2026 Joint Ectopic Calcification Meeting



ABSTRACT BOOK

WELCOME!

2026 Joint Ectopic Calcification Meeting

On behalf of all members of the Organising and Scientific Committees, we are delighted to welcome you to Stockholm for the second edition of the Joint Ectopic Calcification Meeting.

There is a saying that the second edition is always the most challenging, as expectations are shaped by the success of the first. One must not only match the previous edition, but ideally surpass it. We believe that, with this year's venue and scientific programme, we have already achieved this in two important respects. For the third key ingredient—the audience—we rely on you, and we therefore warmly invite you to join us in Stockholm and help make this year's meeting another great success.

While its core strengths remain unchanged—a relaxed and collegial atmosphere, a high scientific standard, and a strong balance between early-career researchers and senior investigators—we have carefully considered the feedback and suggestions received after last year's edition. In addition to the clinical Pedro Valdivielso Keynote Lecture, this year we are also introducing a keynote lecture focused on fundamental research. Furthermore, we have expanded the number of presentation opportunities for early-career and established researchers. We therefore strongly encourage you to motivate your colleagues - junior and senior - to submit an abstract for the meeting.

We hope you will enjoy the programme we have prepared for this year's Joint Ectopic Calcification Meeting, and we very much look forward to welcoming you to Stockholm.

Sven-Christian Pawelzik - Local Organiser
Olivier Vanakker – INTEC Coordinator
Hervé Kempf – ISSEC President


Sarah Beck


Francesco Demetrio Lofaro

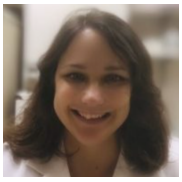

Hervé Kempf


Georges Lefthériotis


Ola Nilsson


Lukas Nollet


Sven-Christian Pawelzik


Flora Szeri


Olivier Vanakker


Andras Varadi

MEETING **PROGRAM**

Stockholm, Sweden

September 28-30th 2026

MONDAY, SEPTEMBER 28TH 2026

08:30 - 09:00	REGISTRATION
09:00 - 09:15	WELCOME
09:15 - 10:00	PEDRO VALDIVIELSO LECTURE ON CLINICAL RESEARCH **Rare genetic calcification disorders: lessons learned over three decades** *FRANK RUTSCH (Münster, Germany)*
10:00 - 13:00	SESSION 1 *(Chair: Hervé Kempf)*
10:00 - 10:30	**To mineralize or not to mineralize in rare conditions associated to pathogenic *FAM20A* variants** *ARIANE BERDAL (Paris, France)*
10:30 - 11:00	**Ectopic hydroxyapatite formation in age-related macular degeneration** *JOHN HULLEMAN (Minneapolis, USA)*
11:00 - 11:30	COFFEE BREAK & POSTER VIEW
	SESSION 1 *continued*
11:30 - 12:00	**Hypophosphatasia - mechanisms and treatment outcomes** *RAJA PADIDELA (Manchester, UK)*

12:00 - 12:20		**The TERC/HSP90/TERT Axis Regulates TERT Non-Canonical Function in Calcific Aortic Valve Disease** *CYNTHIA ST. HILAIRE (Pittsburgh, USA)*
12:20 - 12:40		**Tissue-nonspecific alkaline phosphatase dephosphorylates nicotinamide mononucleotide to regulate NAD metabolism** *DAVID MAGNE (Lyon, France)*
12:40 - 13:00		**Dynamics of mineralization reveal functional heterogeneity of matrix vesicles isolated from human osteoarthritic cartilage** *JESSEM LANDOULSI (Paris, France)*
13:00 - 14:00		LUNCH BREAK & POSTER VIEW
14:00 - 15:30		SESSION 2 *(Chair: Tamas Aranyi)*
14:00 - 14:30		**Obstructive sleep apnea symptom clusters as determinants of coronary artery calcification in a middle-aged Swedish population.** *DING ZOU (Gothenburg, Sweden)*
14:30 - 15:00		**The complex mechanisms of elastic fibres calcification** *DANIELA QUAGLINO (Modena, Italy)*
15:00 - 15:30		**Lipid dysregulation as a driver of vascular calcification** *MAGNUS BÄCK (Stockholm, Sweden)*
15:30 - 16:00		COFFEE BREAK & POSTER VIEW
16:00 - 17:00		SESSION 3 *(Chair: Jessica Bertrand)*
16:00 - 16:30		**Zebrafish models for disease-associated abnormal ossifications: two case studies** *LEONOR CANCELA (Faro, Portugal)*
16:30 - 17:00		**From dynamite to discovery: the legacy of Alfred Nobel** KLAS KÄRRE (Stockholm, Sweden)
17:00 – 17:45		INTEC ANNUAL WORK MEETING
		IN PARALLEL (for early career researchers):
		INTEC EARLY CAREER WORKSHOP 1 **Design an attractive graphical abstract** *JENNIFER SALOMONSSON (Stockholm, Sweden)* *HAMPUS PETRÉN (Stockholm, Sweden)*

TUESDAY, SEPTEMBER 29TH 2026

09:15 - 10:00 — KEYNOTE LECTURE ON BASIC RESEARCH
Arterial calcifications: produced by vascular smooth muscle cells, modulated by endothelial cells
ANJA VERHULST (Antwerp, Belgium)

10:00 – 13:00 — SESSION 4: SELECTED ORAL PRESENTATIONS (*Chair: Ola Nilsson*)

10:00 – 10:15 — **Ferroptosis promotes aortic stenosis through 5-lipoxygenase**
ZIHAN QIN (Stockholm, Sweden)

10:15 – 10:30 — **Hardening of the arteries: why does arterial medial calcification preferentially develop in certain arterial locations?**
JORDAN ELBADRY (London, UK)

10:30 – 10:45 — **Calcium shape characteristics in carotid atherosclerosis and its association to underlying biological signatures**
ARISA KITAGISHI (Stockholm, Sweden)

10:45 – 11:15 — COFFEE BREAK & POSTER VIEW

SESSION 4 *continued*

11:15 – 11:30 — **Endothelial exosomes mediate TMAO-induced vascular calcification through microRNA-dependent Wnt/β-catenin signaling**
JOUMANA AL AKHDAR (Istanbul, Türkiye)

11:30 – 11:45 — **Functional divergence of SWI/SNF chromatin-remodelling subcomplexes during VSMC phenotypic switching in vascular calcification**
MENG-YING WU (London, UK)

11:45 – 12:00 — **AAV-mediated liver-targeted human *ABCC6* gene therapy for PXE**
DORA OBIRI-YEBOAH (Philadelphia, USA)

12:00 – 12:15 — **Defining the temporal dynamics of early cardiovascular calcification through live imaging**
MAYRA MARTINEZ-LOPEZ (Toulouse, France)

12:15 – 12:30 — ***Ggcx* knock-out zebrafish as a model for PXE-like syndrome suffer midfacial hypoplasia, impaired vascular integrity and fatal hemorrhages**
JUDITH VAN WYNSBERGHE (Ghent, Belgium)

12:30 – 12:45 — **ENPP1 loss disrupts mitochondrial calcium homeostasis and promotes osteoblast senescence and mineralisation**
QIYU TANG (Edinburgh, UK)

	12:45 – 13:00	**Soluble epoxide hydrolase in calcified aortic stenosis: from selective modulation to gender-specific personalized therapy** *CLOTHILDE HENNION (Rouen, France)*
13:00 - 14:00		LUNCH BREAK & POSTER VIEW
14:00 - 15:30		SESSION 5 *(Chair: Flora Szeri)*
	14:00 - 14:30	**A new perspective on intervertebral disc calcification - from bench to bedside** *MAKARAND RISBUD (Philadelphia, USA)*
	14:30 - 15:00	**Calcifying and non-calcifying manifestations in PXE - Potential Link to Extracellular Purine Metabolism** *GILLES KAUFFENSTEIN (Strasbourg, France)*
	15:00 - 15:20	**Genetic mapping of joint ectopic calcification identifies KIF26B as a therapeutic target** *MUHAMMAD FAROOQ RAI (Abu Dhabi, UAE)*
	15:20 - 15:30	**The study ended. The patient didn't: reconnecting 30 years of PXE data** *IAN TERRY (PXE International, USA)*
15:30 - 16:00		COFFEE BREAK & POSTER VIEW
16:00 - 17:00		SESSION 6 *(Chair: Sarah Beck)*
	16:00 - 16:30	**Phenotypic and genetic aspects of primary brain calcification** *GAEL NICOLAS (Rouen, France)*
	16:30 - 17:00	**Clinical spectrum of primary brain calcifications** *MARTIN PAUCAR ARCE (Stockholm, Sweden)*
17:00 - 17:45		ISSEC GENERAL ASSEMBLY
		IN PARALLEL (for early career researchers): INTEC EARLY CAREER WORKSHOP 2 **Use of artificial intelligence in systematic reviews** *EMMA-LOTTA SÄÄTELA (Stockholm, Sweden)*
19:30		GALA DINER *(optional)* *M/S Mälar Victoria* *Klara Mälarstrand (Kajplats 3)*



WEDNESDAY, SEPTEMBER 30TH 2026

09:00 - 10:00		SESSION 7 *(Chair: Georges Lefthériotis)*
	09:00 - 09:30	**Calciprotein particles as a therapeutic target of ectopic calcification** *MAKOTO KURO-O (Shimotsuke, Japan)*
	09:30 - 10:00	**How is phosphate sensed? The known, the unknown, and its emerging role in vascular calcification** *LAURENT BECK (Nantes, France)*
10:00 - 10:30		COFFEE BREAK & POSTER VIEW
10:30 - 12:20		SESSION 8 *(Chair: Olivier Vanakker)*
	10:30 – 11:00	**Soft tissue mineralization viewed through the lens of skeletal growth** PHILLIP TREVOR NEWTON (Stockholm, Sweden)
	11:00 – 11:20	**Role of ANKH channel in crystal-induced arthropathies** *CHRISTOPHE DURANTON (Nice, France)*
	11:20 – 11:40	**Phosphate-driven cardiac valve calcification is triggered by endothelial apoptosis in α-klotho zebrafish mutants** *ANABELA BENSIMON-BRITO (Toulouse, France)*
	11:40 – 12:00	**Pyrophosphate homeostasis: from systemic regulation to ectopic calcification** *FLORA SZERI (Budapest, Hungary)*
	12:00 – 12:20	**Macrophage-mediated inflammation: a likely driver of ectopic calcification in pseudoxanthoma elasticum** *OLIVIER LE SAUX (Honolulu, USA)*
12:20 – 13:00		CLOSING REMARKS & AWARD CEREMONY

- Presentations marked with this symbol have been selected from the abstracts submitted to the meeting.
- All meeting activities will take place at Karolinska University Hospital, Solna, in the Sune Bergström Auditorium. The INTEC Early Career Workshop will be held in separate rooms, which will be announced during the conference.
- Registration is required for the meeting, as well as for participation in the Gala Dinner and the INTEC Workshop.
- The registration fee covers access to all sessions and workshops, as well as lunches and coffee breaks. The Gala Dinner is optional and requires an additional fee.
- The Gala Dinner will be held on Tuesday, 29 September, at 7:30 PM. Venue: *M/S Mälar Victoria, Klara Mälarstrand (Kajplats 3)*




















INVITED SPEAKERS

FRANK RUTSCH



Frank Rutsch, MD, is Professor of Pediatrics and Consultant in Metabolic Medicine at the University of Münster, Germany. For almost 30 years, his research has focused on rare genetic calcification disorders, spanning gene discovery, disease mechanisms, and the development of innovative therapeutic approaches.

RARE GENETIC CALCIFICATION DISORDERS: LESSONS LEARNED OVER THREE DECADES

Over the past three decades, rare genetic disorders of ectopic calcification have evolved from little-known clinical curiosities into powerful models for understanding the regulation of extracellular mineralization. This lecture reflects on a scientific journey that began with the clinical recognition of children affected by devastating calcification disorders and progressed through gene discovery, disease modelling, and the identification of disturbed pyrophosphate metabolism as a central mechanism of pathological calcification.

Using disorders such as generalized arterial calcification of infancy (GACI), pseudoxanthoma elasticum (PXE), Singleton–Merten syndrome, arterial calcification due to CD73 deficiency, and related conditions as examples, the lecture will illustrate how careful clinical observation, international collaboration, and advances in molecular genetics have repeatedly challenged existing concepts and opened unexpected avenues of research. Along the way, the field has revealed surprising connections between extracellular nucleotide metabolism, innate immunity, connective tissue biology, and tissue-specific mineralization.

Beyond presenting key scientific discoveries, the lecture will focus on the lessons learned from translational research: the value of long-term patient cohorts, the importance of interdisciplinary collaboration, the role of unexpected findings in shaping new hypotheses, and the challenges of translating mechanistic insights into therapeutic strategies.

Ultimately, this story illustrates how studying a small number of patients with exceptionally rare diseases has fundamentally changed our understanding of pathological calcification and has provided biological principles that extend far beyond the rare disorders in which they were first discovered.

ARIANE BERDAL



Prof. Ariane Berdal is a researcher specializing in bone and dental pathophysiology, with a particular focus on translational research into rare diseases. With 238 international publications (H-index 42) have been produced in line with the coordination of a research lab, a Master program, a hospital center for oral and dental rare diseases, the dental faculty (dean) and management of reasearch networks. Her research has notably contributed to the understanding of mineralization mechanisms, the molecular diagnosis of oral and dental rare diseases, and the role of environmental factors in disease, with current research focusing on Enamel Renal Syndrome and its underlying mechanisms, natural history, and diagnosis and treatment.

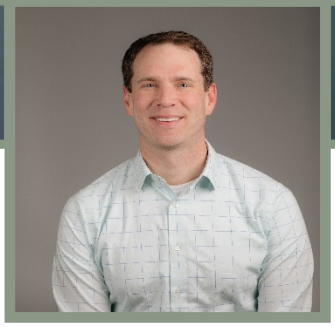
TO MINERALIZE OR NOT TO MINERALIZE IN RARE CONDITIONS ASSOCIATED TO PATHOGENIC *FAM20A* VARIANTS

Context: FAM20A is a cis-Golgi pseudokinase stimulating phosphorylation of secreted proteins (such as enamel proteins EMPs) by FAM20C kinase. *FAM20A* bio-inactivation induces amelogenesis imperfecta and ectopic mineralization: gingiva-AIGF, kidney-ERS (OMIM#204690, *fam20a*-KO mice). The jaws, hosting several tooth & bone, mineralized and soft tissues, are exemplary for AIGF-ERS. Clinical oral, cell, mineral, molecular profiles were explored in a patient cohort (+ paired human controls, *fam20a*-KO mice), its biobank, by in vitro and RNA-Seq studies.

Results-Discussion: Oral natural history of AIGF-ERS disease emerged here: **1)** Soft mesenchymal cells had increased osteogenic capacities (ectopic in pulp, gingiva, follicular sac), relied to cell-autonomous [TGFb]-paths. **2)** EMPs were reversely secreted: not into enamel - which thus was defective - but into the mesenchyme. Polarity loss of enamel-producing cells would be relied to Golgi disruption [*fam20a*-KO TEM data]. Ectopic EMPs would locally trigger soft mesenchyme mineralization properties as visualized on AIGF-ERS including a nanocrystal signature of ectopic minerals [high resolution TEM]. Epithelio-mesenchymal cross-talks were modelized in vitro in EMP-amelogenin assays (Sahi's presentation). **3)** Craniofacial AIGF-ERS bone was defective (Nowwarote, submitted). **4)** In contact with defective enamel, ectopic mineralized tissues and jawbone expanded and fused blocking tooth eruption. In permanent dentition, these events jointly with a progressive gingival hypertrophy end in adults with an edentulous and limited buccal space, requiring mutilating surgeries. **5)** Jaw malformation is the patient priority.

Conclusion 1) One study delivery is clinical: an innovative interceptive protocol, presently tested. **2)** Some cell-autonomous FAM20A pathways would be identified in oral, but also extraoral sites - to be screened systematically. **3)** Aside ABCC6, ENPP1 or MGP, FAM20A would be a rare disease player in ectopic mineralization to consider, with some diagnosis and therapeutic prospects of significance in the craniofacial complex which host major EMP phosphorylated proteins (Nowwarote's presentation).

JOHN HULLEMAN



Dr. Hulleman is an Associate Professor and the Larson Endowed Chair in Macular Degeneration at the University of Minnesota. His research has focused on aspects related to the pathobiology of age-related macular degeneration (AMD), including protein misfolding, extracellular matrix homeostasis, and mineralization of the retina.

ECTOPIC HYDROXYAPATITE FORMATION IN AGE-RELATED MACULAR DEGENERATION

Introduction: Age-related macular degeneration (AMD) is a leading cause of blindness affecting millions worldwide and is characterized by extracellular deposits (called drusen) composed of proteins, lipids, and trace elements under the retina. Of these components, trace elements/minerals, particularly hydroxyapatite (HAP), remain underexplored despite their potential role in coalescing drusen. We sought to further understand the causes and consequences of HAP formation in the eye by utilizing cell culture-based mineralizing models and human donor tissue.

Materials and methods: ARPE-19 (immortalized retinal pigmented epithelial [RPE] cells) and primary porcine RPE cells were used to study the formation of HAP in culture. Osteogenic supplement was used to induce HAP deposit formation in ARPE-19 cells. Mineralization was followed longitudinally by brightfield imaing and NIR autofluorescence. Bulk RNA-seq was utilized to profile transcriptional changes associated with increased mineralization. BoneTag680/Osteosense and SEM-EDS were used to confirm HAP presence. Human donor eyes and eye sections from control and AMD patients were obtained.

Results: HAP formation in ARPE-19 cells required osteogenic supplementation. In contrast, primary porcine RPE cells spontaneously formed HAP deposits with increased culture age, which were accelerated by osteogenic supplementation. RNA-seq of mineralized RPE cells revealed an absence of canonical mineralization genes (e.g., ENPP1, ALPL, RUNX2), but instead hint at a potential role of inflammatory processes. A series of small molecules were identified which prevented HAP formation, but were unable to reverse existing deposits. Analysis of human donor tissue confirmed the presence of HAP in some, but not all intermediate/advanced AMD samples.

Conclusion: RPE cultures can readily form HAP deposits in induced or spontaneous fashion, but seemingly through a mechanism distinct from canonical mineralization. Surprisingly, isolated RPE cells appear quite tolerant of even large HAP deposits. Further analysis and standardized fixation/processing of AMD donor tissue is warranted to gain a clearer picture of the prevalence and role of HAP in this disease.

RAJA PADIDELA



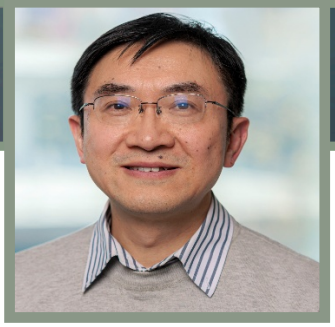
Prof. Padidela is a consultant in Paediatric Endocrinology and Metabolic Bone Disorders at Royal Manchester Children's Hospital, UK, and an Honorary Professor at the University of Manchester. Prof. Padidela is actively involved in managing clinical trials for new medications targeting bone disorders.

He is renowned nationally and internationally for his teaching and training in this field and has authored numerous peer-reviewed journal articles and book chapters.

HYPOPHOSPHATASIA - MECHANISMS AND TREATMENT OUTCOMES

Hypophosphatasia (HPP) is a rare inherited metabolic disorder characterised by deficient tissue-nonspecific alkaline phosphatase (TNSALP) activity and persistently low serum alkaline phosphatase (ALP). Reduced TNSALP activity results in accumulation of substrates including inorganic pyrophosphate and pyridoxal 5′-phosphate, impairing skeletal mineralisation and contributing to multisystem disease. Clinical severity is highly heterogeneous, ranging from life-threatening perinatal and infantile disease with respiratory failure and seizures to childhood and later-onset presentations characterised by rickets, fractures, musculoskeletal pain, muscle weakness, impaired mobility and dental abnormalities. Diagnosis depends on recognising persistently low age- and sex-adjusted ALP in an appropriate clinical context. Contemporary diagnostic criteria incorporate characteristic clinical manifestations, elevation of natural TNSALP substrates and, where informative, identification of pathogenic ALPL variants. Early recognition is particularly important in severe paediatric disease. Asfotase alfa is a bone-targeted recombinant alkaline phosphatase developed to replace deficient TNSALP activity. Clinical studies in paediatric HPP have demonstrated improved survival in severe perinatal and infantile disease, together with sustained improvements in rickets severity, skeletal mineralisation, growth, mobility and muscle function. Clinical cases further illustrate substantial biochemical, skeletal and functional improvement, although complications such as craniosynostosis may persist. Long-term treatment appears important for maintaining skeletal benefit. Five-year UK managed-access experience provides additional real-world evidence of sustained functional benefit in treated children, including improvements in walking and motor function. HPP therefore requires early diagnosis, specialist multidisciplinary assessment and longitudinal monitoring. Emerging therapies aim to improve treatment durability and convenience. Efzimfotase alfa, a longer-acting mineral-targeted TNSALP construct administered every two weeks, is undergoing phase 3 evaluation in children, while gene-delivery, cell-based and alternative enzyme approaches are also being investigated. These developments may broaden future therapeutic options across the heterogeneous HPP spectrum.

DING ZOU



Dr. Ding Zou is a senior researcher at the Center for Sleep Research, University of Gothenburg, Sweden. He has been working with the Swedish CArdioPulmonary BioImage Study (SCAPIS) since 2012. His research focuses on cardiovascular and autonomic regulation during sleep, as well as multidimensional sleep health and cardiovascular risk. He currently serves as Co-Chair of the European Sleep Research Society Scientific Committee.

