela ML[2,3,9]

[1] *PhD Program in Biomedical Sciences, University of Algarve, Faro, Portugal*
[2] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[3] *Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal*
[4] *Department of Laboratory Animal Center, College of Medicine, National Taiwan University, Taiwan*
[5] *3B's Research Group, University of Minho, Guimarães, Portugal*
[6] *ICVS/3B's PT Government Associated Laboratory, Braga/Guimarães, Portugal*
[7] *Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taiwan*
[8] *UMR 7365 CNRS-Université de Lorraine, IMoPA, Vandoeuvre-lès-Nancy, France*
[9] *Algarve Biomedical Center, University of Algarve, Faro, Portugal*

Gla-rich protein (GRP), also known as UCMA (upper zone of growth plate and cartilage matrix associated protein), is the most recent member of the vitamin K-dependent protein family and presents the highest ratio of Gla residues over total residues. Although UCMA was initially considered as a new marker of the resting zone of cartilage, it was later detected in additional tissues including bone and skin. Molecular function is not fully understood, but recent studies have suggested that UCMA may have a physiological role in cartilage development, calcification process during vertebrate skeleton formation and in prevention of ectopic calcifications. This study aimed to characterize an *Ucma*$^{-/-}$ mice phenotype. For that a histological analysis of *Ucma*$^{-/-}$ mice at different ages was done and complemented with microCT analysis. In addition, we have isolated primary chondrocyte cultures from *Ucma*$^{-/-}$ mice and identified changes in gene and protein expression. The results revealed an acceleration of growth plate maturation, leading to a significant increase in tibia hypertrophic region and length of *Ucma*$^{-/-}$ tibias between 8 and 36 days old. The analysis of *Ucma*$^{-/-}$ primary chondrocyte gene expression corroborated this phenomenon, showing an upregulation of genes associated with an increase in the extracellular matrix production and rigidity like collagens I, II, X and aggrecan. In addition, we observed the upregulation of both matrix Gla protein (*Mgp*), likely as a compensatory response to UCMA loss of function, and SRY-Box Transcription Factor 9 (*Sox9*) known to be associated with chondrocyte commitment and maintenance. The analysis of articular cartilage of *Ucma*$^{-/-}$ mice at different ages showed a susceptibility to onset and progression of osteoarthritis. The microCT analysis revealed an early significant increase in bone mineralization parameters until 6 months of age, followed by a premature increase of bone resorption at 12 months of age. In conclusion, this study showed evidence that UCMA may function as a regulator of skeletal growth and contribute to bone mineralization and articular cartilage maintenance.

Acknowledgments: Foundation for Science and Technology for the financing of PhysPath-KS project (EJPRD/0004/2020) "Understanding the pathophysiology of Keutel Syndrome: A path towards cure" and the Centre of Marine Sciences through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

Tracheal calcification: mechanisms and potential respiratory implications in mice with Matrix Gla Protein deficiency

Hugon R[1*], Baptista E[1*], Tomassone J[1], Bianchi A[1], Murshed M[2], Kempf H[1]

[1] *UMR 7365 CNRS-Université de Lorraine, Ingénierie Moléculaire, Cellulaire et Physiopathologie (IMoPA), Vandœuvre-lès- Nancy, France*

[2] *Department of Medicine, Faculty of Dentistry, McGill University, Montreal, Canada*

* *Co-contributors*

Introduction. Tracheal calcification is a rare condition affecting the elderly, but also appears in younger individuals with Keutel Syndrome (KS), a genetic disorder caused by mutations in the calcification inhibitor gene, Matrix Gla Protein (MGP). While elderly individuals are often asymptomatic, KS patients experience respiratory complications, including breathing difficulties and airway infections. Investigating the mechanisms behind tracheal calcification and its impact on respiratory health is essential for improving patient management.

Material & Methods. Alcian blue/Alizarin red and Von Kossa/Alcian blue double stainings were conducted on whole tracheas and 5-µm thick sections of tracheal samples from wild-type ($Mgp^{+/+}$) and MGP-deficient ($Mgp^{-/-}$) mice at various ages. Gene expression analysis was performed using qPCR. Mucin detection was done via ELISA and bronchoalveolar lavage. Mucociliary clearance was evaluated using a Phenol Red dye velocity test.

