45TH EUROPEAN CALCIFIED TISSUE SOCIETY CONGRESS

ECTS 2018

26–29 MAY 2018

where scientific research and clinical practice meet

VALENCIA ★ SPAIN

CONGRESS REPORT

A PRODUCT OF ECTS European Calcified Tissue Society

THIS PUBLICATION IS SUPPORTED BY ucb Inspired by patients. Driven by science.

Foreword from Anna Teti, ECTS president

The ECTS 2018 congress was again exciting and successful. Around 1000 delegates attended the meeting in Valencia, for teaching and learning in a relaxed and lively atmosphere. Our pre-congress day was also very successful and included the Mellanby training course, the first ECTS/ICCBH workshop on Rare Bone Diseases, the East meets West events, 10 very successful Working Groups and the ECTS Academy events. Scientists were able to take advantage of the networking opportunities, clinicians could hear about the latest developments and best clinical practice, and young investigators could benefit from contact with experienced colleagues and opinion leaders, including through our Meet the Expert sessions. ECTS aims at promoting crosstalk between the generations and we believe this goal was fully achieved in Valencia, particularly as a result of the tremendous efforts of our ECTS Academy members.

This report will walk you through the congress highlights. If you have missed anything or want to review the presentations, this is an excellent tool for you. We are very grateful to our delegates, sponsors and staff who made ECTS 2018 a great congress, and to Michael Baldwin and the editorial team for preparing such a useful and interesting report.

The next ECTS congress in 2019 will be in Budapest, Hungary. Our excellent pre-congress will be held on May 10th, followed by the main programme during May 11th-14th 2019. Meanwhile, take advantage of the many activities organized by ECTS during the year. To know more, please visit the ECTS website, download the ECTS app and register to receive the ECTS Newsletter. Stay tuned, we care about you all year long!

Professor Anna Teti
ECTS President

Contents

Published by the **European Calcified Tissues Society**
ECTS Executive Director: **Roberta Mugnai**
Website: www.ectsoc.org
Twitter: @ECTS_soc

Editor: **Michael Baldwin**
Other contributors: **Natalie Butterfield, Alfredo Cappariello, Kashmala Carys, Annabel Curle, Melanie Haffner-Luntzer, Antonio Maurizi, Hanna Taipaleenmäki**
Design/production: **Sublime Creative**
Photography: **Valencia Conference Centre**
Video production: **The Informed Scientists**
Congress Secretariat: **Interplan**

SUPPORTED BY: ucb Inspired by **patients.** Driven by **science.**

Progress for rare diseases at ECTS

ECTS 2018
26-29 MAY 2018

Results of clinical trials for treatment for X-Linked Hypophosphatemia

At ECTS 2018 Robin Lachmann (London), presented the results of a multi-centre phase 3 study to determine the efficacy and safety of burosumab, an anti-FGF23 antibody, for treatment of adult XLH. This rare inherited disorder results from mutations in PHEX leading to raised phosphate excretion, osteomalacia, fractures and dental complications.

A group of 134 adult XLH patients ceased their usual phosphate and vitamin D supplementation and were treated with burosumab or placebo every 4 weeks. After 24 weeks, the placebo group was transferred also to burosumab and the study continued a further 24 weeks. The primary endpoint of reaching the normal range of phosphate levels (LLN) was achieved for 94.1 % of subjects in the burosumab group in the first 24 weeks, with benefits continuing during the rest of the study, also in the crossover group. Healing of fractures and pseudofractures was more likely with burosumab treatment and scoring of pain, stiffness and physical functioning all improved. Injections were well tolerated with no drug-related adverse events.

Also, Wolfgang Högler (Birmingham, UK) presented the results of two phase 2 pediatric clinical studies of burosumab. One study was conducted in 52 children with XLH between the ages of 5 and 12 years with treatment either every two or four weeks, and the other was performed in 13 children aged 1 to 4 years receiving two-weekly treatment. Across all age groups, burosumab treatment was found to consistently raise mean serum phosphorus levels after 40 weeks of treatment, while the Total Rickets Severity Score decreased over 40 weeks, as did the serum alkaline phosphatase levels. None of the subjects discontinued therapy or developed hyperphosphatemia. Results demonstrate substantial healing of rickets in children treated with burosumab.

The results from one of the pediatric trials have already been published. Burosumab is now licenced for treatment of children with XLH in Europe, and in the USA for both children and adults, and may well be life-transforming for people with XLH.