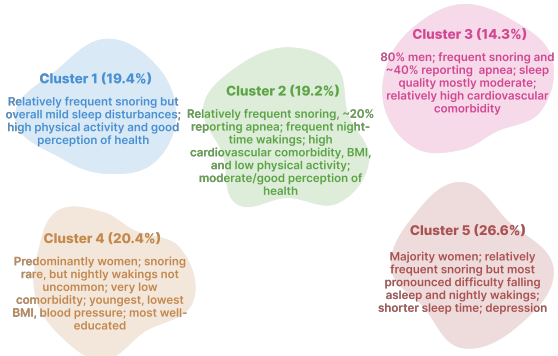
OBSTRUCTIVE SLEEP APNEA SYMPTOM CLUSTERS AS DETERMINANTS OF CORONARY ARTERY CALCIFICATION IN A MIDDLE-AGED SWEDISH POPULATION

Introduction: The newly released EU Protocol on Health Checks for Cardiovascular Diseases excludes obstructive sleep apnea (OSA) screening. We hypothesized that OSA symptom-related phenotypes can improve cardiovascular risk stratification in the general population.

Materials and methods: Using Swedish CArdioPulmonary bioImage Study (SCAPIS) database (n=28,930, 48.1% men, 57±4 years, body mass index 26.9±4.5 kg/m2), anthropometrics, socioeconomic/lifestyle factors, comorbidities, and symptoms of sleep disturbances were analyzed using an explainable artificial intelligence (XAI) clustering algorithm. Coronary artery calcification (CAC) was assessed by non-contrast computed tomography to evaluate subclinical atherosclerosis.

Results: Five distinct clusters were identified (see figure). High CAC burden (CAC score >100) prevalence was 12.7%, 18.7%, 16.7%, 4.1%, and 11.3% in clusters 1 to 5, respectively. The estimated 10-year risk of cardiovascular disease (SCORE2) was 3.6±1.6%, 4.8±2.2%, 4.0±1.8%, 2.1±1.0%, and 3.7±1.9% (ANOVA, P<0.001). XAI demonstrated that specific OSA symptoms (e.g., snoring, witnessed apnea) were key drivers of cluster separation.

Conclusion: XAI-driven clustering reveals distinct clinical phenotypes that correlate strongly with cardiovascular risk. Our results highlight a critical gap in current EU cardiovascular screening guidelines.



DANIELA QUAGLINO



QUAGLINO Daniela is full Professor of General Pathology at the University of Modena and Reggio Emilia (Italy). Along the years, she has investigated the mechanisms leading to altered elastic tissue homeostasis during aging or in genetic diseases. In particular, she has highlighted the role of human fibroblasts in the occurrence of ectopic calcification in the pathogenesis of Pseudoxanthoma elasticum.

THE COMPLEX MECHANISMS OF ELASTIC FIBRES CALCIFICATION

Introduction: Elastin is synthesized by mesenchymal cells providing connective tissues with elasticity, resilience, and long-lasting mechanical efficiency throughout life. After secretion, the elastin precursor, interacting with matrix components, assembles on fibrillin-rich microfibrils to form mature crosslinked elastic fibres (EF). Calcification is a complex, actively regulated process, occurring in several heritable disorders as well as in chronic age-related conditions, which involves the elastin component severely altering the tissue functional compliance.

Materials and methods: Ultrastructural evaluations on tissue samples, proteomic analyses on cultured fibroblasts as well as reductionist approaches using isolated elastin fibrils were applied.

Results: Normally, EF remain resistant to calcification, however proteolytic degradation and structural modifications, such as those observed during aging, inflammation or under oxidative stress, can expose negatively charged sites with high Ca^{2+} binding properties. Moreover, changes in the composition of EF (e.g. associated proteoglycans, glycoproteins, collagens and microfibrillar proteins) can increase the propensity of the fibres to attract calcium and phosphate promoting the nucleation of mineral precipitates. These changes are favoured by calcifying environments where mesenchymal cells respond to pro-osteogenic signals altering their protein profile. Interestingly, comparison of the secretome of cells from tissues differently calcified revealed significant differences in proteolytic activities, matrix components and signalling pathways which contribute to an altered cellular remodelling. Moreover, recent evidence supports the role of mitochondria in contributing to ectopic calcification by modulating calcium-phosphate balance.

Conclusion: Elastic fibres represent an excellent model for studying the complexity of ectopic calcification because they are among the earliest targets of pathological mineralization Their composition and structural organization, susceptibility to age- and disease-related damage, interactions with glycosaminoglycans and calcification regulators provide a valuable system to better understand the role of the extracellular matrix and of changes in cellular behaviour favouring mineral deposition.

MAGNUS BÄCK



Magnus Bäck is Senior Consultant and Professor of Cardiology at the Karolinska University Hospital and Karolinska Institutet in Stockholm (Sweden). At the same time, he is the Director of the cardiovascular INSERM unit in Nancy (France) and Professor at the University of Lorraine. In the European Society of Cardiology (ESC), Magnus Bäck currently acts as Editor-in-Chief for European Heart Journal Open.

LIPID DYSREGULATION AS A DRIVER OF VASCULAR CALCIFICATION

Cardiovascular calcification is closely linked to both lipid- and inflammation-associated residual risk. Beyond the well-established role of lipoprotein retention as a key initiator of atherosclerosis and vascular inflammation, direct lipid-driven mechanisms contribute to the pathogenesis of cardiovascular calcification and its clinical consequences, in large part through inflammatory pathways. Lipids serve as substrates for the generation of both pro-inflammatory and pro-resolving mediators, suggesting that lipid dysregulation may promote a maladaptive inflammatory response with direct implications for ectopic mineralization. Our mechanistic studies demonstrate that inflammation is a major upstream driver of osteogenic reprogramming in structural cardiovascular cells, with bioactive lipid mediators emerging as critical modulators of this process. In particular, omega-3 polyunsaturated fatty acids and their downstream mediators influence both inflammatory activation and its resolution and may represent promising therapeutic targets. Furthermore, evidence linking intraleaflet hemorrhage to erythrophagocytosis, iron-dependent lipid peroxidation, and ferroptosis identifies additional lipid pathways contributing to calcification progression. Together, these findings provide novel mechanistic insights into cardiovascular calcification and highlight lipid dysregulation as a central driver of disease initiation and progression.

LEONOR CANCELA



Leonor Cancela (LC) is Professor Emerita at the University of Algarve and remains active at the Algarve Biomedical Centre and the Centre for Marine Sciences (CCMAR), which she co-founded. She completed her PhD at INSERM/Hôpital Lariboisière in Paris and conducted postdoctoral research at the University of California. She has published over 200 peer-reviewed articles, 15 book chapters, and co-authored 7 international patents. Her research interests focus on molecular, environmental, and epigenetic determinants of skeletogenesis in health and disease and the use of zebrafish as a model for human pathologies, with special incidence on rare diseases.

ZEBRAFISH MODELS FOR DISEASE-ASSOCIATED ABNORMAL OSSIFICATIONS: TWO CASE STUDIES

Zebrafish (Danio rerio) is increasingly used for studying bone pathologies due to the similarity of its axial skeleton to humans and high genomic conservation. In BIOSKEL lab, we developed zebrafish models for two skeletal pathologies to better understand underlying mechanisms and provide a tool to identify therapeutic targets and screen molecules able to rescue disease phenotypes.

Ankylosing spondylitis (AS) is an autoimmune bone disease characterized by fusions and inflammation of the vertebral and sacroiliac joints, loss of mobility, pain, and postural alterations. Although the pathogenesis of AS is not fully understood, polymorphisms in the ERAP1 gene (Endoplasmic Reticulum Aminopeptidase 1) increase disease susceptibility. ERAP1 is essential for immune regulation and antigen presentation by Major Histocompatibility Complex class I molecules. We used CRISPR/Cas9 to induce mutations in zebrafish erap1 genes (erap1a and erap1b). Homozygous mutants producing a truncated Erap1a present abnormal phenotypes and gene expression patterns characteristic of human AS pathology.

CDKL5 deficiency disorder (CDD) is an early-onset epileptic encephalopathy characterised by drug-resistant seizures, motor, visual and neurodevelopmental impairments as well as a progressive defect in mineralization leading to onset of osteoporosis and spondylosis. Zebrafish cdkl5 mutant, previously validated as a model for human CDD, showed deficits in motor behavior and neurodevelopment, and also decreased bone formation leading to lower bone density, craniofacial malformations and microcephaly. Using two available molecule libraries, we have identified molecules capable of rescuing in part or totally the abnormal phenotype of mutant cdkl5 fish.

Conclusion: Homozygous zebrafish mutant mimicking human pathologies can accelerate the identification of disease-specific genes and metabolic pathways, through transcriptomics and proteomics, and can also be used in first line screening pipelines for the identification of molecules capable of rescuing, totally or partially, disease phenotypes in fish, laying down the basis for therapeutic applications in human.

KLAS KÄRRE



Klas Kärre, MD, PhD, Professor emeritus of Molecular immunology, Karolinska Institutet, Stockholm. Member of the Royal Swedish Academy of Sciences, The US National Academy of Sciences and former Chairperson of the Nobel Committee for Physiology or Medicine.

FROM DYNAMITE TO DISCOVERY: THE LEGACY OF ALFRED NOBEL

The lecture will cover a time span from the 17th to the 21st century, describing the family background and life history of Alfred Nobel as well as his legacy: the Nobel prizes. Examples of questions that will be covered are: Is the Nobel family an old aristocratic family? Which academic degrees were held by Alfred Nobel, did he ever do a postdoc? How did a dynamic father pave the way for the invention of dynamite? Which contemporary factors contributed to the perfect timing of this invention? Why were these particular five prize areas selected in the will of Alfred Nobel - Physics, Chemistry, Physiology or Medicine, Literature and Peace? Why was not Economy included in the previous question? Why did it take so long time after the death and the will before the first Prizes were awarded? Who can be awarded a prize, who can nominate, who decides the laureates and how? How many families and how many individuals were awarded more than one Nobel Prize? Some more more Nobel curiosities and statistics, including gender and nationality, and last but not least – why is the Nobel prize so prestigious?

ANJA VERHULST



Anja Verhulst at the University of Antwerp (Belgium) investigates comorbidities of chronic kidney disease (CKD), with a special focus on ectopic vascular calcification and its link with compromised bone health.

ARTERIAL CALCIFICATIONS: PRODUCED BY VASCULAR SMOOTH MUSCLE CELLS, MODULATED BY ENDOTHELIAL CELLS

As the protector of vascular integrity, could the endothelium paradoxically set the stage for its deterioration by actively participating to ectopic calcifications? Ectopic calcifications in the vasculature are characterized by the accumulation of calcium-phosphate crystals and contribute to cardiovascular morbidity and mortality. Vascular smooth muscle cells (VSMCs) are the primary mediators of these calcification, given their capacity to undergo a phenotypic switch toward an osteochondrogenic bone-forming phenotype in response to pathological triggers. On the other hand, emerging evidence recognizes the endothelium as an active participant to this pathological process in valves as well as medial layer tissue, underscoring its central role in the ectopic cardiovascular mineralization processes. The endothelium is a multifunctional monolayer of cells, actively maintaining the vascular homeostasis by regulating the vascular tone, permeability, coagulation and inflammation. Pathological conditions trigger endothelial dysfunction, compromising vascular health and facilitating disease progression beyond the endothelial layer. This lecture will highlight how endothelial dysfunction potentially contributes to the progression of ectopic calcifications, with a focus on arterial media calcifications. Key mechanisms to be discussed include reduced vasodilation, endothelial to mesenchymal transition, endothelial cell senescence and cell death. Importantly, shifting the focus beyond the VSMC-centric perspective could unlock novel therapeutic strategies and would have the advantage to save bone health, which is often compromised with treatment strategies targeting the osteochondrogenic phenotype of VSMCs.

MAKARAND RISBUD



Risbud lab studies the adaptation of nucleus pulposus cells to the unique hypoxic microenvironmental niche of the intervertebral disc. Our work is focused on understanding how aberrant changes in the microenvironment compromise cell function and promote the development of degenerative disc disease, a leading cause of chronic back/neck pain and disability.

A NEW PERSPECTIVE ON INTERVERTEBRAL DISC CALCIFICATION - FROM BENCH TO BEDSIDE

Intervertebral disc degeneration represents a spectrum of distinct, mechanistically driven phenotypes, each representing a unique pathological axis with specific molecular determinants. Among these, disc calcification remains the least studied despite its potentially profound biological and clinical implications. Clinically, disc calcification is associated with poor surgical outcomes and pain that is often refractory to conservative management. At the molecular level, this phenotype is characterized by hypertrophic chondrocyte-like differentiation, increased cell death, inflammation, and disruption of inorganic phosphate (Pi) and pyrophosphate (PPi) homeostasis. Notably, mineralization patterns differ across AF, NP, and EP compartments, indicating strong spatial regulation. Emerging evidence demonstrates that local control of PPi homeostasis, mediated by the ANK–ENPP1 axis, dominates over systemic mineral metabolism in driving calcification. Furthermore, genetic predisposition, aging, and biomechanical stressors such as trauma collectively shape susceptibility to this phenotype. Recent work from others and our laboratory, using various mouse models, has begun to elucidate the intricacies of the pathways that govern disc tissue mineralization. These findings will be sumarized and presented in this keynote lecture.

GILLES KAUFFENSTEIN



Gilles Kauffenstein is a researcher (PhD) investigating the role of purinergic signalling and ectonucleotidases in cardiovascular pathophysiology.

His research aims to elucidate the mechanisms underlying vascular disease associated with pseudoxanthoma elasticum (PXE) and to identify potential therapeutic targets for ectopic calcification.

CALCIFYING AND NON-CALCIFYING MANIFESTATIONS IN PXE - POTENTIAL LINK TO EXTRACELLULAR PURINE METABOLISM

Pseudoxanthoma elasticum (PXE) is a rare inherited disorder caused by pathogenic variants in the ABCC6 gene and is characterized by progressive ectopic calcification affecting the skin, retina, and cardiovascular system. Beyond pathological mineralization, PXE is associated with vascular dysfunction, arterial remodeling, altered hemostasis, and other manifestations whose underlying mechanisms remain incompletely understood. Extracellular purine metabolism and purinergic signalling have emerged as key regulators of mineralization, vascular homeostasis, inflammation, and thrombosis. The ATP/PPi pathway, in which ABCC6 plays a central role, suggests that alterations in purinergic signalling may contribute not only to ectopic calcification but also to the broader cardiovascular phenotype of PXE.

Using complementary investigations in PXE patients and the Abcc6-/- mouse model, we explore the contribution of purinergic signalling to both calcifying and non-calcifying manifestations of the disease. Our work focuses on changes in extracellular nucleotide metabolism by ectonucleotidases, purinergic receptor signalling, vascular remodelling, ectopic calcification, and hemostatic alterations.

By integrating clinical observations with mechanistic studies in experimental models, we aim to identify common pathways linking these various manifestations and to evaluate the therapeutic potential of targeting purinergic signalling.

A better understanding of the interplay between ABCC6 deficiency, extracellular purine metabolism, and vascular homeostasis may provide insights into PXE pathophysiology and reveal novel therapeutic strategies capable of targeting ectopic calcification and PXE-associated cardiovascular complications.

IAN TERRY



Ian F. Terry is CEO and CTO of Aretetic Solutions, where he develops research infrastructure for rare disease and patient-led organizations. He served as Chief Experience Officer at PXE International and Head of User Experience at LunaDNA. Terry also lives with pseudoxanthoma elasticum (PXE); his DNA was included in research that helped identify ABCC6 as the gene responsible for PXE. His work focuses on participant-centered research systems, longitudinal data continuity, and preserving the value of patient-generated data across studies and technology platforms.

THE STUDY ENDED. THE PATIENT DIDN'T: RECONNECTING 30 YEARS OF PXE DATA

Rare disease research is often described as a problem of insufficient data. Yet in long-standing disease communities, valuable data may instead accumulate across studies, platforms, investigators, and decades without being designed to remain longitudinally connected to the participant.

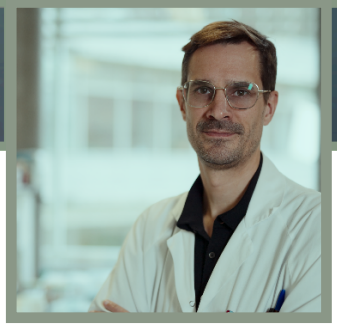
PXE International has collected participant-reported data from individuals with pseudoxanthoma elasticum (PXE) since the late 1990s. Between 1998 and 2024, data were collected through four distinct research periods using paper questionnaires, REDCap, the Platform for Engaging Everyone Responsibly (PEER), and LunaDNA. Each period introduced different survey structures, identifiers, technologies, and patterns of participation. Although many participants contributed repeatedly over time, the resulting datasets were not constructed as a single longitudinal research resource.

We recently reconstructed and harmonized these data by mapping participant identifiers across systems, reconciling changing variable definitions, identifying repeated observations, preserving provenance, and determining where apparently similar questions could—and could not—be treated as equivalent. The resulting resource includes approximately 1,700 unique individuals with usable ophthalmic data and spans nearly three decades of PXE research.

The first analysis examined age-related patterns of reported detection of ocular manifestations in PXE. Findings largely confirmed the expected clinical sequence: peau d'orange was detected earliest, followed by angioid streaks, with retinal hemorrhage and scarring occurring predominantly later in life. More importantly, the analysis demonstrated that decades of community-generated data could be reconstructed into a scientifically coherent research resource despite substantial changes in technology and study design.

Rare disease research infrastructure should be designed around the participant's lifespan rather than the lifespan of an individual study. In small populations, where observations are difficult to replace, preserving participant identity, provenance, and relationships between data across time may be as important as collecting new data.

GAËL NICOLAS



Gaël Nicolas is neurologist and professor of genetics based in Rouen, Normandy, France. In the field of primary brain calcification, he has identified PDGFRB as an autosomal dominant gene and contributed to the identification of several other genes (PDGFB, XPR1, NAA60). He has built a large series of patients in France, which contributed to describe the large clinical and radiological diversity of PBC.

PHENOTYPIC AND GENETIC ASPECTS OF PRIMARY BRAIN CALCIFICATION

Primary Brain Calcification (PBC) is a rare disease with remarkable diversity of neurological or psychiatric symptoms. It is caused by pathogenic variants in genes encoding phosphate transporters, SLC20A2 or XPR1, the platelet-derived growth factor B (PDGFB) or its main receptor (PDGFRB), all with autosomal dominant inheritance, and the MYORG, NAA60 and JAM2 genes, with autosomal recessive inheritance.
We will overview the current knowledge on disease mechanisms associated with PBC as well as the phenotypic spectrum, based on the analysis of data from 269 individuals from 187 families with a definite diagnosis of PBC, confirmed by the identification of a pathogenic variant.

MARTIN PAUCAR ARCE



Dr. Martin Paucar (MD, PhD) is a neurologist and researcher at Karolinska University Hospital and Karolinska Institutet. His expertise lies in movement disorders and neurogenetics, with a special interest in rare neurological diseases, including primary brain calcification. In this talk Dr Paucar will cover the clinical spectrum of PBC and diagnostic challenges.

CLINICAL SPECTRUM OF PRIMARY BRAIN CALCIFICATIONS (PBC) AND EXPERIENCES FROM A LOCAL COHORT

Introduction: The differential diagnosis of brain calcifications is broad. Fahr's disease is the historical term to describe them, but it does not provide information regarding the underlying etiology. On the other hand, primary brain calcification (PBC) constitutes a group of heterogeneous genetic disorders characterized by bilateral calcium deposits in the brain, most commonly affecting the basal ganglia, thalamus, cerebellum, and subcortical white matter. The radiological penetrance in PBC is high, but the clinical penetrance is reduced (44-73%) in several studies. Presentations range from asymptomatic to movement disorders, cognitive, and psychiatric manifestations. So far seven PBC genes have been identified. Treatment is largely symptomatic.

Materials and methods: Data on a PBC cohort from the Karolinska University Hospital with emphasis on its movement disorders will be presented. The current MDS guidelines on PBC discussed. Biomarkers and diagnostic pitfalls are illustrated with clinical vignettes.

Results: In our cohort, 34/52 (65.4%) patients were symptomatic. Gene variants were found in *SLC20A2* (8 variants). followed by *PDGFB* (3 variants). Only one case with a recessive form (*RRP12*) was identified. Radiological progression is generally slow, and the burden of brain calcification is higher in the recessive forms of PBC, followed by *SLC20A2* variants. Phosphate in the CSF is elevated in most patients with *SLC20A2* variants. The main PBC phenocopies in our cohort are DiGeorge syndrome, mitochondrial disorders and Raine syndrome. Some patients meet the criteria for PBC, PD and different forms of atypical parkinsonism.

Conclusion: Our findings are in line with previous studies. Although substantial progress has been made in elucidating its genetic basis, additional PBC genes remain undiscovered. Current MDS guidelines provide a useful framework for diagnosis; however, clinicians should remain aware of diagnostic pitfalls, and the impact of comorbidities. These challenges underscore the need for biomarkers to improve diagnostic accuracy and patient stratification.

MAKOTO KURO-O



Dr. Makoto Kuro-o received his M.D. (1985) and Ph.D. (1991) from the University of Tokyo. He joined the University of Texas Southwestern Medical Center in 1998, where he rose from Assistant Professor to Professor of Pathology, before returning to Japan in 2013 as Professor of Anti-Aging Medicine at Jichi Medical University.

CALCIPROTEIN PARTICLES AS A THERAPEUTIC TARGET OF ECTOPIC CALCIFICATION

Introduction: Calciprotein particles (CPPs) are colloidal nanoparticles containing calcium-phosphate precipitates that form when phosphate concentrations exceed solubility limits in blood or urine. Circulating CPPs induce the production of FGF23, a bone-derived hormone that enhances urinary phosphate excretion. Although FGF23 lowers serum phosphate levels and suppresses CPP formation in blood, it increases phosphate concentrations in renal tubular fluid, thereby promoting CPP formation within the kidney and inducing tubular injury. Progressive tubular damage impairs phosphate excretion, leading to CPP accumulation in the circulation, which contributes to chronic inflammation and vascular calcification in chronic kidney disease (CKD). To test this hypothesis, we developed a CPP adsorption column for incorporation into the hemodialysis circuit and evaluated its ability to prevent vascular calcification in miniature pigs. In addition, we sought to determine the threshold blood FGF23 level above which CPPs appear in renal tubular fluid and accelerate CKD progression through tubular injury.