Results. Our analysis revealed unexpected tracheal calcification in WT mice starting at 30 days after birth, progressing rostro-caudally with age due to terminal differentiation of tracheal chondrocytes. In $Mgp^{-/-}$ mice, calcification began at 14 days and was more extensive, including a surprising calcification in the lamina propria of the tracheal mucosa, which altered the overlying epithelium. This mucosal calcification was specific to the trachea and absent in WT mice, even in old age. Preliminary data suggest that calcification of the mucosa may impair epithelial function, leading to overexpression of the Muc5AC gene and reduced mucociliary clearance.

Conclusion. This study is the first to comprehensively describe tracheal calcification in mice showing that calcification of the cartilage rings is a early physiologic event in mice. It also identifies MGP as a key factor of the tracheobronchial calcification, since its deficiency accelerates cartilage calcification and causes an additional calcification in the tracheal mucosal layer, potentially contributing to the respiratory problems seen in KS patients, which were previously attributed to tracheal ring calcification.

Significance of Premature Vertebral Mineralization in Zebrafish Models in Mechanistic and Pharmaceutical Research on Hereditary Multisystem Diseases

Van Wynsberghe J[1,2,3], Vanakker OM[1,2,3]

[1] *Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium*
[2] *Department of Biomolecular Medicine, Ghent University, Ghent, Belgium*
[3] *Ectopic Mineralization Research Group, Ghent, Belgium*

Zebrafish are increasingly becoming an important model organism to study the pathophysiological mechanisms of human diseases and to investigate how these mechanisms can be effectively targeted using compounds that may open avenues to novel treatments in patients. The zebrafish skeleton has been particularly instrumental as – contrary to other model organisms – the lower load on the skeleton of the aquatic animal enables the possibility for mutants to survive to early adulthood. Several zebrafish models have been generated to study skeletal disorders involving premature vertebral mineralization. Interestingly, vertebral mineralization is also found in zebrafish models of several multisystemic hereditary diseases that in human patients do not affect the vertebral column. Among these models, disturbance of the TGF-β and BMP signalling pathways appear to be a recurrent theme. These pathways signal through a canonical SMAD and non-canonical SMAD independent pathway to downstream osteogenic transcription factors such as RUNX2. In the discussed models, the pathway is disrupted at different levels due to lower levels of important inhibitors, which leads to an upregulation of RUNX2 and subsequently initiates mineralization and osteoblast differentiation. The axial skeletal phenotype of zebrafish models offers a good read-out for compound detection and preclinical evaluation in hereditary multisystemic diseases. This clear and great potential of zebrafish should lead to increased efforts to identify and screen drugs for these complex disorders that are currently often intractable. Furthermore, they can be of valuable help to untangle the mechanisms – in general but also tissue and cell specific – that contribute to physiological and pathological mineralization.

Kinetics of pyrophosphate homeostasis in mouse and humans

Tamatey VC[1,2], Varhegyi M[1,3], Juhász D[3,4], Van Wynsberghe J[5], Nagy AI[4], Vanakker OM[5], Arányi T[1,4], Szeri F[1]

[1] *Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest, Hungary*
[2] *Doctoral School of Biology, ELTE Eotvos Lorand University, Budapest, Hungary*
[3] *Doctoral School of Semmelweis University, Budapest, Hungary*
[4] *Heart and Vascular Center, Semmelweis University, Budapest, Hungary*
[5] *Center for Medical Genetics Ghent, Department of Biomolecular Medicine, Ghent University Hospital, Ghent, Belgium*
[6] *Department of Molecular Biology, Semmelweis University, Budapest, Hungary*

The in vivo levels of plasma inorganic pyrophosphate (PPi), a potent calcification inhibitor might be regulated by tissue non-specific alkaline phosphate-derived ALP activity, hence TNAP is able to hydrolyse PPi. Abcc6 is known to be responsible for the cellular efflux of ATP and subsequent generation of PPi in the circulation; therefore, its deficiency leads to a significant deficit in circulating PPi concentration. The kinetics of ALP activity, PPi and inorganic phosphate (Pi) circulatory concentrations were investigated in both Abcc6 knockout (KO) and wild-type (WT) mice across different ages, as well as in human subjects. Using a specific enzymatic method and standardized colorimetric assays to analyze plasma and serum samples respectively, our findings revealed significant correlations: a strong inverse relationship between ALP activity and age in both mouse groups ($r = -0.7184$ for WT and $r = -0.5752$ for KO), and a notable inverse correlation of the Pi/PPi ratio with age ($r = -0.5026$). Additionally, an inverse correlation ($r = -0.4094$) was observed between plasma PPi levels and serum ALP activity. These kinetic results, unprecedented in literature, suggest that the physiological mechanisms in Abcc6 KO mice are similar to what we measured in patients with pseudoxanthoma elasticum (PXE), and patients with zero coronary artery calcium score, highlighting potential pathways for therapeutic interventions in mineralization disorders.