Oliver Gardiner, founder of XLH UK who attended ECTS 2018, told us "Burosumab is life-changing for patients with XLH as it is the first and only treatment that targets the underlying mechanism of their hypophosphatemia. The trial results suggest improvements in the areas that matter to patients, including reductions in daily pain and stiffness as well as improvements in healing of fractures and rickets severity, which may limit the need for invasive, corrective surgeries. We at XLH UK are currently working closely with the patient community and NICE, as the cost-effectiveness of burosomab is currently being evaluated with guidance expected for England and Wales in late October 2018."

What is new in musculoskeletal research around the world

Over 17,000 publications on bone-related research appeared in the last year, generating a flood of new data. Hans Van Leeuwen (Erasmus Medical Centre) used his **What is New in Basic Science?** talk to ask how we can get the most out of this data. Firstly, we need to be sure that the data itself is high quality, published with sufficient methodological details to allow the results to be replicated, and meeting the FAIR data principles. Hans van Leeuwen then highlighted the growing trend for using artificial intelligence (AI) in basic and clinical research, which could help us make better use of the data we generate, and to also fully "digest" the data generated in previous years. One of the most established uses of AI is analysis of massive datasets describing biochemical and genomic profiles. Also, AI is being explored for *in silico* simulation of biological processes, a recently developed example being the D-cell simulation that is using machine learning to compute how gene mutations and other changes could affect cell growth.

Despite the growing availability of AI tools, and the appearance already of some clinical applications, none of the abstracts submitted to ECTS 2018 involves such approaches, but we can probably expect to see AI feature much more in the coming years.

In other news... Look out through the rest of the report for other interesting papers highlighted in our **What is New?** session by Hans van Leeuwen (for basic science) and Franz Jakob (for clinical science).

ECTS meets with rare disease patient groups

ECTS is organising a new initiative to support rare bone disease patient groups in networking and mutual support.

To kick off this network, a meeting was organised in Valencia, alongside the ECTS 2018 congress, for patient group leaders to discuss their priorities. These included representatives of OIFE (osteogenesis imperfecta), the Spanish Association for Camurati-Engelmann patients, Hypophosphatasie Deutschland and the UK and Spanish patient groups for X-linked hypophosphatasia. Also participating in the meeting were representatives of ECTS, Luca Sangiorgi from the European reference network ERN-BONE, and companies involved in developing new therapies for rare bone diseases.

In a breakout discussion session the patient group leaders identified current challenges for their work, where they see a new network as being helpful. Several points were agreed. As many of the groups are for patients with very rare diseases, small numbers of people are involved in each group, spread out geographically, and this makes organising and membership engagement quite difficult. It would be interesting to share experience of how to handle having a rare disease from a psychological point of view. Communication was also raised as a major issue. Social media provides many opportunities for misinformation to circulate, and it is also not easy for patients to always understand the latest developments in research – patient groups can play a key role here in providing information. Access to medical professionals who are familiar with rare bone diseases is not always easy.

The new European reference networks such as ERN-BONE will also address some of these issues, and patient groups are keen to interact with these other networks as they develop.

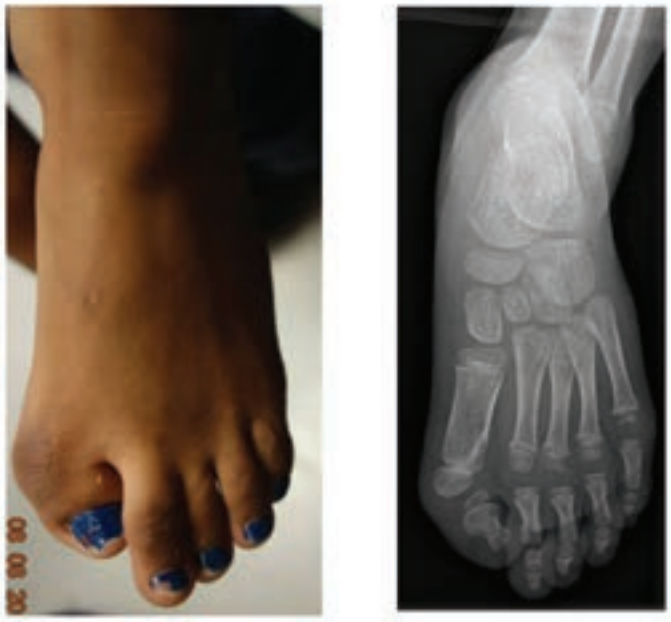
The meeting also included presentations on the development of new therapies for rare bone diseases from the companies present, and a summary of the rare bone diseases workshop also held during the precongress. Further development of the network is expected with teleconferences, a meeting next year and the participation of further patient organisations.