Materials and methods: Miniature pigs underwent bilateral nephrectomy followed by maintenance hemodialysis for 28 days using either a CPP adsorption column (treatment group) or a control column lacking CPP adsorption capacity (control group). The FGF23 threshold was determined by analysis of a cohort of approximately 5,000 individuals.

Results: Compared with controls, animals treated with the CPP adsorption column showed improved survival, reduced coronary artery and pulmonary calcification, lower left ventricular mass index, and reduced blood CRP levels. Cohort analysis identified a blood FGF23 threshold of 53 pg/mL above which renal tubular damage occurred and CKD progression accelerated.

Conclusion: These findings support a pathogenic role for CPPs in ectopic calcification and other CKD complications and identify CPP adsorption as a promising therapeutic strategy. The identification of an FGF23 threshold associated with intratubular CPP formation suggests that FGF23 may serve as a biomarker for selecting patients likely to benefit from CPP-targeted interventions.

LAURENT BECK



Laurent Beck is a Senior Research Director at Inserm in Nantes. His research focuses on phosphate sensing, from its role in bone and FGF23 regulation to its emerging contribution to vascular calcification in CKD.

HOW IS PHOSPHATE SENSED? THE KNOWN, THE UNKNOWN, AND ITS EMERGING ROLE IN VASCULAR CALCIFICATION

Phosphate (Pi) homeostasis is maintained by a tight endocrine network centered on fibroblast growth factor 23 (FGF23), its co-receptor αKlotho, 1,25-dihydroxyvitamin D and PTH. Hereditary hypophosphatemic rickets and tumoral calcinosis illustrate how narrow this range must be kept, and how severe its disruption becomes in chronic kidney disease (CKD), where sustained hyperphosphatemia aggravates vascular calcification and increases cardiovascular mortality. Maintaining this balance requires early detection of changes in extracellular Pi. While Pi sensing is well characterised in unicellular organisms and plants — notably via SPX domains coupled to inositol pyrophosphates — its mammalian equivalent remains far less understood, largely because it must operate through inter-organ endocrine loops rather than intracellular adaptation alone. Two candidates have been proposed to control Pi-dependent FGF23 secretion: Pi-regulated heterodimerization of the transporters PiT1/PiT2, which signals independently of Pi uptake, and FGFR1c, activated by extracellular Pi through a still-debated mechanism. Whether and how these two systems interact remains unknown. Critically, nearly all mechanistic data on PiT- and FGFR1c-dependent sensing derive from bone. Yet chronic hyperphosphatemia acts directly on the vascular wall, where endothelial cells form the first barrier in contact with circulating Pi or other Pi-containing ligands such as calciprotein particles. Although PiT1/2 and FGFR1c are expressed in endothelial and smooth muscle cells, their functional role in vascular Pi detection is almost never addressed: research on Pi-induced vascular calcification focuses mostly on downstream osteochondrogenic transitions, endothelial-to-mesenchymal transition, and paracrine crosstalk toward smooth muscle cells — all bypassing the Pi-sensing step itself. The aim of this presentation is to define and clarify the concept of Pi sensing, to explain why it is necessary, to describe the identified mechanisms of Pi detection while highlighting their unresolved shortcomings, and to place this knowledge within the context of vascular calcification. A better understanding of this process is a prerequisite for identifying therapeutic targets acting upstream of the vascular consequences of hyperphosphatemia.

PHILLIP TREVOR NEWTON



Dr. Newton is an Associate Professor at the Department of Women's and Children's Health, KI. His lab focusses on skeletal growth and regeneration.

SOFT TISSUE MINERALISATION VIEWED THROUGH THE LENS OF SKELETAL GROWTH

Introduction: Calcification in physiologically mineralised vertebrate tissues begins within nanoscale, membrane-delimited extracellular vesicles, often termed matrix vesicles (MVs). *SGMS2*, which encodes the plasma membrane-resident sphingomyelin synthase 2 (SMS2), has emerged as an important determinant of skeletal ossification. Heterozygous *SGMS2* variants are associated with a broad spectrum of bone phenotypes, ranging from rickets-like deformities in childhood to early-onset osteoporosis, implicating SMS2 in mineralisation, although the underlying mechanisms remain incompletely understood.

Materials and methods: Spatially resolved transcriptomics; size-exclusion chromatography; electron microscopy; nanoparticle tracking analysis; mass spectrometry; western blotting.

Results: We applied spatially resolved transcriptomics to human growth plate biopsies from individuals with idiopathic tall stature, identifying novel genes expressed throughout the growth plate, including *SGMS2* expression in hypertrophic zone (HZ) chondrocytes. Given the growth plate abnormalities observed in individuals carrying *SGMS2* variants, and the fact that mineralisation occurs within the HZ, we prioritised *SGMS2* for further investigation. As MV-associated Sphingomyelin phosphodiesterase 3 (SMPD3) utilises sphingomyelin as a substrate from which phosphate ions are ultimately generated for mineral formation, we hypothesised that SMS2 may be directly involved in MV-mediated mineralisation. To test this hypothesis, we isolated MVs from murine growth plates and found them to contain relatively abundant SMS2 by liquid chromatography-electrospray ionisation tandem mass spectrometry. Using an established turbidity-based MV mineralisation assay, pharmacological inhibition of SMS2 enzymatic activity delayed mineral formation, supporting a functional role for SMS2 in promoting MV-initiated mineralisation.

Conclusion: We propose that SMS2 forms part of a sphingomyelin metabolism pathway that is functionally coupled to phosphate transfer from MV membrane phospholipids to hydroxyapatite during MV-mediated mineralisation. This framework provides new insight into the phenotypes associated with *SGMS2* variants and advances our understanding of the physiological mechanisms underlying vertebrate mineralisation.



SELECTED ORAL PRESENTATIONS

THE TERC/HSP90/TERT AXIS REGULATES TERT NON-CANONICAL FUNCTION IN CALCIFIC AORTIC VALVE DISEASE

Rolando A. Cuevas[1], Kenny Mackenzie[1], Nina Gakii[1], Parya Behzadi[1], Kristin A. Konopka[3], Derek Serna-Gallegos[3], Cynthia St. Hilaire[1,2,3]

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2. Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA
3. Department of Cardiothoracic Surgery, Pittsburgh, PA, USA

Introduction: Calcific aortic valve disease (CAVD) is a highly prevalent disorder characterized by the osteogenic reprogramming of valve cells, yet the underlying molecular mechanism remains poorly defined. We previously discovered a novel non-canonical role for telomerase reverse transcriptase (TERT). Our data show that TERT acts as a co-transcriptional regulator to promote the osteogenic transition of valve interstitial cells (VICs) in early CAVD pathogenesis. Structural modeling suggests TERT's RNA-binding domain interacts with both the long non-coding RNA TERC and the transcription factor STAT5, indicating TERC dissociation may enable TERT's functional switch.

Materials and methods: We integrated in vitro, in vivo, and molecular approaches utilizing human valve interstitial cells (VICs), *Tert*$^{-/-}$ and *lncTerc*$^{-/-}$ mouse models, and diet-induced calcification. SplitFAST imaging, RNA immunoprecipitation, RNAscope, and confocal microscopy monitored TERT/STAT5, TERT/TERC, and TERT/HSP90 dynamics. Small-molecule inhibitors Luminespib (global) and Pimitespib (cytosolic) were leveraged to evaluate the functional necessity of HSP90.

Results: TERT expression is induced by inflammatory signaling and is required for valve cell calcification, whereas *Tert*$^{-/-}$ models exhibit impaired calcification. Conversely, *lncTerc*$^{-/-}$ cells display elevated calcification and increased expression of the osteogenic gene RUNX2. Mechanistically, *TERC* dissociates from TERT during osteogenic differentiation and redistributes to the cytosol, allowing TERT to assemble with STAT5 and drive RUNX2 expression. Crucially, immunoprecipitation confirmed that the physical TERT/HSP90 interaction is enhanced during osteogenic differentiation. Furthermore, both global and cytosolic HSP90 inhibition alter transcription permissive H3K9 acetylation patterns and significantly reduced the expression of key osteogenic and fibrotic markers, including *RUNX2*, *ALPL*, and *ACTA2/SMA*.

Conclusion: Our findings demonstrate that lncTERC inhibits TERT's co-transcriptional activity. The physical separation of TERC from TERT, facilitated by HSP90-mediated cytosolic retention, shifts TERT from canonical telomere maintenance to non-canonical TERT/STAT5 co-transcriptional control. This positions the TERC/HSP90/TERT axis as a novel, pharmacologically targetable mechanism for treating cardiovascular calcification.

TISSUE-NONSPECIFIC ALKALINE PHOSPHATASE DEPHOSPHORYLATES NICOTINAMIDE MONONUCLEOTIDE TO REGULATE NAD METABOLISM

Dalia Gerardin[1], Eva Drevet Mulard[1], Iliass Imam[2], Laurence Bessueille[1], Anne Briolay[3], Sébastien Violot[2], Gilles Jean Philippe Rautureau[1], Pierre Damien Coureux[4], Lionel Ballut[2], David Magne[1]

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2. Univ Lyon, Université Claude Bernard Lyon 1, UMR CNRS 5086, MMSB, Lyon, France.
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4. Univ Paris-Saclay, UMR CNRS 9198, I2BC, Gif-sur-Yvette, France

Introduction: Tissue-nonspecific alkaline phosphatase (TNAP) is essential for physiological mineralization through hydrolysis of inorganic pyrophosphate (PPi), a potent inhibitor of apatite formation. Beyond mineralization, TNAP hydrolyzes several phosphorylated extracellular metabolites, suggesting broader metabolic functions that remain poorly understood. Recently, TNAP was shown to contribute to FAD metabolism by dephosphorylating flavin mononucleotide (FMN), thereby promoting riboflavin uptake. We therefore hypothesized that TNAP similarly hydrolyzes nicotinamide mononucleotide (NMN) into nicotinamide riboside (NR), contributing to NAD metabolism. This hypothesis was further supported by our observation that pharmacological TNAP inhibition with SBI-425 markedly reduced hepatic NAD levels in ApoE-deficient mice.

Materials and methods: NMN hydrolysis was investigated by NMR spectroscopy in serum from four healthy adult donors and in the osteoblastic cell lines MG63 (low TNAP expression) and Saos-2 (high TNAP expression). Recombinant human TNAP was used to characterize NMN hydrolysis in vitro. Finally, cryo-electron microscopy and molecular docking were performed to identify the interactions between NMN and the TNAP active site.

Results: Using the inhibitors SBI-425 and AOPCP, we found that NMN hydrolysis in human serum depends on the combined activities of circulating TNAP and CD73. The same enzymes mediated NMN dephosphorylation at the surface of osteoblastic cells, with relative contributions reflecting their expression levels. TNAP efficiently hydrolyzed NMN, displaying catalytic activity comparable to, or greater than, that observed with PPi and without evidence of substrate inhibition. Cryo-EM and docking analyses revealed that NMN interacts with key residues within the TNAP active site, including residues distinct from those interacting with PPi and known to be mutated in hypophosphatasia.

Conclusion: These findings identify NMN as a novel TNAP substrate and uncover a previously unrecognized role for TNAP in NAD metabolism, considerably expanding the metabolic functions of this enzyme beyond extracellular PPi hydrolysis.

JESSEM LANDOULSI

DYNAMICS OF MINERALIZATION REVEAL FUNCTIONAL HETEROGENEITY OF MATRIX VESICLES ISOLATED FROM HUMAN OSTEOARTHRITIC CARTILAGE

Fanny Duhalde[1,2], Célia Orengo[1], Elise Pajot[2], Jihye Lee[2], Sophie Galier[3], Clément Guibert[2], Sandra Casale[2], François Guyot[4], François-Paul Ehkirch[1,5], Maryse Guerin[3], Jérémie Sellam[1,6], Xavier Houard[1]* and Jessem Landoulsi[2]*

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Ectopic calcification results from highly localized calcium phosphate mineralization events that develop within confined extracellular microenvironments before propagating into the surrounding extracellular matrix (ECM). Matrix vesicles (MVs), specialized extracellular vesicles, are recognized as key initiators of this process during both physiological and pathological mineralization. Although intravesicular and extravesicular mineralization pathways are believed to coexist, the respective contribution of individual MVs remains poorly understood because most studies rely on ensemble-averaged measurements performed after mineralization.

Herein, we introduce a single-vesicle approach to investigate the mineralization behavior of native human osteoarthritic cartilage-derived MVs under near-physiological conditions. Isolated MVs were immobilized on a biomimetic ECM-like interface, enabling atomic force microscopy (AFM) imaging and force spectroscopy to monitor the same individual vesicles at successive stages of mineralization.

Although isolated MVs appear morphologically indistinguishable before mineralization, single-vesicle analysis reveals marked functional heterogeneity. Only a subset of MVs undergoes mineral accumulation, whereas others remain mechanically and structurally unchanged, a distinction that is completely masked by conventional ensemble measurements. AFM nanomechanical measurements further reveal a progressive increase in the apparent stiffness of mineralizing MVs, consistent with confined mineral deposition within intact vesicles. The correlation between vesicle stiffness and dimensional changes further supports a mineralization process dominated by intravesicular mineral accumulation prior to the emergence of extracellular mineral.

These findings complement our recent work on collagen mineralization, where real-time AFM demonstrated that controlling the spatial localization of phosphate generation enables the sequential development of intra- and extrafibrillar mineralization. Together, these studies illustrate how nanoscale AFM approaches can directly visualize confined mineralization events and provide new mechanistic insight into the spatial regulation of calcium phosphate formation during physiological and ectopic calcification.

ZIHAN QIN

FERROPTOSIS PROMOTES AORTIC STENOSIS THROUGH 5-LIPOXYGENASE

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5. Laboratory of Physiopharmacology, Faculty of Medicine and Health Sciences and Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Campus Drie Eiken, Antwerp, Belgium.
6. Department of Cardiology, Anzhen Hospital, Beijing, China.
7. Department of Clinical Physiology, Region Västra Götaland, Sahlgrenska University Hospital, Gothenburg, Sweden.
8. Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

Introduction: Calcific aortic valve disease (CAVD) culminates in severe aortic stenosis, currently lacking pharmacological treatment. Intra-leaflet haemorrhage-induced iron overload promotes valvular ferroptosis. This study identifies a druggable ferroptosis pathway and validates its translational relevance across large-scale population cohorts.

Materials and methods: Bulk- and single-cell transcriptomics, whole-mount histology, immunohistochemistry, and primary human valvular interstitial cell (VIC) models of 212 aortic valves from surgical patients were integrated for mechanistic insights. In vivo, valvular ferroptosis and thickness were assessed in a doxorubicin-induced ferroptosis mice model, and a wire injury model with ferroptosis inhibition was utilized to evaluate remodelling and haemodynamic obstruction. A total of 4874 participants of the Swedish CArdioPulmonary bioImage Study (SCAPIS) underwent cardiac computed tomography for aortic valve calcification determination and 273 550 individuals from the UK Biobank were followed for aortic stenosis incidence.

Results: Intra-leaflet haemorrhage was prevalent in calcified aortic valves and correlated positively with calcification and circulating ferroptosis biomarker. Bulk transcriptomics and single-cell RNA sequencing identified lipid peroxidation as the dominating valvular ferroptosis pathway centred on arachidonate 5-lipoxygenase (ALOX5). Inducing ferroptosis in VIC triggered lipid peroxidation and calcification while reducing viability, which were reversed by targeting the ALOX5-ACSL4 axis. In vivo, doxorubicin-induced valvular ferroptosis exacerbated valvular thickening in apoE-/- mice through ALOX5-ACSL4 upregulation. ALOX5 inhibition reduced valve thickness and haemodynamically improved valve function in the wire injury model. Arachidonic acid levels independently predicted aortic valve calcification and incident aortic stenosis in SCAPIS and UK Biobank, respectively, and positively correlated with circulating ferroptotic marker.

Conclusion: These findings establish the ALOX5-ACSL4 axis as a critical mediator of ferroptosis in CAVD and promising repurposing target for clinical intervention.

HARDENING OF THE ARTERIES: WHY DOES ARTERIAL MEDIAL CALCIFICATION PREFERENTIALLY DEVELOP IN CERTAIN ARTERIAL LOCATIONS?

Jordan ElBadry[1], Maciej Cejrowski[1], Abby Chun Yan Tam[1], Rohan Master[1], Amalia Moore[1], Caroline Wheeler-Jones[1], Isabel R Orriss[1]

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Introduction: Arterial medial calcification (AMC) does not develop uniformly, with certain vessels more likely to calcify. This study investigated the mechanisms underpinning this location-specific AMC development.

Materials and methods: AMC was investigated using *in vivo* (rats fed a warfarin/vitamin K1 diet)*, ex vivo* (aortic rings) and *in vitro* (VSMCs) approaches. Samples were analysed using microCT, histology, RT-PCR, western blotting and cell-based assays.

Results: MicroCT analysis of whole aortas showed the most AMC in the abdominal region (14% calcified); arch and thoracic regions were 5% and ≤1% calcified, respectively ($p<0.001$). Individual calcifications were largest in the abdominal ($0.005mm^2$) > arch ($0.0025mm^2$) > thoracic ($0.0003mm^2$, $p<0.001$) aorta. Compared to regions experiencing laminar flow, areas associated with disturbed flow had ≤30-fold more AMC ($p<0.001$). Histology revealed that, in all regions, AMC developed first at the interface of the tunica media and external elastic membrane before spreading longitudinally along the elastin fibres and towards the lumen. Early calcifications developed without matrix changes. Elastin breakdown was only evident in regions of established AMC and highest in the abdominal aorta (8.7%) > arch (7.2%) > thoracic (3.3%). *Ex vivo* aortic rings initially (≤7 days) showed the same variation: abdominal (12% calcified) > arch (11%) > thoracic (3%). After 28 days, no differences were evident. *In vitro*, calcification and alkaline phosphatase activity were 49% and 60% ($p<0.01$) lower, respectively, in thoracic VSMCs compared to arch and abdominal cells. RNA/protein analysis revealed thoracic VSMCs displayed less phenotypic changes retaining higher expression of VSMC markers (MYH11, Smtn, αSMA, TAGLN) and reduced osteogenic markers (Runx2, TNSALP, OPN).

Conclusion: These data show that, whilst all aortic regions can calcify, the thoracic aorta consistently shows the lowest susceptibility. This variation is due to fundamental differences in the VSMCs, and potentially shear stress, which directly influence the onset, extent and location of AMC.

CALCIUM SHAPE CHARACTERISTICS IN CAROTID ATHEROSCLEROSIS AND ITS ASSOCIATION TO UNDERLYING BIOLOGICAL SIGNATURES

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Introduction: Intraplaque calcification is a common morphological component of atherosclerotic plaque. While diverse calcium shapes have been described, most rely on qualitative descriptions, with limited understanding of the relationship between calcium phenotypes and underlying biological signatures. To address this, we introduce novel quantitative markers of spatial calcium morphology and identify their association with underlying molecular transcriptomic profiles.

Materials and methods: Carotid endarterectomy specimens were identified in the Biobank of Karolinska Endarterectomies with pre-interventional computed tomography angiography (CTA) and post-interventional bulk RNA sequencing available (n=68). Reconstructed CTA images were analyzed using ElucidVivo software, including 3D reconstruction of intraplaque calcium. Geometric radiomic descriptors quantified calcium shape, size, and distribution, focusing on elongation, flatness, and sphericity: entities statistically describing calcium topology (sphericity quantifying 3D roundness; elongation and flatness measuring geometric spread along principal axes). Associated up- and downregulated biological expressions were identified using differential gene expression (DGE) and gene set enrichment analysis, coupling morphological characteristics to biological vulnerability.

Results: High sphericity associated with localized inflammation and reduced focal adhesion (upregulation: IL36RN, IL36B, IGFN1, SLC25A48; downregulation: EPYC, CCL17, TRIM55, PCSK9, SPHKAP, **Figure 1.b**), possibly contributing to vulnerable characteristics. Conversely, elongated calcifications were associated with enhanced cellular trafficking, actin cytoskeletal organization, and reduced contractility (upregulation: MMP3, MEPE, CLEC3A; downregulation: ADRA1D, UTS2, **Figure 1.c**), reflective of changes in vascular smooth muscle cell phenotype and processes influencing the spatial organization of intraplaque calcification. Flatness exhibited several co-expressive signals as elongation, alongside increased oxidative stress responses (upregulation: SIGLEC12, CCL17, CLEC3A; downregulation: CDH1, UTS2, **Figure 1.d**), suggesting additional cellular stress environments accompanying mineralization. Further validation of these trends are required; however, results provide initial quantitative coupling between calcium morphology and associated biology.

Conclusion: Quantitative calcium shape descriptors are associated with distinct molecular signatures relating to plaque stability, providing novel insight into the underlying biological mechanisms of intraplaque calcium.