Keywords: serum alkaline phosphate, plasma pyrophosphate, Abcc6 knockout mice, pseudoxanthoma elasticum

Analysis of mineralization phenotypes in zebrafish larvae expressing a dominant negative *mgp* mutant

Sares D[2], Simão M[1,2], Martins G[1,2], Cucchiarini M[3], Venki J[3], Cancela ML[1,2], Gavaia P[1,2]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal*
[3] *Center of Experimental Orthopaedics, Saarland University Medical Center, Homburg/Saar, Germany*

MGP (Matrix Gla Protein) is a vitamin K-dependent protein, exerting an inhibitory function of bone mineralization and vascular calcification in vivo. Loss of function of MGP in humans, caused by mutations or genetic polymorphisms, is responsible for Keutel syndrome, characterized by extensive calcifications in soft tissues like lungs, blood vessels and skin. Still, the molecular mechanisms underlying MGP activity in preventing calcification of cartilage and blood vessels are not fully understood. Due to the advantages of zebrafish (Danio rerio) as a model, larvae were subjected to a "dominant negative" competition for expression of mutant forms of MGP by microinjection of recombinant adeno-associated virus - rAAV. Several viral vectors were generated, containing constructs coding for human MGP or two mutated forms of MGP affecting the Gla domain or the serine domain. Despite some mortality and developmental deformities observed in the embryos microinjected with rAAV vectors, the larvae that did not show deformations manifested distinct mineralization phenotypes for each mutant form of MGP expressed. Due to the high affinity for calcium ions of the Gla domain of MGP, its mutation was shown to have an impact on bone mineralization in the operculum, namely by increasing the extent and intensity of calcification of the mineralized area. The absence of the functional domain of phosphoserines also showed a similar effect, but without altering the pattern of operculum calcification. Our results reveal the possible importance of the MGP Gla domain and phosphoserine domain in preventing the extracellular matrix mineralization phenotype and ectopic calcifications present in Keutel Syndrome.

Acknowledgments: Foundation for Science and Technology for the financing of PhysPath-KS project (EJPRD/0004/2020) "Understanding the pathophysiology of Keutel Syndrome: A path towards cure" and the Centre of Marine Sciences through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

rAAV-mediated transduction of ATDC5 and Saos-2 cells with mutant Matrix Gla Protein (MGP) transcripts showed the impact of Gla domain and Serine phosphorylation on the inhibition of mineralization

Simão M[1,2], Geraldo L[1], Rodrigues C[1], Conceição N[1,2,3], Gavaia PJ[1,2], Venkatesan JK[4], Cucchiarini M[4], Cancela ML[1,2,3]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Faculty of Medicine and Biomedical Sciences, University of Algarve, Faro, Portugal*
[3] *Algarve Biomedical Center, University of Algarve, Faro, Portugal*
[4] *Center of Experimental Orthopaedics, Saarland University and Saarland University Medical Center, Homburg/Saar, Germany*

Vitamin K dependent proteins are known by their post-translation modification of γ-carboxylation catalysed by γ-glutamyl carboxylase and assisted by reduced vitamin K as a cofactor. Matrix Gla Protein (MGP) is one of these proteins and mainly secreted by chondrocytes and vascular smooth muscle cells, and functions as a physiological inhibitor of mineralization. Mutations in the human *MGP* gene are associated with a rare hereditary disease (Keutel syndrome), characterized by abnormal cartilage calcification, peripheral pulmonary stenosis, and facial hypoplasia. In addition, deregulation of MGP function can contribute to the formation of ectopic calcifications in soft tissues. With this study, we aimed to transduce in Saos-2 and ATDC5 cell lines, with genetically engineered rAAV virus encoding cDNAs for a wild type (WT), a Gla-deficient mutant (*MGP*_Gla_mut) and a serine-deficient (*MGP*_Ser_mut) *MGP*. The mineralization levels were evaluated by staining the cells with Alizarin Red S and quantification achieved by solubilizing the staining salt with cetylpyridinium chloride. In addition, we evaluated the expression of genes associated with mineralization deposition by qPCR in cells. Different results were observed in the two cell types after treatment with rAAVs *MGP*_Gla_mut and *MGP*_Ser_mut. In ATDC5 cells, MGP_*WT* promoted inhibition of mineralization relative to the control, with MGP_Gla_mut partially rescuing this effect. On the contrary, in Saos-2 cells, MGP_WT treatment did not affect mineralization compared to the control, however *MGP*_Gla_mut significantly increased mineralization relative to both MGP_WT and control. The gene expression was done only for Saos-2 cells, and we observe in cells treated with *MGP*_Ser_mut the upregulation of osteocalcin and osterix. Also, *UCMA (*upper zone of growth plate and cartilage matrix associated protein) and *MGP* were upregulated as a compensatory effect. In conclusion, our data provides further evidence of the different impact of loss of function of the Gla domain and phosphorylation sites on the mineralization process.