ECTS are grateful to the following companies for their financial support of the meeting: Mereo BioPharma, Kyowa Kirin and Alexion Pharmaceuticals Inc.

Moving forward in understanding and treating Fibrodysplasia Ossificans Progressiva

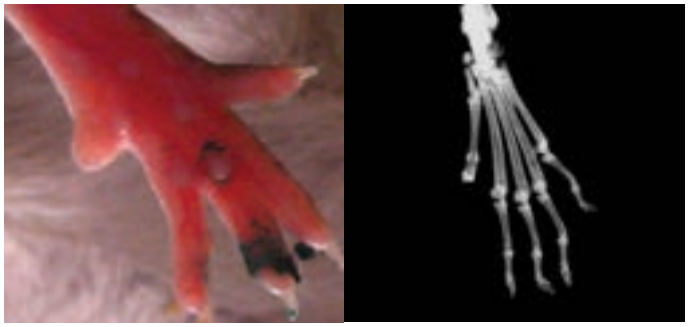
The ECTS 2018 educational symposium on Fibrodysplasia Ossificans Progressiva (FOP) featured as speakers **Eileen Shore** (Philadelphia), a key contributor to the genetic discovery of the disease, and **Genevieve Baujat**, from the reference centre for skeletal dysplasias in Paris, who is involved in the development of the FOP Connection Registry.

FOP is a very rare genetic condition featuring skeletal dysplasia and heterotopic ossifications. Estimates of prevalence range between 1 in 2 million and 1 in 700,000. FOP manifests at birth with a malformation of the big toe. The disease progresses in "flare ups" with bone forming in soft connective tissue, skeletal muscle, tendons and ligaments in particular. Loss of independence typically occurs by the third decade of life with a high risk of early death during the fourth decade due to thoracic insufficiency syndrome.



Left: a four-year old child showing the characteristic congenital hallux valgus (short big toe). Right: anteroposterior radiograph of the foot shows the broad first metatarsal with monophalangism. (G. Baujat)

In her talk (and also in a Meet the Expert session), Dr Shore described the basic science of heterotopic ossification in FOP and paths to treatment. The autosomal dominant gain-of-function mutation responsible for FOP has been identified in the AVCR1 gene (ALK2), which encodes a type 1 BMP receptor, stimulating mesenchymal cells via dysregulated BMP signalling towards chondrogenesis and osteogenesis. More than 95% of analysed cases are due to a single nucleotide substitution that causes the amino acid substitution R206H. The R206H mutation causes enhancement of downstream signalling at a very low level, without the presence of the ligand. High or constitutively active expression is lethal.



An Acrv1R206H/+ mouse model (representative images at 4 weeks old) had shortened or absent proximal and distal phalanges, similar to malformations in FOP patients. (S. Chakkalakal)

Whilst the mechanisms of the initiation of both FOP and trauma-induced heterotrophic ossification are still largely unknown, it is thought that the bone formation is a replacement of the hosting tissue, in which osteogenic progenitor cells are induced to differentiate by an incorrect signal or by misinterpretation of a signal. However, once the ectopic bone formation is initiated, the bone that forms is remarkably normal, with no apparent differences in the basic structure, development or remodeling relative to skeletal bone tissue. The progenitor cell identity has not yet been confirmed, however tissue resident mesenchymal stem cells and endothelial precursors have been implicated. Unfortunately, the process of surgical removal of heterotopic ossifications in a FOP patient is likely to prompt further bone development and is thereby not a viable option.

A knock-in mouse model for the R206H mutation has similar malformations of the first digit of hind limb and extraskeletal bone formation, recapitulating the human disease. As exogenous retinoid agonists can block chondrogenesis, Eileen Shore and colleagues have investigated an oral RARγ agonist palovarotene as a potential therapeutic strategy for FOP, and shown that it not only prevents spontaneous heterotopic ossification but also restores long bone growth and mobility, alleviating concerns that the RARg agonist treatment would have negative effects on the growth plates of young children with FOP.