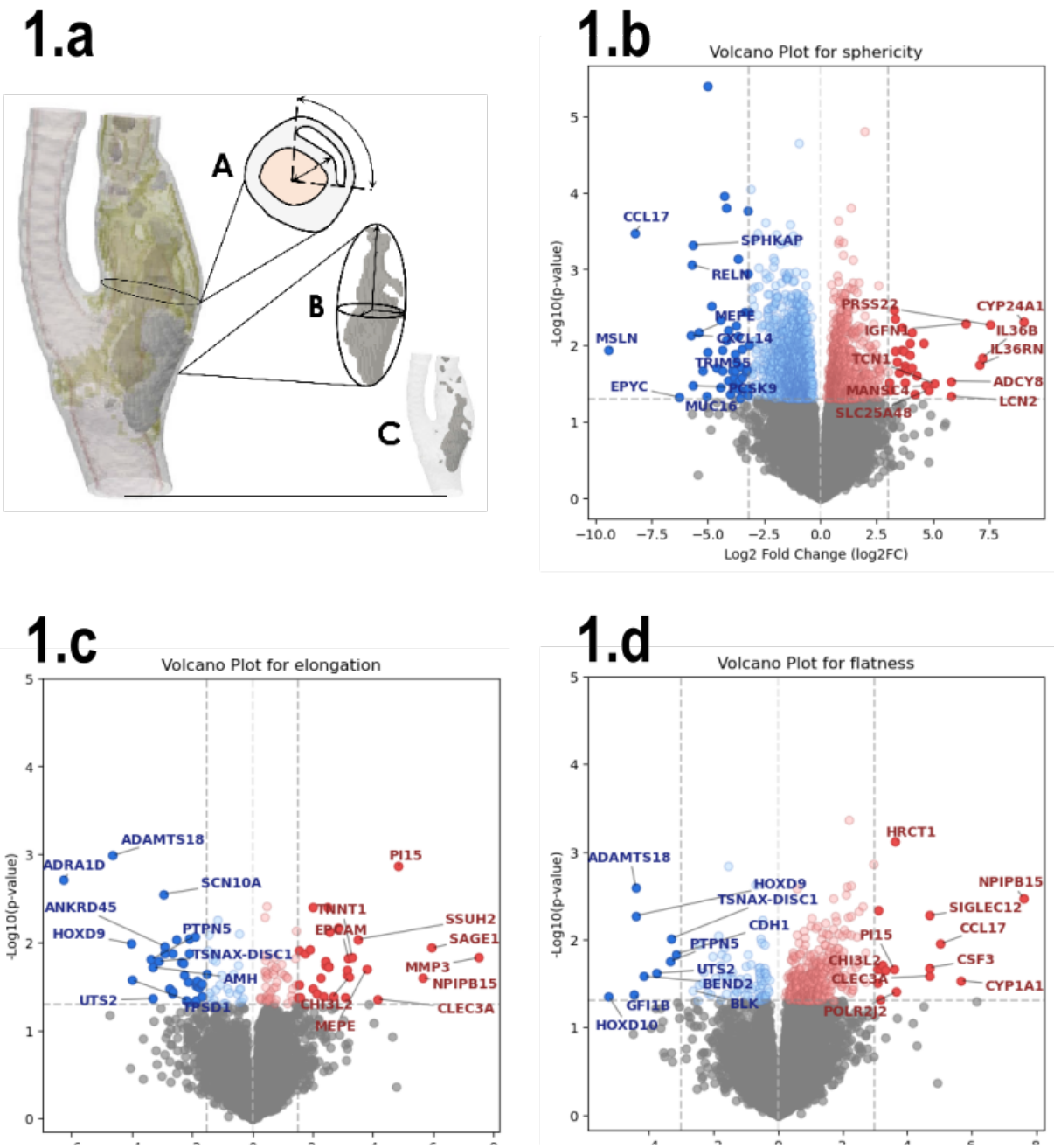


Figure 1 - Spatial calcium morphology and DGE volcano plots: (a) Quantification of intraplaque components based on their sizes (A), shapes (B), and distributions (C) to represent calcium morphology of an individual. (b-d) Volcano plots showing differential gene expression associated with sphericity, elongation, and flatness.

JOUMANA AL AKHDAR

ENDOTHELIAL EXOSOMES MEDIATE TMAO-INDUCED VASCULAR CALCIFICATION THROUGH MICRORNA-DEPENDENT WNT/B-CATENIN SIGNALING

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Introduction: Vascular calcification (VC) is an actively regulated process contributing substantially to cardiovascular morbidity and mortality. Although vascular smooth muscle cells (VSMCs) are key mediators of calcification, the contribution of endothelial cells (ECs) through endothelial-to-mesenchymal transition (EndMT) and extracellular vesicle signaling remains incompletely understood. Trimethylamine-N-oxide (TMAO), a gut microbiota-derived metabolite linked to cardiovascular disease, promotes vascular dysfunction; however, its role in EndMT-associated exosomal signaling and VSMC osteogenic reprogramming remains unclear. This study investigated whether clinically relevant TMAO concentrations induce EndMT and whether endothelial-derived exosomes promote vascular calcification through microRNA-mediated Wnt/β-catenin activation.

Materials and methods: Human umbilical vein endothelial cells (HUVECs) were exposed to TMAO (1–100 µM). EndMT was assessed by qPCR and Western blotting. Endothelial-derived exosomes were isolated by differential ultracentrifugation and characterized by transmission electron microscopy, nanoparticle tracking analysis, and exosomal marker profiling. Human aortic vascular smooth muscle cells (HAVSMCs) were treated with isolated exosomes, and phenotypic modulation, osteogenic differentiation, and calcification were evaluated through gene expression analysis, β-catenin activation, and Alizarin Red staining. Exosomal miR-30a-5p and miR-222-3p levels were quantified, while β-catenin inhibition and miR-222-3p overexpression studies were performed.

Results: TMAO induced dose-dependent EndMT, evidenced by reduced CD31 and VE-cadherin expression and increased α-SMA, vimentin, N-cadherin, and SNAIL1 levels. Exosomes from TMAO-treated ECs promoted VSMC phenotypic switching, suppressing contractile markers while increasing RUNX2, osteopontin, TNAP, β-catenin expression, and calcium deposition. These exosomes exhibited reduced miR-30a-5p and elevated miR-222-3p levels, favoring Wnt/β-catenin activation. Inhibition of β-catenin signaling or endothelial exosome biogenesis significantly attenuated osteogenic reprogramming and calcification, while miR-222-3p overexpression independently induced osteogenic signaling in HAVSMCs.

Conclusion: These findings identify a novel gut microbiota-vascular calcification axis in which TMAO-induced EndMT reprograms endothelial exosomal microRNA cargo, promoting VSMC osteogenic differentiation and calcification through Wnt/β-catenin signaling. Targeting endothelial exosome-mediated communication may provide a therapeutic strategy against vascular calcification.

MENG-YING WU

FUNCTIONAL DIVERGENCE OF SWI/SNF CHROMATIN-REMODELLING SUBCOMPLEXES DURING VSMC PHENOTYPIC SWITCHING IN VASCULAR CALCIFICATION

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Introduction: Chromatin remodelling regulates transcription by modulating chromatin accessibility, thereby driving the transcriptional reprogramming underlying osteogenic differentiation of vascular smooth muscle cells (VSMCs). Although osteogenic differentiation is a key driver of vascular calcification, the epigenetic mechanisms by which SWI/SNF chromatin-remodelling complexes regulate VSMC phenotypic switching remain poorly understood. Here, we investigated the roles of distinct SWI/SNF subcomplexes in this process.

Methods: Primary human aortic VSMCs were cultured under calcifying conditions. SWI/SNF components were profiled using an epigenetic qPCR array, followed by siRNA-mediated gene silencing and calcification assays. CUT&RUN-qPCR and CUT&RUN sequencing defined BRD7 and BRD9 chromatin occupancy and downstream transcriptional programmes. Protein interactions were analysed by immunoprecipitation–mass spectrometry, and findings were integrated with human single-cell RNA sequencing and genetic analyses. Selected observations are undergoing further mechanistic validation.

Results: SWI/SNF subcomplexes exhibited opposing functions during VSMC calcification, with ncBAF promoting and PBAF suppressing osteogenic differentiation. BRD9 depletion attenuated mineral deposition, whereas loss of BRD7 or PBRM1 accelerated calcification. BRD7 occupancy at the RUNX2 promoter decreased during calcification, consistent with derepression of osteogenic transcription. CUT&RUN sequencing identified BRD7-regulated pathways associated with inflammation, senescence, and RASSF1A–YAP1 signalling. Human single-cell transcriptomic data revealed cell type-specific activation of BRD7 target genes during VSMC phenotypic switching. In parallel, the ncBAF–ETS2 axis promoted osteogenic transcriptional programmes, while ETS2 loss-of-function variants were associated with reduced cardiovascular and coronary artery disease risk. Ongoing mechanism and in vivo studies are validating these regulatory pathways.

Conclusions: These findings suggest that SWI/SNF subcomplexes exert distinct regulatory functions during VSMC phenotypic switching and identify chromatin-remodelling pathways as potential therapeutic targets for vascular calcification. Ongoing studies are investigating whether subunit switching between SWI/SNF subcomplexes is a critical event that primes VSMC phenotypic switching while further defining the underlying molecular mechanisms.

AAV-MEDIATED LIVER-TARGETED HUMAN ABCC6 GENE THERAPY FOR PXE

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Introduction: Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder caused by loss-of-function mutations in ABCC6, a transmembrane transporter protein predominantly expressed in the liver. ABCC6 deficiency reduces plasma PPi, a potent endogenous inhibitor of calcium crystal deposition. We hypothesize that AAV-mediated, liver-targeted delivery of human *ABCC6* would restore plasma PPi and counteract ectopic calcification in an *Abcc6*$^{-/-}$ mouse model of PXE.

Materials and methods: Two doses of AAV serotype 8 vector encoding codon-optimised human *ABCC6* under the control of the liver-specific promoter HLP (1×10^{12} and 5×10^{12} vg/mouse) were compared for hepatic transgene expression in *Abcc6*$^{-/-}$ mice; the higher dose (5×10^{12} vg/mouse) achieved superior expression and was used for subsequent studies. To assess prevention and reversal of ectopic calcification, *Abcc6*$^{-/-}$ mice received a single retro-orbital injection of AAV8-HLP-ABCC6co at 4 weeks of age (prevention study) or 13 weeks of age (reversal study). All mice were sacrificed at 22 weeks of age. The therapeutic efficacy was assessed by hepatic ABCC6 transgene expression, plasma PPi levels, and ectopic calcification of the muzzle skin.

Results: Immunofluorescent staining and Western blot confirmed robust hepatic ABCC6 expression following AAV administration. Treated mice showed significantly elevated plasma PPi levels similar to those observed in asymptomatic *Abcc6*$^{+/-}$ mice. In the prevention study, AAV administration significantly prevented muzzle skin calcification. In the reversal study, the same treatment halted further progression of calcification.

Conclusion: AAV-mediated liver-targeted human *ABCC6* gene therapy restores plasma PPi, effectively prevents ectopic calcification, and stabilizes established calcification in a mouse model of PXE. These findings support continued development of AAV-based gene therapy as a therapeutic strategy for PXE.

MAYRA MARTINEZ-LOPEZ

DEFINING THE TEMPORAL DYNAMICS OF EARLY CARDIOVASCULAR CALCIFICATION THROUGH LIVE IMAGING

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Cardiovascular calcification (CVC) is a major cause of heart valve and vascular dysfunction and contributes substantially to cardiovascular morbidity, mortality, healthcare costs, and reduced quality of life worldwide. Despite its clinical burden, current therapies remain limited to slowing disease progression, largely because the cellular and molecular mechanisms driving disease initiation remain poorly understood.

We use the zebrafish *enpp1* mutant, a model of congenital CVC that develops reproducible calcification in the cardiac outflow tract (OFT). Optical transparency, fluorescent reporter lines, and vital calcium dyes enable live imaging of calcification onset and associated extracellular matrix remodeling and cellular interactions.

Live imaging showed the earliest detectable OFT calcification at 19 days post fertilization (dpf). By 22 dpf, the number and size of calcified foci had increased, and by 30 dpf the OFT was extensively calcified. 3D imaging indicates that early calcifications develop within the OFT tissue, away from the lumen, and consistently localize to similar regions, suggesting that local mechanical forces may contribute to calcification initiation. Early OFT calcification was observed in only a subset of homozygous *enpp1* mutants and did not correlate with the severity of ectopic calcification in other tissues, further suggesting that local mechanisms contribute to tissue-specific susceptibility to CVC.

Preliminary single-cell transcriptomic analysis of calcifying OFTs from adult *α-klotho* mutants, a model of aging-related CVC, revealed enrichment of immune-associated biological processes. The cellular and molecular mechanisms associated with calcification initiation, including these immune responses, are currently being assessed *in vivo*. We hypothesize that conserved mechanisms govern congenital and aging-related CVC. A single-cell transcriptomic atlas of the *enpp1* OFT will be integrated with the *α-klotho* dataset to identify shared candidate regulators for functional validation in both models.

By integrating longitudinal live imaging with single-cell transcriptomics, this work aims to define the molecular and cellular events driving CVC initiation, identify mechanisms of tissue susceptibility, and uncover potential therapeutic targets acting before irreversible calcification is established.

GGCX KNOCK-OUT ZEBRAFISH AS A MODEL FOR PXE-LIKE SYNDROME SUFFER MIDFACIAL HYPOPLASIA, IMPAIRED VASCULAR INTEGRITY AND FATAL HEMORRHAGES

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Introduction: Gamma-glutamyl carboxylase, encoded by *GGCX*, catalyzes vitamin K (VK)-dependent gamma-carboxylation of glutamate residues, a posttranslational modification essential for various biological processes, including bone metabolism and blood coagulation. Biallelic pathogenic *GGCX* variants cause Pseudoxanthoma elasticum-like disorder with multiple coagulation factor deficiency (PXE-like; OMIM# 610842), characterized by elastic fiber calcification and fragmentation, severe skin laxity, midfacial hypoplasia, decreased VK-dependent coagulation factors levels, and, in some patients, cerebral aneurysms. As the *Ggcx-/-* mouse model shows embryonic lethality, we aimed to establish and characterize a zebrafish model of GGCX deficiency.

Materials and methods: The zebrafish *ggcx* gene was knocked-out using CRISPR/Cas9. Phenotypic characterization was done by histochemical staining and fluorescent imaging of transgenic lines for endothelial cells (*Tg(kdrl:GFP)*), erythrocytes (*Tg(globin:GFP)*) and osteoblasts (*Tg(osx:Kaede)*). Coagulation was assessed by nicking the caudal vein plexus in 5-day-old larvae and measuring clotting time compared to controls.

Results: The *ggcx-/-* zebrafish were viable but developed premature lethality at ~1 month of age. Alcian blue staining revealed altered skull morphology in larvae, recapitulating midfacial hypoplasia observed in patients. Although bone formation was affected, alizarin red staining showed no significant differences in mineralization levels or patterns. Sudden death juvenile zebrafish exhibited spontaneous intracranial and pericardial hemorrhages. Exposure to 34°C induced intracranial hemorrhage in 3-day-old mutants due to decreased vascular integrity and aneurysm formation. Additionally, 5-day-old *ggcx-/-* larvae showed prolonged clotting times compared to controls.

Conclusion: We established the first viable *ggcx-/-* zebrafish model. Loss of *ggcx* causes craniofacial abnormalities, impaired vascular integrity and delayed blood clotting, resulting in fatal hemorrhagic events. The absence of detectable ectopic mineralization may reflect the juvenile lethality of the model. This zebrafish model provides a valuable platform to investigate the roles of GGCX in skeletal development, vascular integrity and hemorrhagic disease, including cerebral aneurysm formation.

ENPP1 LOSS DISRUPTS MITOCHONDRIAL CALCIUM HOMEOSTASIS AND PROMOTES OSTEOBLAST SENESCENCE AND MINERALISATION

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Introduction: ENPP1 is an extracellular mineralisation regulator that hydrolyses ATP to generate inorganic pyrophosphate, an inhibitor of ectopic mineralisation and regulator of skeletal mineralisation. Additionally, ENPP1 has been implicated in cellular energy metabolism; however, whether it directly regulates mitochondrial homeostasis remains unclear.

Materials and methods: ENPP1 expression was silenced by siRNA in MC3T3-E1 osteoblast-like cells. Mitochondrial function was assessed using fluorescent probes MitoSOX and H2DCFDA to measure mitochondrial superoxide and reactive oxygen species, while mitochondrial membrane potential was assessed using fluorescent dyes JC-1 and TMRM. Mitochondrial quality control was examined by TOM20–LC3 immunofluorescence colocalisation and a tandem mCherry–eGFP mito-QC reporter of mitochondrial delivery to acidic lysosomes. Mitochondrial Ca^{2+} was measured using the Rhod-5N fluorescent indicator, while PDHE1α phosphorylation was assessed by immunoblotting as a biochemical readout of mitochondrial matrix Ca^{2+}. Senescence was assessed by senescence-associated β-galactosidase staining and p21/p16 immunoblotting. Mitochondrial calcium uniporter (MCU)-dependent mitochondrial Ca^{2+} uptake was examined using the MCU inhibitor Ru360. Mineralisation was evaluated by Alizarin Red staining and calcium quantification.

Results: ENPP1 knockdown increased mitochondrial superoxide and intracellular oxidant burden, while reducing mitochondrial membrane potential. It also decreased TOM20–LC3 colocalisation and mito-QC red-only puncta, indicating impaired mitochondrial delivery to lysosomes. These changes were accompanied by increased senescence-associated β-galactosidase activity, p21/p16 accumulation and mineral deposition. Mechanistically, ENPP1 knockdown increased mitochondrial Ca^{2+} accumulation and reduced PDHE1α phosphorylation. Inhibition of mitochondrial Ca^{2+} uptake with Ru360 restored PDHE1α phosphorylation and attenuated the accumulation of senescence-associated proteins, supporting MCU-dependent calcium overload.

Conclusion: These findings identify ENPP1 as a previously unrecognised regulator of mitochondrial homeostasis. ENPP1 loss promotes MCU-dependent calcium overload, oxidative stress, mitochondrial depolarisation, impaired mitophagy, senescence and increased mineral deposition. This work links ENPP1-dependent mitochondrial dysfunction to osteoblast mineralising behaviour and advances understanding of the cellular mechanisms underlying both ectopic and skeletal mineralisation.

CLOTHILDE HENNION

SOLUBLE EPOXIDE HYDROLASE IN CALCIFIED AORTIC STENOSIS: FROM SELECTIVE MODULATION TO GENDER-SPECIFIC PERSONALIZED THERAPY

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Introduction: There is no available treatment to delay the development of calcified aortic stenosis (CAS). Soluble epoxide hydrolase (sEH) could be an innovative target because its hydrolase (sEH-H) and phosphatase (sEH-P) activities metabolize lipid mediators and pyrophosphate anions (Pi) known to regulate inflammatory and calcification mechanisms. We aim to characterize the role of sEH in the development of CAS and evaluate the impact of its inhibition in various preclinical models.

Materials and methods: Valvular endothelial (VECs) and interstitial (VICs) cells were isolated from aortic valves of CAS patients. sEH substrates and metabolites were measured in static or flow culture supernatants by LC-MS/MS and bioluminescence. In a Pi-induced calcification model on VICs and isolated rat aortic valves, the effect of sEH-H and sEH-P inhibition was determined by colorimetry (o-cresolphtalein calcium quantification) and immunochemistry (Osteosense® staining of hydroxyapatite deposits). Finally, in a CAS model of nephrectomised rat model fed with phosphorous-rich diet (Nx+P), the impact of a genetic inhibition of sEH-P was evaluated by echocardiography and immunochemistry.

Results: In VECs, the release of sEH substrates and metabolites is not different between laminar or oscillating flow conditions. VICs calcification is reduced by pharmacological sEH inhibition, with a more pronounced effect of sEH-H inhibition in women and sEH-P inhibition in men. sEH-P inhibition also increases PPi levels in VICs supernatants. Furthermore, pharmacological and/or genetic inhibition of sEH-P reduces hydroxyapatite deposits *in vivo* and *ex vivo* in the aortic valve. This effect is *associated in vivo* with the prevention of intercusp distance reduction and increased aortic valve flow velocity in Nx+P rats.

Conclusion: Taken together, those results show that sEH is an innovative therapeutic target to prevent CAS development. Gender-specific effects, with modulation of the inflammatory axis via sEH-H in women and calcification mechanisms via sEH-P in men should be deeper investigated.

GENETIC MAPPING OF JOINT ECTOPIC CALCIFICATION IDENTIFIES KIF26B AS A THERAPEUTIC TARGET

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Introduction: Joint ectopic calcification (EC) is a pathological response to injury characterized by inappropriate mineralization of intra-articular soft tissues, including the meniscus and synovium. The genetic architecture of EC in humans remains poorly understood. Here, we combined genetic mapping with functional validation to identify genetic determinants of joint EC and define mechanisms contributing to disease susceptibility.

Materials and methods: EC was induced by destabilization of the medial meniscus in the LG/J × SM/J advanced intercross line (maintained through 44 generations of random mating) and quantified by micro-CT. QTL analysis was performed to map meniscal and synovial EC phenotypes to discrete genomic loci. Candidate genes within these QTLs were prioritized based on SNP annotation, gene expression, and biological relevance and prior human genetic evidence, with KIF26B selected for functional analysis. The effects of KIF26B knockdown were examined using RNAi-mediated silencing in a murine model of EC and in human and murine progenitor/stem cells.

Results: QTL analysis identified 20 loci associated with meniscal and synovial EC, encompassing 141 positional candidate genes. Functional annotation of EC-associated SNPs implicated genes involved in skeletal development, bone metabolism, pathological mineralization, and osteoarthritis. KIF26B mapped to a high-susceptibility EC locus and has previously been identified in human GWAS of heterotopic ossification. Mechanistically, loss of KIF26B impaired osteogenic differentiation of stem/progenitor cells through canonical Wnt/β-catenin signaling. Functional validation of Kif26b revealed that intra-articular Kif26b silencing attenuated injury-induced EC in mice.

Conclusion: EC is a genetically complex trait regulated by multiple susceptibility loci and biological pathways, with KIF26B emerging as a promising therapeutic target for the treatment of EC. By linking genetic mapping to functional validation, this study illustrates how susceptibility loci for complex traits can reveal disease mechanisms that may ultimately advance our understanding of human disease and facilitate the identification of therapeutic targets.

ROLE OF ANKH CHANNEL IN CRYSTAL-INDUCED ARTHROPATHIES

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Introduction: Crystal-induced arthropathies are characterized by the deposition of microcrystals in joints and periarticular tissues, resulting in inflammation and progressive joint damage. Calcium pyrophosphate (CPP) and monosodium urate (MSU) crystals cause calcium pyrophosphate deposition disease (CPPD, chondrocalcinosis) and gout, respectively. Gout can be controlled by urate-lowering therapies, whereas no disease-modifying treatment is currently available to prevent CPP crystal formation or promote crystal dissolution. CPP crystal formation remains poorly understood and likely results from the combined effects of aging, tissue injury, metabolic disorders, and disturbances in the balance between calcium, phosphate (Pi), and pyrophosphate (PPi). Genetic studies have identified key regulators of PPi homeostasis, including **ANKH, ENPP1,** and **TNAP** in both age-related and familial forms of CPPD. Recent evidence indicates that the plasma membrane protein **ANKH** exports ATP, a precursor of extracellular PPi, as well as other anions such as citrate and malate. By controlling extracellular PPi levels, ANKH may play a central role in CPP crystal formation. Mutations in **ANKH** are associated with two autosomal dominant disorders: **craniometaphyseal dysplasia (CMD)**, characterized by abnormal bone growth, and a rare familial form of CPPD marked by early and extensive crystal deposition, recurrent inflammation, joint destruction, and ankylosis.