Acknowledgments: Foundation for Science and Technology for the financing of PhysPath-KS project (EJPRD/0004/2020) "Understanding the pathophysiology of Keutel Syndrome: A path towards cure" and the Centre of Marine Sciences through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020.

Workshops

Talking and writing science

Pedro Quintino de Sousa

University of Algarve, Faro, Portugal

The Science Communication Workshop aims to optimise PhD students, researchers' and professors' skills to convey complex scientific concepts to diverse audiences. In this workshop, participants will learn the fundamentals of language, ethics and science communication; the linguistic and formal components of a scientific talk; and apply basic writing and narrative rules to guarantee peer acceptance and successful communication. The workshop combines a theoretical framework and hands-on activities, practical tips, and personalised feedback to enhance the ability (both for seasoned scientists and budding science communicators) to share scientific knowledge with confidence, and clarity.

Pedro Quintino de Sousa (PhD 2016). Professor at University of Algarve and researcher at CIAC (Centro de Investigação em Artes e Comunicação). Teaching experience and research interests include language teaching in Higher Education, English Science Communication, English for Academic Purposes (writing and speaking), English Cultural Studies and Portuguese as a Foreign Language.

Introduction to presentation skills

Tom De Moor

Ghent University, Ghent, Belgium

In this interactive workshop, we focus on various aspects of a presentation: content, visuals and delivery. We will discuss frequently asked questions and examples from different fields and formats to analyze the development process of a(n academic) presentation:

• What is your main message?

• How do you develop the proper presentation structure to maximize audience retention?

• How can a visual complement your presentation and boost memorability?

• What are do's and don'ts when it comes to delivering the presentation?

Various tips and tricks will be listed, from which you can cherry-pick to fit your personal style, your research topic and the relevant (conference) format.

Tom De Moor has been working for the University Language Centre (UCT) of Ghent University since 2008, where he coordinates, develops and teaches Academic and Professional Language Education. He specializes in academic communication skills for researchers and professorial staff, including writing, lecturing, applying and various aspects of conference skills.

Industry presentations

The T50® Calciprotein Crystallization test (T50 test)

Andreas Pasch

Calciscon, Switzerland

Calciscon AG is a Swiss in vitro diagnostics company, devoted to the development of Calciprotein particle (CPP) based diagnostics. CPP are naturally circulation nanoparticles involved in the transportation and clearance of mineral debris. While the related functional mineral buffer system in serum is of utmost physiological importance, it can become pathophysiologically relevant in certain clinical conditions like chronic kidney disease. The T50 test measures ex vivo the transformation from non-toxic primary CPP (CPP1) to toxic secondary CPP (CPP2) which induces inflammation, oxidative stress and calcification. A large and further growing body of literature demonstrates the clinical relevance of the T50 test.

Discovering FOXO3-based therapeutics to repair, restore and rejuvenate

Wolfgang Link

Refoxy Pharmaceuticals GmbH, Germany

Refoxy Pharmaceuticals GmbH, a German biotechnology startup, is at the forefront of developing innovative therapies targeting FOXO3 transcription factors—key regulators of cellular homeostasis. FOXO3 proteins play a crucial role in maintaining cell health and have significant therapeutic potential for treating age-related diseases. Leveraging this potential, Refoxy has developed a cutting-edge discovery platform designed to identify novel chemical activators of FOXO3. This platform has successfully generated several promising chemical series that activate FOXO3, demonstrating broad therapeutic benefits across various disease models. The company's approach holds promise for advancing new treatments that repair, restore, and rejuvenate cellular function.

Ion Ampliseq technology for targeted resequencing

Marcos Morey Villar

Thermo Fisher Scientific, USA

Information on INTEC

itnintec@ugent.be

www.itnintec.com

@INTECitn

itnINTEC

@itnINTEC