In her presentation, Genevieve Baujat described a recent FOP prevalence study, including the challenges involved in identifying non-diagnosed patients. A further complication for the study is that patients with non-genetic heterotopic ossifications may be diagnosed as false positives. Current management strategies for FOP include avoidance of injections and other potential inducers of flare up, pain management and physiotherapy. Possible drug therapies include immunosuppression, ligand traps and anti-inflammatory drugs. However, the promising results obtained for oral palovarotene in mouse models has led to a clinical trials programme, starting with a FOP natural history study of 114 patients with annual follow-up. This has documented flare-up characteristics and conducted full body CT and MRI or ultrasound scans. The phase 2 clinical trial (NCT02279095) has recruited participants with FOP to study response to treatment. An episodic treatment regimen during a flare-up was first studied, comparing to a placebo group. After 12 weeks of treatment, results show that 65% fewer participants in the treatment group experienced heterotopic ossification during flare-up, and mean volumes of new bone volume were reduced by 60% compared to placebo.

Following evaluation of the results, a second stage has been conducted evaluating the association of low-dose chronic treatment and higher-dose flare-up treatment. This finally treated 53 subjects, including 12 children. All the participants completed the study. Some mild or moderate adverse effects were observed that are normally expected with retinoid agonists, mainly dryness of the skin and lips, but there was no discontinuation of treatment. The results of the chronic treatment stage are to be published shortly.

ECTS WORKING GROUPS

The pre-congress day featured sessions of 10 working groups, designed for discussion of very focused topics in smaller groups, suitable for an informal exchange of ideas and collaborations. Selected working groups are featured in this report (see below).

- WG 1: **Rheumatic Diseases and Bone** (see here)
- WG 2: **Non Mammalian Models** - Potential for the study of common and rare skeletal diseases (see here)
- WG 3: **Bone Bioengineering, Regeneration and Implants** - Bone Biomechanics (see here)
- WG 4: **Cancer and Bone** - Breast and prostate bone metastases (see here)
- WG 5: **Imaging Bone Strength** - Standardization of microCT, QCT and FE data (see here)
- WG 6: **Transgenic Animals in Musculoskeletal Research** (see here)
- WG 7: **Cartilage Pathophysiology** - Common and rare disorders of cartilage (see here)
- WG 8: **Epigenetic Regulation of Bone Formation** - DNA and histone modifications (see here)
- WG 9: **Non Calcified Tissues and Ectopic Calcifications** (see here)
- WG 10: **Rare Bone Diseases** - Advances in therapies for the osteopetroses (see here)

However, plans are already in place for a phase 3 trial of palvarotene (MOVE - NCT03312634) for chronic treatment of pediatric and adult patients, to evaluate its safety and efficacy in preventing new heterotopic ossifications, compared to untreated controls (using external NHS data). The treatment regimen will still involve a daily oral dose plus a temporarily higher dose in case of flare-up. Participants are so far recruited mainly from Europe and North America but the hope is to involve participants from additional countries. If successful, MOVE will provide the data for regulatory approval of a new therapy for FOP.

Meanwhile research on the basic mechanisms of FOP continues, with particular interest in the role of immune cells in inducing flare ups. Heterotopic formation of bone in tissues outside the skeleton can be precipitated in any person by severe trauma, such as injuries to the central nervous system, joint replacements or deep tissue burns. Studying FOP can also help us understand the mechanisms of these trauma-induced heterotopic ossifications.

ECTS/ICCBH Workshop on Rare Bone Disease Pathophysiology, Manifestation and Treatment from Childhood to Adulthood

The session was started by **Wolfang Högler** (Birmingham, UK) who talked about the rare bone diseases affecting mineralization in children, focusing on different forms of hypophosphatasia. Dr Högler gave an overview of the current therapeutic approaches for X-linked hypophosphatemia, showing data from the clinical trials of Burosumab (see above).

The second speaker **Michael Econs** (Indianapolis) spoke about the rare bone diseases affecting mineralization in adults. In particular, he introduced the role of FGF23 in bone homeostasis and mineralization. Dr Econs described different diseases impacting FGF23 levels in human and mouse models including: X-linked hypophosphatemia due to mutation in the *Phex* gene; Autosomal Dominant Hypophosphatemic Rickets caused by mutation in the FGF23 gene; Tumor Induced Osteomalacia in which the tumor produces FGF23 and finally Autosomal Recessive Hypophosphatemic Rickets, a very rare disease in which elevation of FGF23 levels is due to mutations affecting different genes including *DMP1*, *ENPP1* and *FAM20C*.

Outi Mäkitie (Helsinki) then discussed rare bone diseases affecting bone fragility in children, including osteogenesis imperfecta, juvenile and X-linked osteoporosis, spondylo-ocular syndrome and Winchester syndrome. She also talked about the therapeutic approaches for these diseases such as bisphosphonates, denosumab and romosozumab.