Results: We recently generated several ANKH variants to investigate their transport properties. Preliminary studies in HEK293 cells showed that the CPPD-associated mutations **P5L** and **M48T** induce extracellular accumulation of both citrate and PPi, whereas the CMD-associated deletion mutants **ΔS375** and **ΔF377** do not. In parallel, we are examining how local (synovial) and systemic PPi (plasma) dysregulation contributes to crystal formation and joint inflammation. Measurement of synovial fluid samples showed significantly higher PPi concentrations in CPPD patients (6.1±0.8 µM n=22) compared with gout patients (2.1±0.4 µM, n=17, $p<0.01$).

Conclusion: Together, these findings support the hypothesis that CPPD-associated ANKH mutations alter substrate transport and disrupt extracellular PPi and citrate homeostasis, thereby promoting crystal formation and deposition.

PHOSPHATE-DRIVEN CARDIAC VALVE CALCIFICATION IS TRIGGERED BY ENDOTHELIAL APOPTOSIS IN A KLOTHO ZEBRAFISH MUTANTS

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Introduction: Cardiovascular calcification (CVC) is a common disease associated with a number of factors including mineral systemic increase such as hyperphosphatemia, which causes extracellular matrix calcification and progressive dysfunction of arteries and cardiac valves. However, the initiating mechanisms of CVC, particularly in cardiac valves, remain poorly understood. αKlotho is a key regulator of phosphate homeostasis, and its deficiency in mice and zebrafish causes vascular calcification. In this study, we investigated how phosphate imbalance disrupts endothelial integrity and tissue homeostasis to promote cardiovascular calcification.

Materials and methods: Using a zebrafish *α-klotho* mutant model of phosphate-driven CVC, combined with single-cell RNA sequencing and tissue analyses, we characterized the early cellular and molecular events associated with disease initiation. To determine the specific contribution of hyperphosphatemia, we exposed explanted wild-type zebrafish hearts to high phosphate levels *in vitro* and analyzed endothelial integrity, apoptosis, and cardiac valve calcification.

Results: The *α-klotho* mutants developed elevated serum phosphate levels before the onset of cardiovascular calcification, followed by progressive calcification of the cardiac valve. Single-cell RNA sequencing and tissue analysis at early stages of valve calcification showed endothelial apoptosis and disruption of endothelial barrier integrity, followed by increased immune cell infiltration. Calcification initiated specifically in subendothelial regions displaying signs of endothelial apoptosis and barrier compromise. Exposure of explanted wild-type zebrafish hearts to hyperphosphatemic conditions reproduced the endothelial apoptosis and valvular calcification phenotypes observed in *α-klotho* hearts, indicating that the cardiovascular calcification phenotype is primarily driven by elevated phosphate levels and endothelial dysruption.

Conclusion: Our findings establish the zebrafish *α-klotho* mutant as a model to study cardiovascular calcification and identify endothelial apoptosis as an early initiating event in cardiac valve calcification. This model provides a framework for investigating how phosphate imbalance disrupts cardiovascular tissue homeostasis and for defining the cellular interactions that contribute to pathological soft tissue mineralization.

PYROPHOSPHATE HOMEOSTASIS: FROM SYSTEMIC REGULATION TO ECTOPIC CALCIFICATION

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Introduction: Inorganic pyrophosphate (PPi) is a major endogenous inhibitor of ectopic calcification, whereas inorganic phosphate (Pi) promotes mineralization. Yet, systemic PPi regulation, its interplay with Pi, and their relevance to pathological calcification remain incompletely understood. We investigated PPi homeostasis from molecular and physiological regulation to experimental and human cardiovascular calcification.

Materials and methods: Regulation of major PPi homeostasis genes was investigated using molecular, genomic, and mouse approaches. Circulating PPi, Pi, and alkaline phosphatase (AP) were evaluated across physiological conditions. Associations with aortic valve and coronary calcification were assessed in independent cardiovascular cohorts using multivariable and two-part regression models. Mouse studies explored the dynamic regulation and functional consequences of altered Pi–PPi homeostasis.

Results: We identified conserved transcriptional co-regulation of PPi homeostasis genes and age-dependent PPi–Pi–AP relationships shared between mice and humans. In two independent cardiovascular cohorts, Pi/PPi was independently associated with aortic valve calcification burden in 154 patients, while lower PPi was associated with coronary calcification burden, with a corresponding positive association for Pi/PPi. Beyond these associations, an acute change in systemic Pi availability elicited a prompt, approximately threefold PPi response, revisiting a largely overlooked physiological observation and revealing an unexpected layer of regulation. Perturbation of PPi homeostasis also altered tissue mineralization, which could be modified by targeting mineralization pathways.

Conclusion: Our findings challenge the view of Pi and PPi as merely opposing determinants of mineralization and instead suggest a dynamically coupled homeostatic system capable of rapidly responding to changes in mineral availability. Revisiting this overlooked physiology through modern genetic and clinical evidence may reveal previously unrecognized determinants of calcification susceptibility.

MACROPHAGE-MEDIATED INFLAMMATION: A LIKELY DRIVER OF ECTOPIC CALCIFICATION IN PSEUDOXANTHOMA ELASTICUM

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Introduction: Physiological calcification of soft tissues is a common occurrence in aging and various acquired and inherited disorders. ABCC6 mutations cause the calcification disorders_pseudoxanthoma elasticum (PXE) and some cases of generalized arterial calcification of infancy (GACI), which is otherwise caused by defective ENPP1. Both PXE and GACI present significant calcification in the cardiovascular system. *ABCC6* is primarily expressed in liver, which has given the impression that the liver is central to the pathophysiology of PXE/GACI. The recent emergence of inflammation in PXE patients suggested that immune cells play an unexpected role in the calcification phenotype.

Materials and methods: We used for this project *Abcc6*$^{-/-}$ and *Ank*$^{-/-}$*xAbcc6*$^{-/-}$ mice as well as human samples from PXE patients and a variety of methodologies described in recent publications.

Results: We observed significant mineralization in lymph nodes and surrounding connective tissues in *Abcc6*$^{-/-}$ mice. Furthermore, we found evidence of lymphangiogenesis both in the skin of PXE patients and *Abcc6*$^{-/-}$ mice, consistent with an inflammatory process. Furthermore, restoring wild type bone marrow in *Abcc6*$^{-/-}$ mice significantly reduced calcification in several target tissues. We also investigated a 13-year-old patient experiencing an erythematous flareup of his PXE lesions. Histological examination revealed perivascular T cell-infiltrates with Th1 polarity and elevated expression of cytotoxicity markers in RNAseq and RT-qPCR analyses. Macrophages are central regulators in T cell activation and they seem the most likely mediator for this effect. Indeed, we found an abundance of macrophages associated with calcified tissues in *Abcc6*$^{-/-}$ as well as in *Ank*$^{-/-}$;*Abcc6*$^{-/-}$ mice while infusion of wild type macrophages or Prednisolone treatment rescue calcification.

Conclusion: Collectively, these data provide strong evidence of macrophage-mediated inflammation in PXE-related calcification.



POSTER PRESENTATIONS

P1

SAAR ADRIAENSEN

NITRIC OXIDE DONOR SUPPLEMENTATION SHOWS DIVERGENT EFFECTS ON ARTERIAL MEDIA CALCIFICATION AND VASCULAR FUNCTION

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Introduction: Previous research showed that inhibition of nitric oxide (NO) aggravates arterial media calcification (AMC), suggesting a protective role for physiological NO, whereas excessive NO levels have been linked to nitro-oxidative stress. The present study evaluated NO supplementation as therapeutic strategy in AMC.

Materials and methods: Primary rat aortic vascular smooth muscle cells were cultured in calcifying medium (inorganic phosphate (Pi), 3.9mM) and treated with DETA NONOate (DETA; 25 or 50 µM) for 7 or 14 days. Calcium deposition was assessed with o-cresolphthalein assay and alizarin red staining.
Additionally, the effect of the NO-donor molsidomine (120mg/L in drinking water) on AMC was evaluated *in vivo* in both DBA2 mice and Sprague Dawley rats exposed to warfarin. Aortic calcification was assessed by atomic absorption spectrometry, o-cresolphthalein assay and Von Kossa staining. Aortic gene expression was analyzed by RT-qPCR and vascular reactivity by organ baths.

Results: *In vitro*, 25µM DETA reduced calcium deposition by 40.8% after 7 days, but protective effects were lost after 14 days. In contrast, 50µM DETA provided no additional benefit compared with 25µM DETA after 7 days, whilst after 14 days it elicited a heterogenous response, with calcium deposition increasing up to ten-fold versus Pi.
In vivo, molsidomine did not affect AMC levels in both mice and rat but remarkably, it increased aortic wall thickness, indicative of vascular remodeling. Furthermore, mice aortic RNA levels of lipocalin-2, associated with inflammation and oxidative stress, were increased by three-fold. Finally, molsidomine-treated aortic segments showed an increased contractility in response to phenylephrine and K^+, together with a reduced basal NO index.

Conclusion: These findings indicate that NO donor therapy is not uniformly effective against AMC, suggesting that maintaining physiological NO homeostasis rather than increasing NO levels may be essential. Excessive NO exposure may induce nitro-oxidative stress and desensitize NO signaling, thereby promoting vascular dysfunction.

ZAHIRA AHMADI

INORGANIC PYROPHOSPHATE HOMEOSTASIS IN GESTATIONAL DIABETES MELLITUS

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Introduction: Inorganic pyrophosphate (PPi) is an endogenous regulator of skeletal mineralization and a major inhibitor of ectopic calcification. We previously demonstrated that circulating PPi progressively decreases during pregnancy and is significantly reduced at term, potentially due, at least in part, to increasing placental alkaline phosphatase (ALP) activity. In patients with pseudoxanthoma elasticum, who have chronically low PPi, multiparity was associated with increased vascular calcification. Reduced PPi levels have also recently been reported in diabetes. Whether gestational diabetes mellitus (GDM) further alters PPi homeostasis during pregnancy remains unknown.

Materials and methods: Plasma, serum, and urine samples are collected at term from a pilot cohort of women with GDM and healthy pregnant controls. Plasma and urinary PPi concentrations and serum ALP activity are determined and evaluated together with relevant clinical and metabolic parameters.

Results: Plasma and urinary PPi levels are compared between women with GDM and healthy pregnant controls. Their relationships with ALP activity and clinical and metabolic parameters are assessed to determine whether GDM is associated with an additional reduction in PPi beyond the physiological decrease observed during pregnancy.

Conclusion: This study will provide an initial indication of whether GDM represents an additional modifier of PPi homeostasis during pregnancy and may provide a basis for understanding the potential consequences of combined pregnancy- and diabetes-associated alterations in PPi metabolism.

TAMÁS ARÁNYI

EPIGENETIC PROFILING REVEALS DNA METHYLATION DIFFERENCES IN PXE FIBROBLASTS

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Introduction: Pseudoxanthoma Elasticum (PXE) is a heritable disorder marked by progressive calcification of elastic fiber–rich tissues due to loss-of-function mutations in the *ABCC6* gene. While *ABCC6* is predominantly expressed in hepatocytes and only at negligible levels, in fibroblasts, PXE fibroblasts exhibit disease-associated phenotypic changes. This suggests that secondary, cell-autonomous mechanisms—potentially involving epigenetic regulation—contribute to disease manifestation in non-hepatic tissues.

Materials and methods: We conducted genome-wide DNA methylation profiling of primary dermal fibroblasts from PXE patients (n=15) and age- and sex-matched controls (n=12) using the Illumina EPIC Array v2 (~930,000 CpG sites, >99% of genes).

Results: We identified 15 differentially methylated CpG sites (DMS) ($p < 10^{-5}$) 3 of which are $p < 10^{-6}$. Over 65% of the DMS exhibited >10% methylation change. Notably, many DMSs mapped to loci associated with transcriptional regulation or signal transduction.

Conclusion: PXE fibroblasts exhibit distinct DNA methylation profiles compared to controls, implicating epigenetic dysregulation in PXE pathogenesis. Therefore, despite the small cohort size, the robustness of the methylation differences highlights the potential of epigenetic profiling in uncovering disease-related pathways and potential therapeutic targets in PXE.

TAMÁS ARÁNYI

CALCIPAT: AN ACCESSIBLE WEB TOOL FOR TRANSPARENT ABCC6 VARIANT ASSESSMENT IN PXE

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Introduction: Pseudoxanthoma elasticum (PXE) is caused by the biallelic loss-of-function variants of ABCC6. To date >1000 variants of the gene were reported. However, their classification is challenging and may even change over time as new evidence emerge. Manual application of the ABCC6-adapted Sherloc framework for variant classification is time-consuming. We developed CalciPat, a freely accessible web tool that enables clinicians and researchers to query or enter ABCC6 variants and transparently assess the evidence supporting their classification.

Materials and methods: CalciPat implements an *ABCC6*-adapted Sherloc decision tree integrating population frequency, patient observations, segregation, variant type, curated functional studies, and computational predictions. Related evidence is evaluated collectively. The tool incorporates the published reassessment of 286 variants (Verschuere et al., 2021), European PXE cohorts, and evidence on incomplete penetrance. Users can analyse individual variants or both alleles, with a separate recessive genotype summary. Each curator-approved release records the underlying rules and database versions, enabling classification changes to be tracked over time.

Results: The current release contains 411 curated variants represented by 1,142 aliases. For each query, CalciPat reports pathogenic and benign evidence, the five-tier classification, VUS subclass, and a step-by-step rationale. The implementation passed all predefined validation checks (546/546) and reproduced expected classifications for 12 reference and challenging variants, including the incompletely penetrant p.Arg391Gly and p.Val787Ile. These results demonstrate consistent implementation of the classification rules but do not establish clinical diagnostic accuracy.

Conclusion: CalciPat provides an accessible, transparent, and reproducible framework for *ABCC6* variant assessment, clearly showing how classifications are derived and why they may change as evidence evolves. It is intended for research and expert-review support instead of stand-alone clinical diagnosis. A modular next-generation successor, RarePath, is currently under development.

JUSTINE BERGEON

AUTONOMIC NERVOUS SYSTEM DYSREGULATION AND THE CHOLINERGIC ANTI-INFLAMMATORY PATHWAY IN CALCIFIC AORTIC VALVE STENOSIS: RATIONALE FOR TRANSCUTANEOUS VAGUS

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Introduction: Calcific aortic valve stenosis (CAVS) is an active fibrocalcific disease characterized by chronic inflammation, autonomic nervous system (ANS) dysregulation, and progressive valvular calcification. Postoperative atrial fibrillation (POAF) complicates 38–63% of surgical aortic valve replacements. Its pathogenesis involves pre-existing sympathetic hyperactivity, inflammation, and an arrhythmogenic substrate combined with a surgery-related trigger, resulting in the development of POAF. Inflammation is associated with autonomic nervous system (ANS) imbalance, which reduces the parasympathetic tone. These observations provide the rationale for targeting autonomic dysfunction through transcutaneous vagus nerve stimulation (tVNS).

Materials and methods: To investigate both the clinical and tissue-level relevance of autonomic dysfunction we conducted a retrospective analysis of 223 patients who underwent surgical aortic valve replacement and cardiac rehabilitation at the Saint-Étienne University Hospital. Heart rate variability was assessed using 24-hour Holter monitoring to evaluate the autonomic nervous system and POAF incidence. In parallel, we investigated the cholinergic anti-inflammatory pathway with α7nAChR in immunofluorescence with light-sheet microscopy in calcified aortic valves from the DAVAACA cohort at Karolinska Institutet in Sweden.

Results: The overall POAF incidence in this French cohort was 53%. The mean SDNN was significantly higher in patients without POAF (90.14 ± 22.36 ms) than in those with POAF (62.90 ± 28.22 ms) (p=.004). Similarly, the mean SDANN was higher in patients without POAF (80.04 ± 20.94 ms) than in those with POAF (56.86 ± 11.17 ms) (p=.009). Qualitative light-sheet microscopy demonstrated stronger α7nAChR immunofluorescence signals in non-stenotic valve tissues than in stenotic valve tissues in the DAVAACA cohort.

Conclusion: These findings support the central role of autonomic imbalance in the development of POAF after surgical treatment of CAVS and suggest a role for the cholinergic anti-inflammatory pathway in valvular calcification. Together, these observations provide the rationale for a multicenter, randomized, sham-controlled trial evaluating transcutaneous vagus nerve stimulation (tVNS) as a preventive strategy against POAF and as a potential modulator of neuroimmune mechanisms in CAVS.

MARTA CERRUTI

THE COMPLEX INTERPLAY OF SEX, UNDERLYING PATHOLOGY AND MINERAL PROGRESSION IN CALCIFIC AORTIC VALVE DISEASE

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Introduction: During calcific aortic valve disease (CAVD) calcium phosphate minerals accumulate on heart valve leaflets, increasing risk of heart failure. Diabetes is a risk factor. Sex affects CAVD prognosis and progression.

Fetuin, a potent mineralization inhibitor, is more abundant in female than male serum in healthy subjects, but fetuin levels in CAVD patients are unknown.

To find out, and to evaluate proteins correlated with differences in CAVD mechanism, we collected heart valves and blood samples from 44 CAVD patients (50% diabetic) and analyzed minerals, tissues and serum with advanced mineral characterization techniques and proteomics.

Materials and methods: Excised heart valves were frozen in liquid nitrogen and stored at –80 °C for protein analysis or placed in ethanol for 24 hours and vacuum dried overnight for mineral analysis. Frozen valve tissues were ground in liquid nitrogen and treated with EDTA to dissolve the minerals. After EDTA extraction, tissues were washed in PBS and protein was extracted from the organic component

Results: Minerals deposited in female heart valves had a more fibrous morphology than in male valves. Minerals mostly included hydroxyapatite and whitlockite, and diabetic patients had less whitlockite.

Contrary to the general population, male serum showed more fetuin than female serum. Fetuin levels in both minerals and valve tissue extracts did not show any correlation with sex or with diabetes. Proteomic data showed clear differences between protein types found in mineral vs tissue extracts, with proteins related to neutrophil-mediated immunity – an early event in CAVD progression - upregulated in EDTA leachates compared to tissue extracts. There was no specific clustering related to sex or diabetes.

Conclusion: Differences in minerals in diabetic patients may indicate a different underlying mechanism. Proteins included in minerals may reveal early disease events. Tissue and mineral fetuin levels suggest a complex expression and accumulation mechanism. Ongoing analyses aim to identify additional mineral- and patient-related factors underlying the observed results.

NATÉRCIA CONCEIÇÃO

BEYOND NEURODEVELOPMENT: CDKL5 LOSS IMPAIRS SKELETAL DEVELOPMENT AND MINERALIZATION IN ZEBRAFISH

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CDKL5 Deficiency Disorder (CDD) is a rare X-linked neurodevelopmental disorder caused by pathogenic variants in the *CDKL5* gene. It is characterized by early-onset epilepsy, severe developmental delay, and motor impairment, with additional features including microcephaly, dysmorphic facial features, and scoliosis. While the neurological aspects of CDD are well studied, the role of CDKL5 in skeletal development and mineralization remains poorly understood. Zebrafish (*Danio rerio*) is a well-established model for studying genetic disorders due to its genetic conservation and suitability for developmental studies. This study investigated the effects of Cdkl5 loss on skeletal development and mineralization using a zebrafish mutant model.

The *cdkl5* ^{sa21938} zebrafish mutant line was analysed using skeletal staining, imaging, quantitative RT-PCR, and whole-transcriptome RNA sequencing at 5 and 35 days post-fertilization (dpf).

Mutant larvae exhibited reduced head size, consistent with the microcephaly observed in CDD. Skeletal staining revealed shortened craniofacial cartilage structures and reduced bone mineralization, indicating impaired craniofacial and skeletal development. Quantitative RT-PCR confirmed dysregulation of skeletal marker genes in mutant early larvae. Transcriptome analysis identified a development delay in mutants and revealed downregulation of genes associated with cartilage maturation at both 5 and 35 dpf, while genes involved in bone formation were downregulated at 35 dpf, providing a molecular basis for the observed skeletal abnormalities. Functional enrichment analysis further showed impaired craniofacial neural crest development accompanied by disrupted cartilage and bone development.

These findings demonstrate that Cdkl5 loss disrupts molecular pathways essential for skeletal development, highlighting functions beyond neurodevelopment. The zebrafish *cdkl5* ^{sa21938} model provides a valuable platform for investigating the role of Cdkl5 in tissue mineralization and may reveal mechanisms linking neurodevelopmental disorders with skeletal abnormalities.

PAUL COULOT

SCANOGRAPHIC PHENTOTYPE DESCRIPTION OF PSEUDOXANTHOMA ELASTICUM PATIENT : RISK MAJORATION OF RENAL CALCIFICATION AND KIDNEY STONES

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Introduction: Pseudoxanthoma elasticum (PXE) is a rare autosomal recessive disorder caused by loss-of-function mutations in the *ABCC6* gene, leading to ectopic calcifications affecting the skin, vasculature, and eyes. In *Abcc6* knockout mouse models, extensive kidney calcifications are consistently observed. However, human data remain inconsistent. Recent studies in French and American cohorts suggest an increased risk of nephrolithiasis in PXE patients, whereas a Dutch study based on computed tomography (CT) imaging did not confirm an increased prevalence.

Materials and Methods: We analyzed whole-body low-dose non-contrast CT scans from 50 PXE patients enrolled in the prospective PROPHECI cohort. Images were acquired with a slice thickness of 0.625 mm. A systematic evaluation of kidney structures was performed using a density threshold of 130 Hounsfield units (HU), derived from the Agatston scoring method, to identify pathological calcifications. Additional abdominal organs were also assessed.

Results: Using this approach, 84% of patients exhibited renal calcifications, and 32% had overt nephrolithiasis. Calcifications were localized to the papillary region in 61% of cases and within the renal parenchyma in 66%. Some of these calcifications were subtle and would likely be missed without application of the 130 HU threshold. Extra-renal findings included hepatic microcalcifications in 90% of patients and testicular macrocalcifications in 94% of male patients.

Conclusion: Renal calcifications, including nephrolithiasis and extensive Randall's plaque-like lesions, as well as probable renal arterial calcifications, appear highly prevalent in PXE. To our knowledge, this study provides the first detailed CT-based characterization of the renal phenotype in PXE patients. Comparative analysis with a matched control population is ongoing.

ROBBE DERUDDER

TELOMERE LENGTH IS PRESERVED DESPITE CELLULAR SENESCENCE IN PSEUDOXANTHOMA ELASTICUM

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Introduction: Pseudoxanthoma elasticum (PXE) is a rare hereditary disorder caused by biallelic *ABCC6* mutations, leading to pathological calcification of the skin, arteries and eyes. Increasing evidence implicates oxidative stress, DNA damage and cellular senescence in PXE pathophysiology. Because telomere shortening is a hallmark of biological ageing and cellular senescence, leukocyte telomere length (TL) may provide insight as whether the reported accelerated biological ageing in PXE is systemic or rather tissue-specific.

Materials and methods: Relative leukocyte TL was assessed in 50 molecularly confirmed PXE patients and 50 age-, sex- and sample collection date-matched healthy controls using a modified singleplex quantitative PCR assay based on Cawthon's method. Samples with a DNA Integrity Number (DIN) < 6.5 were excluded, followed by additional outlier exclusion, resulting in 45 PXE patients and 45 controls. Relative TL was compared using a paired t-test in complete matched pairs (n = 41). Multivariable linear regression was performed to assess the independent association between PXE and TL after adjustment for age, sex, collection period and DIN.

Results: Mean leukocyte TL did not differ between PXE patients and matched controls, with a mean paired difference of was 0.009 T/S units (95% CI −0.099 to 0.117; p = 0.869). Multivariable linear regression likewise showed no independent association between PXE and TL (β = 0.028, 95% CI −0.066 to 0.123; p = 0.552). Increasing age (β = −0.0047/year; p < 0.001) and more recent sample collection period (β = −0.392; p < 0.001) were independently associated with shorter TL.

Conclusion: Leukocyte TL is not reduced in PXE patients compared with matched healthy controls, suggesting that the accelerated cellular senescence in PXE is unlikely to be driven by systemic telomere attrition. These findings support the hypothesis that the cellular senescence observed in PXE may predominantly involve telomere-independent, tissue-specific mechanisms, potentially driven by persistent DNA damage signaling and oxidative stress.

P10

ROBBE DERUDDER

THE AUTOTAXIN – LYSOPHOSPHATIDIC ACID RECEPTOR AXIS CONTRIBUTES TO ECTOPIC CALCIFICATION IN PSEUDOXANTHOMA ELASTICUM

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Introduction: Pseudoxanthoma elasticum (PXE), caused by bi-allelic *ABCC6* mutations, is characterized by ectopic calcification of the skin, arteries, and eyes. Autotaxin (ATX/ENPP2), a lysophospholipase D enzyme, generates lysophosphatidic acid (LPA), which activates LPA receptors (LPARs) and promotes inflammatory and pro-calcific pathways, including IL-6 and BMP-2. We investigated the involvement of ATX/LPAR signaling in and its functional contribution to inflammatory and pro-calcific responses in PXE patients.

Materials and methods: ATX/LPAR pathway activity was investigated in dermal fibroblasts and skin tissue from PXE patients and age- and sex-matched controls. ATX, LPARs, and downstream targets were evaluated by immunohistochemistry and RT-qPCR at baseline and during a 20-day calcification assay (days 0, 10, and 20). Calcification was evaluated by Von Kossa staining and Alizarin Red S (ARS) staining. Effects of ATX and LPAR inhibition were assessed using HA130 and Ki16425, respectively. Serum ATX and lipoprotein(a) were measured by ELISA and immunoassay, respectively. PXE fibroblasts were stimulated with lysophosphatidylcholine (LPC) to assess NF-κB activation (p65 immunocytochemistry and nuclear translocation), IL-6 secretion (ELISA), and LPC-induced calcification (ARS).

Results: PXE skin showed increased ATX and LPAR3 expression, with downstream signaling components co-localizing with osteogenic markers and calcified areas. PXE fibroblasts showed increased ATX, LPAR1, PIK3CA, ERK1, and IL-6 expression. During *in vitro* calcification, ATX, LPAR1, ERK1, and IL-6 further increased. Pharmacological inhibition of ATX/LPAR signaling reduced mineralization, supporting a functional role for this pathway in PXE calcification. Circulating ATX levels were two-fold higher in PXE patients, whereas lipoprotein(a) levels were normal. Preliminary experiments showed increased nuclear NF-κB p65 localization following LPC stimulation of PXE fibroblasts.

Conclusion: Our findings implicate ATX/LPAR signaling in PXE-associated ectopic calcification and identify it as a potential therapeutic target. Ongoing studies are determining whether LPC-induced pathway activation promotes calcification through an NF-κB–IL-6–BMP signaling cascade.

P11

JORDAN ELBADRY

CAN WE COMPLETELY HUMANISE RESEARCH METHODS FOR ARTERIAL MEDIAL CALCIFICATION?

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Introduction: There is increasing demand to reduce the use of experimental animals and animal-derived products in biomedical research to improve translation to humans and address the ethical concerns of animal use. This work examined whether human umbilical arteries could be used as an alternative *ex vivo* model for studying arterial medial calcification (AMC). Alongside, it investigated if protocols could be modified to remove all animal products.

Materials and methods: Human VSMCs and umbilical artery rings were cultured in normal and calcifying media for ≤7 and ≤28 days respectively. Foetal bovine serum (FBS) replacements including human serum (HS), human platelet lysate (HPL) and chemically derived FBS alternatives (CDFA) were tested for their capacity to support proliferation and calcification. Samples were analysed using microCT, histology and cell-based assays.

Results: VSMCs cultured in FBS, HS and HPL displayed similar proliferation rates. The proportion of dead cells was also comparable being 6%, 11% and 11% in FBS, HS and HPL, respectively. All CDFA-containing media resulted in widespread cell death in <7 days, meaning they were not viable alternatives to FBS. Levels of calcification were 80% and 90% lower in HS and HPL, respectively, compared to FBS. MicroCT analysis of umbilical artery rings revealed AMC levels were similar in FBS (14.5% calcified) and HS (17%) conditions but lower in CDFA-containing media (5-7%). Histology revealed several key differences between umbilical arteries and the established aortic rat ring model. Firstly, the medial layer was thicker and did not contain any elastin. Secondly, matrix breakdown was not observed and, finally, regions of AMC were smaller and more diffuse.

Conclusions: These data show that converting to completely animal-free protocols is challenging. Whilst umbilical arteries calcify *ex vivo*, the significant structural differences mean that further work is required to establish whether this model effectively recapitulates what occurs in elastic arteries.

MIGUEL DAVID

OPTIMIZATION AND CHARACTERIZATION OF AN EX VIVO PORCINE MODEL OF VALVULAR CALCIFICATION

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Introduction: Cardiovascular calcification is a multifactorial disorder with major health implications worldwide. Among the most common forms are calcified aortic valve disease (CAVD), mitral annular calcification, and, less commonly, pulmonary valve calcification. As no effective therapies are currently available, there is an urgent need to develop reliable experimental models. Although in vitro models are widely used to investigate valvular calcification, they fail to preserve the structural and cellular complexity of native valve tissue. Therefore, the aim of this study was to optimize an ex vivo model of valvular calcification using whole porcine valve leaflets.

Materials and methods: Heart valves (Aortic, mitral and pulmonary) were harvested from sacrificed porcine hearts. Individual valve leaflets were collected and identified by anatomical localization. Then, calcification was induced using inorganic phosphate (PM; 3 mM NaH_2PO_4/Na_2HPO_4). After 14 days of culture, one fragment of the valve leaflet was lyophilized, digested, and its calcium content was determined by Inductively Coupled Plasma Optical Emission Spectroscopy. The other fragment was fixed and stained with Alizarin Red.

Results: The phosphate-rich medium induced calcification in all valves. Pulmonary valve leaflets exposed to PM calcified significantly (44 mg Ca/g tissue) compared to those cultured in the control medium (0.83 mg Ca/g tissue). Aortic valve leaflets also exhibited mineralization (13 vs. 1.34 mg Ca/g tissue). Finally, calcification was observed in the mitral leaflets exposed to PM (10 vs. 2 mg Ca/g tissue). No significant differences in calcification were observed based on the anatomical position of the leaflets. Histological analysis of the aortic valve leaflets revealed a differential pattern of calcification across the different layers.

Conclusion: A robust ex vivo model of multivalvular calcification was established, reproducing key pathological features of human valve calcification. Its reproducibility and anatomical consistency make it a valuable platform for investigating disease mechanisms and evaluating novel anti-calcification therapies.

KRISZTINA FÜLÖP

THE METABOLIC BACKGROUND OF LOW PYROPHOSPHATE IN A MOUSE MODEL OF TYPE I DIABETES

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Introduction: Cardiovascular calcification is one the most serious consequences of diabetes mellitus, thus representing the predominant cause of death among diabetes patients. Earlier we found that the level of plasma pyrophosphate (PPi) - a potent inhibitor of calcification - was decreased in the Type I diabetes mouse model, and the same phenomenon was found in human patients (Fülöp et al, 2025).

Materials and methods: We have induced Type I diabetes in C57Bl/6 mice using streptozotocin and investigated the molecular pathomechanisms leading to lower PPi in diabetes.

Results

1) Plasma alkaline phosphatase (ALP) activity was increased in diabetic C57BI6 mice. We have also found that insulin supplementation keeps ALP activity and plasma PPi level in the normal range.
2) A substantial decrease of plasma ATP level - the source of PPi - was detected in diabetic mice.
3) Plasma adenosine (ADO) levels were measured, and we have found a negative correlation between plasma ADO and ALP activity in diabetic animals.
4) Elevated expression of Alpl was detected in the liver in diabetic mice. Neither the kidney nor the adipose tissues showed higher Alpl expression in the same animals.

Conclusion: As ALP hydrolyses PPi, its higher activity and/or its higher expression in the liver of diabetic mice can be one of the causes of low PPi. Lower ATP level may also contribute to lower PPi in two ways: i) as the source of PPi, its decreased level may directly result is lower PPi levels; ii) ADO - a metabolic product of ATP - down-regulates ALP expression, therefore lower ATP may indirectly lead to higher ALP expression.

P14

SYEDA FATIMA HAMNA

DO CALORIE RESTRICTION MEDIATORS EXERT PROTECTIVE EFFECTS AGAINST VASCULAR CALCIFICATION?

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Introduction: Vascular calcification (VC) is a significant contributor to cardiovascular disease (CVD) events, involving the deposition of hydroxyapatite minerals in vessels and the transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells. Research shows that calorie restriction (CR) may reduce CVD risk by reducing inflammation and regulating mitochondrial health, making it a potential treatment option for VC.

Materials and methods: The study consisted of three parts. Firstly, a CR mouse model was used to determine the relationship between VC and CR. RNA-sequencing and functional clustering were undertaken on aorta isolated from CR (24h-fast) versus *ad libitum*-fed mice. Next, a murine vascular smooth muscle cell (MOVAS) calcification model was characterised following 7-days of culture. Finally, MOVAS cells were treated with different nutrient restriction (NR) approaches including *in-vitro* NR modelling and the use of putative CR mimetics such as AdipoRon, an adiponectin receptor 1/2 (adipoR1/2) agonist.

Results: 24-h CR in mice upregulated ossification (Spp1; 2.5-fold; $p<0.0001$) and mitochondrial (Pdk4; 4.73; $p<0.0001$) markers, as well as the anti-inflammatory peroxisome proliferator-activated receptor (PPAR) pathway ($p<0.0001$). The MOVAS calcification model showed increased calcification using Alizarin red staining (2-fold, $p<0.01$) and a commercial calcium assay (200-fold, $p<0.0001$) along with increased mRNA expression of the osteogenic marker Runx2 (1.2-fold, $p<0.01$). A decline in mitochondrial health was also noted, indicated by an increase in reactive oxygen species (ROS) (1.2-fold, $p<0.05$), and a decrease in mitochondrial membrane potential (0.085-fold, $p<0.0001$) and mitochondrial dysfunction identified by the rate of oxidation reduction of mitochondrial substrates such as L-malic acid (2.7-fold, $p<0.001$) using Omnilog analysis. For NR therapeutic strategies, only AdipoRon treatment reduced calcification (20µM; 1.25-fold; $p<0.05$), but did not alter osteogenic marker expression or mitochondrial function.

Conclusion: Overall, this study suggests a potential role for NR as a therapeutic strategy against vascular calcification, with AdipoRon reducing calcification. Further investigation is required to fully explore its potential.

P15

ROMAIN HUGON

DISTINCT ROLES OF MGP GLA AND SER RESIDUES IN TRACHEAL CALCIFICATION

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Patients with Keutel Syndrome (KS), an extremely rare genetic disorder caused by loss-of-function mutations in Matrix Gla Protein (MGP), develop early and prominent tracheal calcification of the cartilage rings (TCC), associated with respiratory complications including recurrent airway infections and breathing difficulties. MGP is a potent inhibitor of ectopic calcification whose activity depends on two post-translational modifications: γ-carboxylation of glutamate residues into γ-carboxyglutamate (Gla) residues and phosphorylation of serine (Ser) residues. Using MGP-deficient (*Mgp*$^{-/-}$) mice, a well-established model of KS, we previously showed that they recapitulate the precocious TCC observed in patients but also develop an unexpected additional calcification within the tracheal mucosa (TMC), associated with elastin fiber degradation. Since Gla and Ser residues have been shown to play distinct role in vascular calcification, we aimed to investigate their respective contributions to TCC and TMC.

To address this question, we assessed mineral deposition and the elastic fiber network by Von Kossa and Hart's staining respectively in the trachea of several mouse models: *Mgp*$^{-/-}$ mice, *S3mut/S3mut* mice carrying mutated Ser residues, and tissue-specific transgenic models expressing either WT or Gla-mutated MGP in SM22α- or Col2a1-expressing cells.

None of the models examined appeared to fully rescue early TCC. In contrast, local re-expression of functional WT MGP in *Mgp*$^{-/-}$ mice either in SM22α- or Col2a1-expressing cells prevented TMC. This protective effect was not found after re-expression of Gla-mutated MGP, indicating that Gla residues are required to prevent TMC. Conversely, *S3mut/S3mut* mice remained protected from TMC, suggesting that Ser residues are less critical in this compartment.

These findings reveal tissue-specific roles for the functional domains of MGP in the trachea, which are interestingly opposite to those observed in vascular tissue. This suggests that the respective contribution of those residues is highly tissue dependent.

VIKTÓRIA JENEY

GLYCOLYTIC REPROGRAMMING MEDIATES HYPOXIA-INDUCED OSTEOGENIC DIFFERENTIATION AND CALCIFICATION OF VASCULAR SMOOTH MUSCLE CELLS

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Introduction: Hypoxia is a potent inducer of vascular calcification, yet the metabolic mechanisms linking oxygen deprivation to osteogenic differentiation remain incompletely understood. We investigated whether hypoxia-driven glycolytic reprogramming is required for vascular smooth muscle cell (VSMC) calcification.

Materials and methods: Human VSMCs were cultured under normoxic (21% O_2) or hypoxic (1% O_2) conditions in the presence of elevated phosphate. Calcification was quantified by Alizarin Red staining, and osteogenic differentiation was assessed by expression of RUNX2, SOX9, and alkaline phosphatase. The contribution of HIF signaling was examined by siRNA-mediated silencing of HIF-1α and HIF-2α. RNA sequencing, pathway enrichment analysis, immunoblotting, quantitative PCR, and targeted metabolomics were used to characterize hypoxia-induced metabolic remodeling. Functional significance was evaluated using genetic silencing and pharmacological inhibition of key glycolytic enzymes and regulators.

Results: Hypoxia markedly potentiated phosphate-induced VSMC calcification and increased expression of osteogenic markers. Silencing either HIF-1α or HIF-2α significantly attenuated hypoxia-enhanced mineralization, demonstrating that both transcription factors contribute to this response. Transcriptomic analysis identified glycolysis among the most significantly enriched pathways under hypoxia. Consistently, hypoxia increased expression of GLUT1, HK2, PFKFB3, LDHA, and PDK4 while suppressing PDH-E1, indicating reduced pyruvate entry into mitochondrial oxidation. Metabolomic profiling confirmed this metabolic switch, revealing accumulation of glycolytic intermediates accompanied by depletion of tricarboxylic acid cycle metabolites. Functional studies established the requirement for glycolytic reprogramming, as silencing of GLUT1, HK2, PFKFB3, LDHA, PDH, or PDK4, as well as pharmacological inhibition of glucose uptake, glycolysis, or pyruvate metabolism, significantly reduced phosphate-induced calcification.

Conclusion: Hypoxia promotes vascular calcification by inducing a HIF-dependent glycolytic switch that redirects cellular metabolism away from mitochondrial oxidation toward glycolysis. These findings identify metabolic reprogramming as a key determinant of VSMC osteogenic differentiation and suggest that targeting glycolytic metabolism may represent a promising therapeutic strategy to prevent vascular calcification.

ESZTER KOZAK

TETRACYCLINE AND MINOCYCLINE INHIBIT ECTOPIC MINERALIZATION IN A MURINE MODEL OF NEUROGENIC HETEROTOPIC OSSIFICATION

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Introduction: Neurogenic heterotopic ossification (NHO) is a rare, complex complication arising from severe concurrent injuries to the central nervous system and peripheral tissues. This pathological process triggers abnormal formation of mature bone at the peripheral injury sites through mechanisms that remain poorly understood.

Tetracyclines exhibit pleiotropic biological effects beyond their antimicrobial role, and may prevent abnormal tissue remodeling and mineralization. Minocycline has been shown to suppress ectopic mineralization by inhibiting poly-ADP-ribose polymerase (PARP), but it was also shown to prevent mineralization in an acellular model, suggesting an alternative mechanism of anti-calcification effect. Through either of these mechanisms, tetracycline antibiotics may prevent NHO formation, therefore, the effect of 3 tetracycline antibiotics were tested in a mouse model.

Materials and methods: Single dose injections of tetracycline family antibiotics (tetracycline, minocycline and doxycycline) were tested in a murine model of NHO induced by a combination of closed head injury and peripheral muscle trauma of the tibialis anterior by cardiotoxin injection under general anesthesia. Sham treated animals received anesthesia alone. Tested antibiotics were injected within five minutes, or delayed up to 2 hours after trauma.

Calcification after trauma was assessed by acidic extraction of calcium content and colorimetric measurement as well as visualization with in situ Alizarin Red staining. Expression levels of key transcription factors were assessed by qPCR.

Results: Of the 3 tested antibiotics, tetracycline and minocycline were found to inhibit trauma-induced mineralization in a dose-dependent manner, and remained effective even if delayed. Treatment with doxycycline proved ineffective. Gene expression of *Runx2*, which plays a central role in osteoblast differentiation, was shown to be decreased by tetracycline and minocycline treatment.

Conclusion: Tetracycline and minocycline may provide an alternative to established preventive treatments of HO. Doxycycline however was found to be ineffective, and this difference may help shed light on the mechanism of action.

P18

ADRIÁNA KUTÁS

LONGITUDINAL PROFILING OF PLASMA N-GLYCOMIC ALTERATIONS IN A MOUSE MODEL OF TYPE I DIABETES

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Introduction: Type 1 Diabetes (T1D) is a complex metabolic disorder with rising global incidence. The leading cause of death among diabetic patients is cardiovascular complications, often resulting from ectopic calcification. However, the molecular pathomechanism underlying the metabolic complications are still not fully understood.

Materials and methods: In our laboratory we have developped T1D mouse models using streptozotocin induction. We have examined temporal changes in plasma N-glycan profiles at 2, 8, and 20 weeks post-induction using HILIC-UPLC-FLR-MS to investigate the potential role of protein N-glycosylation in the progression of diabetes.

Results: The glycomic analysis revealed significant structural remodeling of 20 individual glycan species, with complex, multi-sialylated structures proving most sensitive to disease progression. Notably, bi-antennary structures such as A2G1S1, A2G2S1, and A2G2S2(2) exhibited a marked decrease in relative abundance that strongly correlated with elevated blood glucose levels. In contrast, highly sialylated and fucosylated glycans like FA2G2S3 and FA3G3S3 showed a progressive increase over the 20-week period, suggesting an adaptive response to chronic metabolic stress and altered hepatic processing.

Conclusion: Our findings demonstrate that chronic hyperglycemia is accompanied by substantial remodeling of the plasma N-glycome, characterized by increased sialylation and fucosylation. These alterations closely track the progression of metabolic dysregulation, suggesting that while they parallel blood glucose trends, they provide a distinct molecular readout of the systemic glycosylation response to glucotoxicity.

GEORGES LEFTHÉRIOTIS

DO STATINS PLAY A ROLE IN CORONARY AND PERIPHERAL ARTERY CALCIFICATION IN PXE?

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Background: Pseudoxanthoma elasticum (PXE) is a rare inherited disorder caused by pathogenic ABCC6 variants. ABCC6 deficiency reduces extracellular inorganic pyrophosphate (PPi), promoting progressive vascular calcification. Although PXE arteriopathy has a distinct pathophysiology, many patients receive statins because of cardiovascular risk. As statins may increase calcification of atherosclerotic plaques despite reducing plaque burden, we investigated whether statin use was associated with vascular calcification in PXE.

Methods: We retrospectively analyzed 80 patients with PXE enrolled in the PROPHECI trial. Non-contrast coronary CT (35/80) and lower-limb CT (54/80) quantified coronary artery calcium (CAC) and lower-limb arterial calcification (LLAC). Because CAC and LLAC were highly skewed, Spearman correlations were used for univariate analyses. For exploratory multivariable analyses, CAC and LLAC were transformed using ln(score+1). Models assessing statin use were adjusted for age and sex; smoking was examined in sensitivity analyses.

Results: Twenty-nine patients received statins. Statin-treated patients were older and had higher PHENODEX, CAC and LLAC values, but lower LDL-C, total and non-HDL cholesterol. CAC>0 was present in 37% and LLAC>0 in 82%. CAC and LLAC were strongly correlated (r=0.679). Age was positively associated with PHENODEX, CAC, LLAC and statin use. PHENODEX correlated with LLAC but not CAC, while LDL-C was inversely associated with LLAC. For LLAC, age explained 12.9% of variance (R^2=0.129, p=0.006); adding sex increased R^2 to 0.239, whereas statin use added only 1.0% (ΔR^2=0.010, p=0.401). For CAC, age explained 28.7% (R^2=0.287, p<0.001); adding sex increased R^2 to 0.499, whereas statin use added 3.0% (ΔR^2=0.030, p=0.173). The PHENODEX–LLAC association attenuated after age adjustment (partial r=-0.064).

Conclusion: In this cohort, the association between statin use and vascular calcification was largely explained by age and sex. No independent association between statin use and CAC or LLAC was demonstrated after adjustment. Larger prospective studies are needed to determine whether statins modify vascular calcification in PXE.

ARE THERE DISTINCT MOLECULAR PATHWAYS UNDERLYING PHYSIOLOGICAL AND PATHOLOGICAL CALCIFICATION?

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Introduction: Mineralization is a highly regulated process by which inorganic minerals, primarily calcium phosphate in the form of hydroxyapatite, are deposited, physiologically, within hard connective tissues (*e.g.,* bone and teeth) providing the required structural tissue strength or, pathologically, within soft connective tissues (*e.g.,* vessels, skin) leading to inflammation and organ failure. To disclose which are the common mechanisms and the specific peculiarities of the mineralization process in these two different contexts, we have investigated, after exposure to calcifying stimuli, the protein profile of human dermal fibroblasts, as representative of soft connective tissues, and of the SaOS-2 cell line, widely used as an *in vitro* model with mature osteoblast behavior and therefore representative of hard connective tissue.

Materials and methods: Fibroblasts and SaOS-2 cells were cultured in standard (DMEM/MEM, respectively) or in calcifying media (CM, supplemented with 10mM beta-glycerophosphate, 50µM/ml ascorbic acid and 100nM dexamethasone), until calcification was clearly evident by staining with Alizarin red. Proteins were extracted in RIPA buffer and processed for LC-MS/MS analysis.

Results: SaOS-2 cells showed abundant calcification already after 7 days in CM, whereas 21 days were required for fibroblasts to form a mineralized matrix. Proteomic analysis revealed a strong response to CM in both cell types, with numerous differentially expressed proteins (DEPs) compared to cells grown in standard media. DEPs common to fibroblasts and SaOS-2 cells were related to the extracellular space, whereas specific DEPs mainly involved energy metabolism in fibroblasts and phosphate metabolism in SaOS-2 cells.

Conclusion: Data highlight that mineral deposition in both cell types is associated to a strong cellular response involving distinct molecular pathways. These findings identify potential targets whose modulation, depending on the biological contest, may enhance or suppress physiological and pathological calcification, respectively.

P21

RHONA LYONS

THE ROLE OF SUBRETINAL PIGMENT EPITHELIUM CALCIFICATION IN AGE-RELATED MACULAR DEGENERATION

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Introduction: Ectopic calcification is a pathological feature of age-related macular degeneration (AMD). A hallmark of AMD is the drusen accumulation between the retinal pigment epithelium (RPE) and Bruch's membrane, which disrupts nutrient and oxygen exchange and impairs waste clearance. Drusen contain proteins, lipids, and calcium phosphate minerals, predominantly hydroxyapatite (HAP) and, whitlockite (WHT). The mechanisms driving calcification and its effects on RPE function remain poorly understood. This project aims to elucidate the intra- and extracellular factors contributing to ectopic calcification in the sub-RPE space.

Materials and methods: Primary human RPE (hRPE) cells were cultured on Transwells and exposed to conditions that promote calcification. These included: 2 weeks in osteogenic media (β-glycerophosphate, ascorbic acid, and dexamethasone); 72 hours exposure to calcium phosphate dibasic; up to 20 weeks in culture; 12 weeks in culture on pre-seeded synthetic calcium phosphate minerals. Transepithelial electrical resistance (TEER) and pigmentation were monitored to assess functionality and maturity, respectively. Changes in gene expression were evaluated using qPCR. Protein level changes were evaluated by immunolabelling. Metabolic function was assessed using the Resipher and Seahorse assays, which measure the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

Results: Calcification was associated with reduced TEER and pigmentation, indicating progressive disruption of RPE barrier function and delayed cell maturation. qPCR and immunostaining demonstrated significant changes in the expression of calcification- and glycolysis-related molecules. Metabolic assays revealed a significant shift towards glycolysis in calcifying hRPE cells, indicating impaired mitochondrial respiration and metabolic dysfunction.

Conclusion: Ectopic calcification adversely affects RPE barrier integrity and metabolic capacity. These findings suggest that calcification directly contributes to retinal dysfunction and may drive progression towards advanced AMD, thereby supporting clinical observations linking mineral deposition to disease severity and vision loss.

P22

VICKY JULIUS MAWUNTU

INVESTIGATING CELL DEATH ORGANELLE STRUCTURES AS NUCLEATION TEMPLATES FOR PATHOLOGICAL CALCIFICATION

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Introduction: Pathological calcification is commonly attributed to extracellular matrix components that provide a scaffold for mineral deposition. However, the contribution of intracellular components released during cell death remains poorly understood. This study investigates whether cell death–derived remnants act as nucleation sites for calcium phosphate mineralization, with a particular focus on mitochondria and nuclei as representative subcellular systems.

Materials and methods: A hydrogel-based in vitro biomineralization model was developed to provide a controllable environment for studying mineral nucleation and growth. Isolated cell debris containing mitochondrial and nuclear fractions was embedded within hydrogel matrices and incubated in simulated body fluid. This system preserves biologically relevant structural and chemical interfaces while minimizing the influence of living cellular processes, allowing template-driven mineralization to be distinguished from solution-mediated ionic effects.

Results: The findings indicate that the hydrogel model successfully supports calcium phosphate mineralization and enables direct assessment of subcellular components as potential nucleation templates. Differences in mineral formation associated with mitochondrial and nuclear fractions suggest that organelle-specific physicochemical properties may influence the initiation of calcification.

Conclusion: This study establishes a framework to investigate the contribution of intracellular components to pathological biomineralization. By examining cell death–derived organelles as potential nucleation sites, this work provides new insights into the early mechanisms of calcium phosphate mineral formation beyond the extracellular matrix paradigm and may reveal new targets for understanding and controlling pathological calcification.

P23

ALESSIA MAZZILLI

MINOCYCLINE CAN SUPPRESS ECTOPIC CALCIFICATION EVEN WHEN IT IS UNCOUPLED FROM DNA DAMAGE AND PARP1 ACTIVATION.

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Introduction: There is growing evidence that DNA damage is not merely a consequence of calcification but can actively promote the osteogenic/calcifying phenotype in several acquired (e.g., diabetes, atherosclerosis) or genetic (e.g., Pseudoxanthoma elasticum, progeria) diseases, thus representing one of the major mechanisms linking aging, oxidative stress and pathological calcification. Within this context, poly(ADP-ribose) polymerase 1 (PARP1), in response to DNA damage, catalyses the synthesis of poly(ADP-ribose) (PAR) chains, potentially promoting ectopic calcification. It has been recently proposed that the antibiotic minocycline (Mi) can act as a calcification inhibitor lowering oxidative stress and PARP1 activation, although the mechanisms of the inhibition remain unclear.

Materials and methods: Human dermal fibroblasts from patients affected by Pseudoxanthoma elasticum, a rare disease characterized by progressive ectopic calcification, were cultured for 21 days in DMEM, in calcifying medium (CM, supplemented with 10mM β-glycerophosphate, 50µg/mL ascorbic acid, 100nM dexamethasone), or in CM + 3µM Mi. DNA damage, PARP1 expression, PARylation and oxidative stress were investigated using morphological, biochemical and proteomic approaches.

Results: CM induced mineral deposition without signs of cell death, nor of DNA damage (i.e., expression of γ-H2AX) or PARP1 activity, while increasing oxidative stress. Mi inhibited mineralization without affecting PARP1 activity during the calcification process until the last time point (i.e., 21 days) when, unexpectedly, PARP1 appeared up-regulated, although PARylation remained unaltered. Moreover, under Mi treatment, oxidative stress levels remained unmodified compared to CM.

Conclusion: Findings indicate that Mi can prevent CM-induced mineral deposition also through PARP1 independent mechanisms, thus suggesting that DNA-damage-induced PARP activation is not universally required for calcification. Rather, it is one of several pathways that can favour calcification, and its importance may depend on cell type, stimulus, and stage of calcification.

P24

NUNTHAWAN NOWWAROTE

FROM SOFT TISSUE TO MINERAL: ECM REMODELING AND PHOSPHATE IMBALANCE IN FAM20A DEFICIENCY

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Introduction: Enamel–renal syndrome (ERS), caused by loss-of-function mutations in FAM20A, is characterized by gingival hyperplasia and ectopic gingival mineralization. Because extracellular matrix (ECM) organization and phosphate/pyrophosphate (Pi/PPi) homeostasis jointly regulate mineral nucleation, we investigated how FAM20A deficiency creates a permissive environment for pathological mineral deposition.

Materials and methods: Primary gingival fibroblasts from three patients with ERS and three healthy controls were cultured either in standard medium supplemented with ascorbic acid to model fibrosis-associated pathology or in osteogenic medium to model ectopic mineralization. Cell-derived ECM was analysed by LC–MS/MS and annotated using matrisome databases. Differentially abundant proteins were selected using an absolute fold change ≥2, adjusted $p \leq 0.05$ and at least three quantified peptides. Functional enrichment and protein–protein interaction analyses were combined with the evaluation of Pi/PPi-regulatory genes. Mineralization was further assessed following Pi/PPi modulation.

Results: The total number of matrisome proteins was similar across groups (288–295), but ERS cells displayed marked qualitative and quantitative ECM remodelling. Under fibrosis-associated conditions, SPP1, EREG, LEP and NRG1 were detected only in controls, whereas EGFL6, SFRP4 and GPC5 were ERS-specific. Under osteogenic conditions, MUC16, FRZB and LEP were control-specific, while CCL7, FGL2 and IL34 were detected only in ERS cultures. Enrichment analyses highlighted collagen biosynthesis and assembly, ECM tensile strength, proteoglycan glycosylation, coagulation-related pathways and calcium-dependent binding. ERS cultures also showed altered expression of Pi/PPi genes, indicating disrupted Pi/PPi homeostasis. Importantly, exogenous PPi suppressed mineral deposition and reversed the mineralizing phenotype toward a non-mineralizing state.

Conclusion: FAM20A deficiency remodels the gingival ECM and perturbs Pi/PPi metabolism. The rescue by PPi supports phosphate imbalance as a functional driver of ectopic mineralization and a potential therapeutic target in ERS.

P25

CÉLIA ORENGO

HETEROGENEITY AND ROLE OF CHONDROCYTES-DERIVED MATRIX VESICLES IN OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA) is the most prevalent musculoskeletal disease, associated with increased mortality and yet no curative treatments exist. OA is characterized by synovitis, pathological subchondral-bone remodelling, and cartilage degradation. At the bone-cartilage interface, the extracellular matrix (ECM) undergoes mineralization, neovascularization, and innervation. Hypertrophic (Hyp) differentiation of chondrocytes plays a key role in these processes: the number of Hyp chondrocytes correlates with the presence of vascular channels. These channels contain microvessels and sensitive nerves and are associated with cartilage degeneration and patient pain. Hyp chondrocytes also release matrix vesicles (MVs,) anchored to the ECM, where they initiate its mineralization. As molecular cargos, MVs are enriched in nucleic acids and proteins.

Our data suggest a heterogeneity of MVs in OA patient cartilage, leading us to hypothesize that during Hyp differentiation, chondrocytes-derived MVs could be involved in OA progression through cartilage mineralization, but also through other processes via their molecular cargo function.

Materials and methods: The aim of this study was to characterize the heterogeneity of MVs at the molecular level. MVs were isolated by differential ultracentrifugation from the ECM of murine chondrocytes differentiated into two stages: prehypertrophic (Prehyp, n=5) and Hyp (n=5). Transcriptomic and proteomic analyses were then conducted on total RNA and protein content at both stages.

Results: We showed that the molecular content of MVs varies depending on the chondrocyte differentiation stage: 67 proteins and 53 miRNAs are differentially expressed between Prehyp and Hyp chondrocytes-derived MVs. Gene set enrichment analyses revealed the involvement of the differentially expressed proteins in biomineralization, bone and cartilage development, and several of the differentially expressed miRNAs have previously been associated with tissue development and remodelling processes.

Conclusion: These results support the hypothesis of heterogeneous populations of MVs in the context of OA, which could play different functions in OA progression beyond mineralization.

MARIA DEL MAR

PHYSICOCHEMICAL CRYSTALLIZATION INHIBITORS PREVENT VASCULAR CALCIFICATION THROUGH DISTINCT MOLECULAR RESPONSES

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Introduction: Cardiovascular calcification is an actively regulated process with limited therapeutic options, in which calcium phosphate crystal nucleation and growth are key final events. Targeting these physicochemical events has emerged as a promising therapeutic strategy. Here, two endogenous calcification inhibitors, inositol hexaphosphate (IP6) and pyrophosphate (PPi), were directly compared in a warfarin-induced rat model. We aimed to evaluate their anti-calcification efficacy together with molecular responses through the analysis of calcification-related gene expression, enzymatic activity, and plasma crystallization potential.

Materials and methods: A six-week warfarin-induced rat model with 32 male rats was used. IP6 and PPi were administered subcutaneously at 15 mg/kg. Calcium content was quantified by ICP-OES in aorta, carotid and femoral arteries and heart. Serum alkaline phosphatase (ALP) activity was determined by a colorimetric assay. Expression of calcification- and extracellular matrix-related genes (Runx2, Bmp2, Bmp1, Spp1 and Mgp) was measured in femoral arteries by RT-qPCR. To assess systemic pharmacodynamic activity, plasma collected near Tmax was evaluated using a validated ex vivo calcium phosphate crystallization assay.

Results: Calcification was mainly restricted to arterial vasculature. Both PPi and IP6 treatments significantly reduced vascular calcium deposition. Interestingly, PPi restored serum ALP activity to levels comparable to healthy control, whereas IP6 did not modify the elevated ALP activity induced by warfarin. Conversely, only plasma from IP6-treated animals significantly inhibited ex vivo calcium phosphate crystallization (>50%). Gene expression profiling revealed convergent suppression of Runx2 by both treatments, together with distinct downstream molecular signatures. IP6 restored Bmp2 expression to healthy levels, whereas PPi normalized Bmp4 and Spp1 expression. Both treatments increased expression of the endogenous calcification inhibitor Mgp, suggesting activation of protective anti-calcification pathways despite their differential regulation of osteogenic markers.

Conclusion: Although both PPi and IP6 effectively prevented warfarin-induced vascular calcification, they exhibited distinct pharmacodynamic and transcriptional responses, supporting different mechanisms underlying a shared anti-calcification effect.

P27

VALENTIN PLATEL

INFLAMMATION AND ENDOTHELIAL DYSFUNCTION IN AORTIC STENOSIS CAN BE MODULATED BY EZH2 INHIBITION IN VALVULAR ENDOTHELIAL CELLS IN FLOW

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Introduction

Calcified aortic stenosis (CAS) is the most common valvular heart disease in industrialized countries and a major cause of death. Currently no pharmacological treatment exists, and its burden on public health increases with the aging population. CAS initiates with endothelial dysfunction. This is primarily caused by the exposure of valvular endothelial cells (VECs) to oscillating shear stress (OSS) which is detrimental to the cells on one side and to laminar shear stress (LSS) identified as protective of the endothelial phenotype on the other side. The epigenetic enzyme EZH2 (Enhancer of Zeste Homolog 2) controls the repression of numerous genes. Its implication in the shear stress response is already well-established.

Materials and methods

We performed a meta-analysis of single-cell RNA-seq data to check the expression of pro-inflammatory chemokines and genes associated with endothelial dysfunction in patients. Also, we built an *in vitro* CAS model using IBIDI® flow culture system. We then looked at gene's expression with qPCR and bulk RNA-seq and chemokines secretion with ELISA test.

Results

We found an increase in pro-inflammatory chemokine genes (e.g. CXCL8) associated with endothelial dysfunction (e.g EDN1) in patients. RT-qPCR and bulk RNA-seq results demonstrate a decrease in the expression of genes associated with endothelial function (e.g. KLAF2/4) and an increase in those associated with endothelial dysfunction (e.g. EDN1) in OSS group compared to LSS. Their secretion is also increased in OSS-treated VECs. Bulk RNA-seq analyses show that 33% of the genes inhibited by OSS were overexpressed after treatment with EZH2 inhibitor, and that, simultaneously, 40% of the genes promoted by OSS returned to an underexpressed state after treatment.

Conclusion

In this context, it appears that EZH2 activity could be a point of convergence for CAS initiation and sustenance mechanisms. EZH2 therefore appears as a potential target in the future development of preventive therapies targeting the progression of CAS.

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ADNAN KHALID SADDIQI

FUNCTIONAL PROFILING REVEALS DISTINCT MOLECULAR DEFECTS IN PATHOGENIC ABCC6 VARIANTS

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Introduction: ABCC6 is a key regulator of extracellular inorganic pyrophosphate (PPi) homeostasis, and loss-of-function variants cause pseudoxanthoma elasticum (PXE), a disorder characterized by progressive ectopic mineralization. Although numerous pathogenic ABCC6 variants have been identified, the molecular mechanisms underlying their functional defects remain incompletely understood. Here, we investigated five PXE-associated variants (R518Q, G992R, R1138Q, R1138W and E1400K) in vitro to characterize their effects on ABCC6 expression, function and cellular localization.

Materials and methods: Multiple independent HEK293T cell clones expressing each variant were analyzed. ABCC6 mRNA and protein expression was assessed by quantitative real-time PCR and Western blotting, respectively. ABCC6 function was evaluated by real-time ATP efflux and extracellular PPi accumulation assays. Selected variants were localized by confocal microscopy, and N-glycosylation-dependent changes in ABCC6 molecular weight were assessed by tunicamycin treatment.

Results: ABCC6 mRNA expression was preserved in all variants, whereas functional activity was severely impaired, with only negligible residual activity detected in some clones. Western blotting revealed distinct protein phenotypes. R518Q protein was almost undetectable and R1138Q markedly reduced, although both showed an apparently wild-type molecular weight. G992R and R1138W showed severely reduced protein abundance and predominantly lower-molecular-weight ABCC6 species, suggesting impaired protein maturation. E1400K largely retained protein expression but showed altered cellular localization. Tunicamycin treatment shifted wild-type ABCC6 toward a lower-molecular-weight species comparable to those detected for G992R and R1138W, supporting defective N-glycosylation/maturation of these variants. E1400K displayed two protein species, one corresponding to the molecular weight of wild-type ABCC6 and a lower-molecular-weight species, indicating a distinct processing pattern.

Conclusion: ABCC6 variants can cause loss of function through distinct post-transcriptional mechanisms, including reduced protein expression, defective protein maturation and altered cellular localization. Integrated analysis of expression, processing, localization, ATP efflux and PPi accumulation provides a functional framework for mechanistic classification of ABCC6 variants.

SABAA SAHI

ENAMEL MATRIX PROTEINS MAY DRIVE PERIODONTAL ECTOPIC MINERALIZATION IN ENAMEL RENAL SYNDROME

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Introduction: Enamel Renal syndrome (ERS - OMIM# 204690) is a rare genetic disorder caused by pathogenic FAM20A gene loss of function. The oral phenotype is severe and characterized by impaired tooth eruption, amelogenesis imperfecta and gingival fibromatosis. The aim of this study was to investigate the suspected periodontal ectopic mineralization process in a human ERS cohort and its biobank.

Materials and methods: A cohort of 29 (24 French and 5 Brazilian) ERS patients was followed (2007-2025) and studied clinically. Genotype–phenotype correlations were explored. Its biobank issued from surgeries enabled the study of dental, gingival and periodontal ERS (and control) samples using histology and immunofluorescence as well as primary cultures. A 3-week follow-up of gingival and follicular sac cells was performed under proliferative and osteoinductive conditions, with or without recombinant full-length amelogenin (1-1000 ng/ml).

Results: The severity of dental and periodontal disorders increased with age and varied between patients. Most pathogenic FAM20A variants were deletions, predominantly located in exon 11. Six previously unreported variants were identified. Histological, immunofluorescence, and cellular analyses consistently demonstrated the presence of ectopic mineral deposits within mesenchymal soft tissues, particularly in the hypertrophic gingiva and the follicular sac of unerupted teeth. These deposits were located inside and nearby epithelial (CK14-positive) dental (amelogenin-positive) cells which expressed other enamel proteins such as the odontogenic ameloblast-associated protein (ODAM). In vitro studies evidenced increased mineralization capacities for both ERS-derived gingival and dental follicle cells compared with controls. In vitro addition of recombinant amelogenin further enhanced these mineralization properties.

Conclusion: These findings support a dual mechanism in which intrinsic pro-mineralizing properties of ERS mesenchymal cells and enamel protein-mediated epithelial–mesenchymal crosstalk promote ectopic mineralization.

MÁRCIO SIMÃO

MATRIX GLA PROTEIN (MGP) DEFICIENCY PROMOTES ECTOPIC CALCIFICATIONS WHILE ELASTIN A (ELNA) LOSS MODULATES THIS SUSCEPTIBILITY IN ZEBRAFISH

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Matrix Gla Protein loss of function was associated with ectopic calcifications in the larynx, the respiratory tract, the ears, the nose and the vascular system, which are phenotypes observed in Keutel syndrome. Elastic fibers are composed by elastin and are responsible for the elasticity in tissues such as skin, lungs, tendons, and blood vessels and fragmented elastic fibers are more prone to hydroxyapatite deposition like in pseudoxanthoma elasticum (PXE) disease, suggesting that fragmentation can precede mineralization. This study aimed to characterize the impact of Matrix Gla Protein (Mgp) and Elastin a (Elna) loss of function on soft tissues and caudal fin abnormal mineral depositions in zebrafish (*Danio rerio*).

Three zebrafish mutant lines were analysed: $mgp^{\Delta 10}$ (10-nucleotide frameshift deletion), $elna^{sa17177}$ (nonsense mutation introducing a premature stop codon), and the corresponding homozygous double mutant. Histological analyses were performed on the eyes, heart, and caudal fin rays using Von Kossa with 0.1% neutral red, hematoxylin and eosin (H&E), and 0.03% Alizarin Red S staining. Ectopic calcifications were quantified across three histological depths and compared among genotypes. In addition, the presence of structural alterations beyond abnormal calcification was investigated in the analysed tissues.

The results indicate that the $mgp^{\Delta 10}$ mutation promoted higher levels of ectopic calcifications compared with the wild-type and $elna^{sa17177}$ genotypes. These calcifications were located primarily in the zebrafish corneas and within the ventricular myocardium of the heart. In addition, we observed abnormal intra-articular ossification in the caudal fin rays. Double homozygous mutants ($mgp^{\Delta 10}$/ $elna^{sa17177}$) presented a lower incidence of calcification compared with the $mgp^{\Delta 10}$ group.

Mgp acts as a major systemic inhibitor of soft tissue calcification in zebrafish. When mutated in the present of an Elna loss-of-function, the ability of Mgp deficiency to promote ectopic calcification is reversed, confirming previous findings in mammals.

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GIORGIA SINIGAGLIA

MITOCHONDRIAL AND METABOLIC CHANGES DURING OSTEOGENIC DIFFERENTIATION OF VALVULAR CELLS

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Introduction: Progressive calcification of the aortic valve (AV) in Calcific Aortic Valve Disease (CAVD) leads to lifethreatening Aortic Stenosis (AS). CAVD is an active process, involving dysfunction of Valvular Endothelial Cells (VEC) and osteogenic transformation of Valvular Interstitial Cells (VIC). Agerelated alterations in mitochondrial function have been linked to enhanced osteogenic signalling; however, this is understudied in the AV. We have determined the metabolic remodelling of VIC and VEC under osteogenic conditions.

Materials and methods: VIC and VEC were isolated from AV tissue obtained from patients undergoing surgical valve replacement. Cells were characterised by qPCR and exposed to osteogenic medium. Cell viability was monitored using Cell Proliferation Reagent WST1, while mitochondrial respiration was evaluated using Seahorse live-cell bioenergetics measurement.

Results: qPCR confirmed identity and purity of the cell populations. Throughout osteogenic treatment, VIC remained viable while VEC viability decreased over time. Both VIC and VEC showed increased basal and ATPlinked respiration under osteogenic conditions compared to control cells, indicating enhanced oxygen consumption associated with ATP production. Maximal respiration remained stable, suggesting that mitochondrial capacity was not increased, while spare respiratory capacity decreased, particularly in VEC, indicating a reduced ability to respond to the additional energetic demand. Proton leak remained low, with an increase in VIC at day 4, suggesting an adaptive response to mitochondrial stress while overall efficiency remained preserved.

Conclusion: Both VIC and VEC exhibited a metabolic shift under osteogenic stimulation, suggesting a metabolic contribution to valvular mineralisation. In VIC, the enhanced energetic demand associated with osteogenic differentiation was met by their mitochondrial capacity; however, VEC couldn't respond to the energetic demand, likely leading to cell death. The differential response of VIC and VEC to osteogenic cues and early metabolic remodelling may contribute to CAVD pathology through endothelial death and valvular calcification by osteoblast-like VIC, respectively.

P32

XIAO TAN

INVESTIGATING THE ROLE OF METABOLISM AND MITOCHONDRIA DURING THE PROCESS OF VASCULAR CALCIFICATION

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Introduction: Vascular calcification (VC) is a key pathological feature of cardiovascular disease. Although mitochondrial dysfunction and metabolic alterations have been implicated in VC, the underlying cellular mechanisms remain incompletely understood.

Materials and methods: An in vitro MOVAS (murine vascular smooth muscle cell line) calcification model was established. Calcification was evaluated by alizarin red staining and quantitative calcium assay. Osteogenic gene expression was analysed by qPCR. Untargeted metabolomics, Seahorse analysis and Biolog MitoPlate-S assays were performed to characterise metabolic and mitochondrial function. Early mitochondrial dynamics were assessed by qPCR.

Results: Calcification was successfully induced after 7 days and was accompanied by significant upregulation of the osteogenic markers Runx2, Alpl, Phospho1 and Opn ($P<0.05$), confirming osteogenic differentiation.

Untargeted metabolomic profiling revealed marked metabolic perturbation accompanying calcification. Intracellular glucose levels were elevated, yet glycolytic intermediates were depleted, with accumulation of pyruvate and lactate, suggesting impaired glucose utilisation and reduced mitochondrial oxidative flux. Altered amino acid metabolism and lipid-associated pathways suggested compensatory non-glucose carbon entry into the tricarboxylic acid (TCA) cycle, despite an overall reduction in TCA-associated metabolites. Collectively, these data support metabolic reprogramming away from mitochondrial oxidative phosphorylation toward a glycolytic phenotype.

Subsequent Seahorse analysis corroborated these findings. Calcifying cells exhibited reduced oxygen consumption rate (OCR), indicative of impaired mitochondrial respiration, alongside increased extracellular acidification rate (ECAR) and proton efflux rate (PER), consistent with enhanced glycolytic flux. Reduced utilisation of oxidative phosphorylation substrates was further confirmed using Biolog MitoPlate-S assays.

In addition, expression of the mitochondrial dynamics regulators Drp1, Opa1, Mfn1 and Mfn2 was significantly increased after 48 hours of calcification, suggesting remodelling of the fission–fusion machinery preceding calcium deposition.

Conclusion: These findings demonstrate that vascular smooth muscle cell calcification is associated with coordinated metabolic reprogramming, mitochondrial dysfunction and early alterations in mitochondrial dynamics. Targeting mitochondrial metabolism and function may provide novel therapeutic opportunities for preventing vascular calcification.

P33

VIRGIL TAMATEY

CONSERVED TRANSCRIPTIONAL CO-REGULATION OF PYROPHOSPHATE HOMEOSTASIS GENES GOVERNS SYSTEMIC MINERALIZATION FACTORS IN MICE AND HUMANS

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Introduction: Inorganic pyrophosphate (PPi) is a major endogenous inhibitor of ectopic calcification. Its extracellular concentration is determined by a network involving ABCC6, ENPP1, ANKH, and tissue-nonspecific alkaline phosphatase (ALPL), linking PPi metabolism to inorganic phosphate (Pi). We investigated whether these major PPi homeostasis genes are coordinately regulated and how this regulatory network is reflected in circulating mineralization factors across the lifespan.

Materials and methods: Transcriptional co-regulation of ABCC6, ENPP1, ANKH, and ALPL was assessed using complementary genomic and transcriptomic approaches and evaluated experimentally in mouse models. Circulating alkaline phosphatase (AP) activity, PPi, and Pi were assessed across maturation and aging in wild-type and Abcc6$^{-/-}$ mice. Corresponding relationships were evaluated in human control and pseudoxanthoma elasticum (PXE) samples.

Results: The four PPi homeostasis genes showed conserved transcriptional co-regulation, with HNF4A emerging as an important regulator of hepatic ABCC6, ENPP1, and ALPL expression. Circulating AP activity declined markedly after weaning, accompanied by increasing plasma PPi during maturation. During aging, AP activity showed a biphasic decline, while PPi increased particularly in Abcc6$^{-/-}$ mice. PPi was inversely associated with AP activity and positively associated with Pi. Similar PPi–AP and PPi–Pi relationships were observed in human controls and individuals with PXE.

Conclusion: Our findings reveal conserved transcriptional coordination of major PPi homeostasis genes and link this regulation to dynamic changes in circulating mineralization factors. The conserved AP–PPi–Pi relationships across mice and humans support coordinated regulation of systemic PPi homeostasis.

ANDREA TÓTH

AGGRAVATION OF ENDOPLASMIC RETICULUM STRESS IMPAIRS ANTIOXIDANT DEFENSE AND ACCELERATES VASCULAR CALCIFICATION IN CHRONIC KIDNEY DISEASE

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Introduction: Vascular calcification is the common complication of chronic kidney disease (CKD). Accumulating evidence suggests that endoplasmic reticulum (ER) stress promotes the osteogenic transdifferentiation of vascular smooth muscle cells (VSMCs). The transcription factor NRF2 orchestrates the cellular antioxidant system; however, sustained ER stress may compromise NRF2 activity. Despite growing evidence, the mechanistic crosstalk between ER stress and NRF2-dependent antioxidant defense in CKD-associated vascular calcification is poorly understood. Therefore, we investigated whether pharmacological modulation of ER stress using the ER stress inducer tunicamycin (Tun) or the chemical chaperone sodium 4-phenylbutyrate (4-PBA), together with the activation of NRF2 using sulforaphane (SFN) or dimethyl fumarate (DMF), influences vascular calcification and antioxidant system in mice with CKD and in calcifying VSMCs.

Materials and methods: C57BL/6 mice were fed with 0.2% adenine to induce CKD. After CKD induction, mice were treated with Tun, 4-PBA and DMF. Vascular calcification was assessed by von Kossa, Alizarin Red and OsteoSense staining. Protein expression was determined by Western blot, while intracellular ROS production was quantified using a fluorescence assay.

Results: Tunicamycin markedly accelerated CKD-induced vascular calcification in mice, whereas 4-PBA attenuated mineral deposition. In VSMCs, tunicamycin activated ER stress response, enhanced phosphate-induced calcification, increased ALP and SOX9 protein expression, and promoted ROS production, while suppressed NRF2-dependent antioxidant signaling. Both ROS scavenging and 4-PBA treatment reduced oxidative stress, restored antioxidant defense, and attenuated calcification together with the osteochondrogenic phenotypic switch of VSMCs. Conversely, pharmacological activation of NRF2 with SFN or DMF alleviated ER stress, restored antioxidant capacity, and reduced calcification in VSMCs. Consistent with these findings, DMF treatment significantly attenuated vascular calcification in mice with CKD.

Conclusion: Our findings identify a crosstalk between ER stress and NRF2-dependent antioxidant defense as a critical regulator of CKD-associated vascular calcification. Targeting ER stress or restoring NRF2 signaling effectively attenuates oxidative stress, osteochondrogenic reprogramming, and vascular calcification.

P35

DORIAN T'SJOEN

ANGIOID STREAKS AS A SHARED PHENOTYPE OF DISTINCT CALCIFICATION MECHANISMS

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Introduction: Angioid streaks (AS) are breaks in a pathologically calcified Bruch membrane. Their recognition is diagnostically important, as more than half of patients with AS harbor an underlying systemic disease. Clinically, AS also warrant careful monitoring because they predispose to choroidal neovascularization (CNV), leading to vision loss if not detected and treated promptly.

Materials and methods: We report on a 60-year-old man presenting with acute unilateral visual decline. Ophthalmological imaging revealed bilateral peripapillary AS with a right-sided subretinal hemorrhage. Biochemical analyses and subsequent genetic testing were performed to determine the underlying etiology, while multimodal imaging was used to monitor choroidal neovascularization.

Results: Pseudoxanthoma elasticum (PXE) was initially suspected based on the ocular phenotype. Systemic evaluation, however, revealed not only mild vessel wall calcification in all lower limb arteries but also chronic hyperphosphatemia, while skin lesions were absent. During the work-up, the patient developed a periarticular hydroxyapatite deposition at the hip joint with bursitis. Molecular analysis identified a homozygous pathogenic missense variant in *GALNT3* (c.902T>A; p.Val301Asp), confirming the diagnosis of hyperphosphatemic familial tumoral calcinosis (HFTC). Although no CNV was seen initially, OCT-angiography later demonstrated subclinical neovascularization, requiring anti-VEGF therapy.

Conclusion: This case highlights the phenotypic overlap among ectopic calcification (EC) disorders and demonstrates that AS, though most frequently associated with PXE, are not specific for this disease. AS may result from distinct pathogenic mechanisms. In dystrophic calcification, mineral deposition occurs in structurally abnormal tissues despite normal calcium and phosphate homeostasis, as classically observed in PXE. In contrast, metastatic calcification reflects systemic disturbances in mineral homeostasis and occurs in otherwise normal tissues, as in HFTC. Although HFTC is characterized predominantly by periarticular calcified masses, extra-articular manifestations, including AS and vascular calcifications, can mimic PXE. Recognition of this overlap is important for accurate diagnosis and appropriate systemic and ophthalmological management.

DORIAN T'SJOEN

BEYOND THE GENOTYPE: MOLECULAR DIAGNOSTIC CHALLENGES IN PSEUDOXANTHOMA ELASTICUM AND THEIR CLINICAL CONSEQUENCES

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Introduction: Pseudoxanthoma elasticum (PXE) is a hereditary ectopic calcification disorder caused by biallelic *ABCC6* or, rarely, *ENPP1* variants. Although molecular analysis is part of the diagnostic criteria, interpretation can be challenging because of variable expressivity, incomplete penetrance, phenotypic overlap, and technical limitations. Diagnostic uncertainty may directly affect clinical surveillance and reproductive decision-making. We present cases from the Ghent PXE Reference Center illustrating how these challenges can be resolved.

Results: Eight cases were selected representing three recurring diagnostic dilemmas. First, patients with mild or atypical phenotypes may remain in a diagnostic grey zone despite molecular testing. One patient had an incomplete *ABCC6* genotype and intermediate inorganic pyrophosphate levels, prompting consideration of undetected (non-coding) variants and deep phenotyping. Another presented with a retinal pattern dystrophy and both a pathogenic and an incompletely penetrant *ABCC6* variant, requiring variant phasing, segregation analysis and integrated phenotyping. Cases involving incidental findings during genetic work-up in prenatal setting or in infants illustrate the diagnostic and ethical complexity. Second, apparently actionable variants may require confirmation and functional interpretation. An *ABCC6* exon deletion identified by exome sequencing in a PXE patient's partner was disproven by MLPA. A *GGCX* VUS in another partner prompted biochemical and functional evaluation because of reported digenic *ABCC6–GGCX* inheritance. Third, genetic findings may have reproductive implications. Partners carrying incompletely penetrant *ABCC6* variants require assessment for subclinical manifestations and individualized counseling regarding reproductive options. In children with PXE, parental segregation analysis is essential to exclude germline mosaicism and accurately assess recurrence risk.

Conclusion: These cases demonstrate that molecular diagnosis in PXE is not a binary genotype-based process. Resolving diagnostic uncertainty may require structured integration of deep phenotyping, biochemical biomarkers, segregation analysis, variant confirmation, functional studies, and ethical considerations. This multidisciplinary approach enables informed decisions regarding diagnosis, surveillance and reproductive counselling, while acknowledging that residual uncertainty may remain.

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XIONG WENG

TRANSCRIPTOMIC REMODELLING IN ENPP1 AND ABCC6 DEFICIENT MURINE AORTAS IDENTIFIES INFLAMMATORY AND STRESS-RESPONSE PROGRAMMES

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Introduction: Loss of ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) and ATP-binding cassette subfamily C member 6 (ABCC6) is associated with the rare diseases (Generalized Arterial Calcification of Infancy (GACI) and Pseudoxanthoma elasticum (PXE), respectively. Both diseases demonstrate arterial calcification, and are underpinned by the loss of extracellular pyrophosphate (PPi). PPi is a key inhibitor of calcification, and directly regulated by ENPP1 and ABCC6.

Materials and methods: Bulk RNA-seq was performed on murine aortas isolated from tiptoe walking (ttw/ttw) mice (that harbour a spontaneous loss-of-function mutation in the Enpp1 gene), Abcc6 deficient (Abcc6-/-) and wild-type (WT) controls (n=3). Differential expression analysis was visualised by volcano plots and unsupervised hierarchical clustering, followed by KEGG pathway enrichment of differentially expressed genes (https://davidbioinformatics.nih.gov).

Results: Both *ttw/ttw* and *Abcc6*$^{-/-}$ aortas showed clear transcriptomic separation from WT. KEGG enrichment highlighted pathways linked to PI3K-Akt, MAPK, JAK-STAT, p53 signalling and cell cycle regulation, alongside extracellular remodelling signatures including extracellular matrix (ECM)-receptor interaction and focal adhesion, as well as stress-associated programmes such as cellular senescence. In parallel, immune- and metabolism-associated pathways were enriched, including AGE-RAGE signalling, cholesterol metabolism, and **T helper 17 cell** differentiation. Interestingly, direct comparison between *ttw/ttw* and *Abcc6*$^{-/-}$ aortas revealed a pronounced shift towards inflammatory and innate/adaptive immune activation, with enrichment of Toll-like receptor and IL-17 signalling, chemokine signalling, complement and coagulation cascades, as well as neutrophil extracellular trap formation.

Conclusion: Loss of *Enpp1* or *Abcc6* is associated with distinct aortic transcriptomic remodelling patterns, combining ECM/stress responses with strong immune activation signatures—particularly in *Enpp1* deficient aorta. These data indicate inflammatory signalling networks as candidate modulators of aortic calcification and potential therapeutic targets.

P38

MERYEM TUĞÇE YAVUZCANLI

REACTIVE OXYGEN SPECIES PRODUCTION IN SMOOTH MUSCLE CELLS DURING VASCULAR CALCIFICATION: EFFECTS OF N-ACETYLCYSTEINE

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Introduction: Oxidative stress is a hallmark of vascular calcification (VC), yet the relative contribution of cytosolic and mitochondrial reactive oxygen species (ROS) during this process remains unclear. An important source of ROS during VC is NADPH oxidase (NOX). We characterized compartment-specific oxidative responses and evaluated the effects of the antioxidant N-acetylcysteine (NAC) on TNF-a or phosphate-induced mineralization in mouse aortic vascular smooth muscle cells (MOVAS).

Materials and methods: Mouse aortic vascular smooth muscle cells (MOVAS) were stimulated with 3 mM phosphate (Pi), 2.5 mM Ca^{2+} and 3 mM Pi (Ca/Pi), or TNF-α (10 ng/mL and 100 ng/mL) for 7 days. Cytosolic and mitochondrial ROS production was quantified using DCFDA and MitoSOX, respectively, while NOX4 (the isoform expressed in mouse cells) gene expression was analyzed by RT-qPCR. In a complementary study, 3 mM Pi-treated MOVAS cells were incubated with 0.5 mM or 2 mM NAC for the same duration. Mineral deposition was quantified by Alizarin Red staining, normalized to protein content.

Results: Compartment-specific ROS analysis identified cytosolic ROS as the predominant oxidative response to pro-calcific stimulation. Consistent with these findings, 3 mM phosphate increased NOX4 expression, supporting activation of oxidative signaling during the early stages of calcification.

To evaluate the functional significance of oxidative stress, cells undergoing phosphate-induced calcification were treated with the antioxidant NAC. Across three independent experiments, NAC reduced phosphate-induced mineralization by approximately 53% at 0.5 mM and 73% at 2 mM. The inhibitory effect was most consistent and statistically significant at 2 mM (paired t-test, $p = 0.014$).

Conclusion: Our findings support a role for compartment-specific oxidative stress in vascular calcification and suggest that cytosolic ROS together with NOX4-associated signaling may contribute to disease initiation. Importantly, antioxidant intervention with NAC consistently attenuated phosphate-induced mineralization, highlighting treatment with antioxidants as a promising therapeutic target for limiting vascular calcification.

P39

RANA HANAFY

DECODING INFLAMMATION-DRIVEN VASCULAR CALCIFICATION: MECHANISTIC INSIGHTS FROM 2D AND NOVEL 3D ENGINEERED VASCULAR TISSUE MODELS

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Introduction: Cardiovascular disease remains the leading cause of morbidity and mortality worldwide, with vascular calcification (VC) contributing to arterial stiffening, plaque instability and adverse cardiovascular events. Although inflammation is increasingly recognized as a key driver of VC, the early molecular events underlying inflammation-induced calcification remain poorly understood. Moreover, conventional two-dimensional (2D) culture systems fail to recapitulate the three-dimensional (3D) extracellular matrix (ECM) microenvironment.

Materials and methods: Mouse aortic vascular smooth muscle cells (MOVAS) were cultured for 7 days under calcifying conditions (3 mM Pi) or stimulated with TNF-α (1, 5, 25, and 100 ng/mL) in 2D culture. Calcification was assessed by Alizarin Red staining, TNAP enzyme activity and quantification of gene expression of key enzymes and transporters of phosphate/pyrophosphate (Pi/PPi) homeostasis; CD73, Pit-1, eNTPD-1 and ANKH. To establish a more physiologically relevant *in-vitro* model, MOVAS cells were embedded in fibrin-based hydrogels to generate 3D-engineered vascular tissues (EVTs).

Results: TNF-α significantly increased TNAP enzyme activity, reaching a 2-fold increase after treatment with 5 ng/mL ($p < 0.001$), despite the absence of detectable mineral deposition by Alizarin Red staining. Under these conditions, qPCR analysis of Pi/PPi axis showed an increase in TNAP mRNA expression, with ENPP1 decreasing in a dose-dependent manner. In 3D-EVTs, VSMCs displayed a more homogeneous cellular organization compared to 2D cultures, with TNF-α significantly affecting EVT dimensions, suggesting changes in cell–ECM interactions. Altered expression of the above-mentioned key genes involved in Pi/PPi homeostasis during early inflammation was also quantified.

Conclusion: Our findings demonstrate that inflammatory stimulation of VSMCs is associated with early changes in the expression of key regulators involved in Pi/PPi homeostasis before detectable mineral deposition. Furthermore, the developed 3D-EVT model captures tissue-level structural and molecular responses that are not observed in conventional 2D cultures, providing a physiologically relevant platform to investigate the initiation of vascular calcification.

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