The last speaker was **Richard Keen** (London) who talked about the rare bone diseases affecting bone fragility in adults. Dr Keen's talk was focused on the different forms of OI affecting adults, describing the different mutations and giving a definition of bone strength. He emphasised that the real challenge is the identification of patients and the increase of new OI subtypes leading to new OI nomenclature. He also spoke about the treatment of OI patients using drugs such as bisphosphonates, denosumab and romosozumab, also highlighting the side effects related to denosumab discontinuation (rebound effect).

In the last part of the workshop there were 3 short talks (selected abstracts). **Kassim Javid** (on behalf of Luca Sangiorgi) talked about the European Reference Networks and BOND ERN on the rare bone diseases, **Heeseog Kang** presented her project "somatic activating mutation in MAP2K1 causes meloreostosis" and **Fabiana Csukasi** talked about the "disruption of a PTHrp-SIK3 mediated pathway alters mTOR signaling".

The workshop ended with a question & answer session.

Working Group on advances in therapies for the osteopetroses

This ECTS Working Group meeting during the pre-congress featured several approaches for innovative therapy for two types of osteopetrosis, malignant autosomal recessive osteopetrosis (ARO) which is usually diagnosed in infancy, and autosomal dominant osteopetrosis type 2 (ADO2), which is less severe and is usually diagnosed in adolescents and sometimes in adults.

Michael Econs (Indianapolis) gave a talk about "Clinical aspects of osteopetrosis". His talk focused on ADO2, presenting clinical features, and showing that only one two thirds of patients are symptomatic. Next, he showed how different the progression of bone phenotype can be, presenting radiographs of two brothers affected by ADO2, one worsening over time, the other not. This aspect is also observed in the first animal model of ADO2.

Anna Villa (Milan) presented on "Somatic gene therapy for autosomal recessive osteopetrosis". She gave an overview on ARO, and differences between osteoclast-rich and osteoclast-poor forms, pointing out the link between genetic basis and clinical features. The main difference impacting on clinical management is the onset of primary or secondary neurological defects. In the case of a primary neurological defect, hematopoietic stem cells transplantation (HSCT), the only therapeutic strategy nowadays available for ARO patients, is precluded. However, HSCT is not a panacea, and Anna Villa spoke from her experience about its limitations, side effects, poor survival and cause of death after HSCT. For these reasons, new therapeutic strategies are urgently required, for example a better conditioning regimen before HSCT and alternatives to classical HSCT. Gene therapy seems to be the best candidate. She presented the state-of-art for gene therapy and the differences between retroviral and lentiviral vector used in gene therapy. Next, she showed results from her research, based on gene therapy on mouse model of ARO, using a clinically-approved lentiviral vector used in mucopolysaccharidoses therapy. She showed results on the treatment on mice, and the rescue of phenotypical features of ARO. Moreover, Anna Villa showed protocol applied to patient's cells and transplanted in mouse model.

Mattia Capulli (L'Aquila) gave a presentation on RNAi-based therapy for ADO2. He explained why chloride channel 7 (CCLN7) dependent ADO2 is a good candidate for RNA interference therapy, since the silencing of the mutated allele could allow to the intact one to work properly. He presented the mouse model for ADO2 they generated, and the strategy they used for preparing the RNA-interference probes. Finally, he showed the results on *in vitro* and *in vivo* experiments. At L'Aquila they used protocols with differing timing and dosage of RNAi probes to obtain a complete rescue of osteopetrotic phenotype. Moreover, their strategy did not showed side effect or off-target events.

Ciro Menale (Milan) presented a talk entitled "MSC-seeded biomimetic scaffolds as factory of human soluble RANKL in Rankl-deficient osteopetrosis". In his work, he developed a 3D culture system on a biocompatible scaffold for treating Rankl-/- mice, a mouse model of osteoclast-poor ARO, lacking osteoclast lineage TRAcP positive cells. Murine Rankl/- MSCs were lentivirally transduced with human soluble RANKL (LvhsRL) and seeded on scaffold. Once implanted subcutaneously in Rankl-/- mice, the scaffolds were well tolerated, colonized also by cells of the host and intensely vascularized. Of note, in the bone of Rankl-/- implanted mice with LvhsRL-transduced Rankl-/- MSCs, he observed the formation of TRAP+ cells, likely due to soluble RANKL released from the scaffolds into the circulation.

Uta Rössler (Berlin) reported on an alternative gene therapy approach for ARO based on gene transfer by the Sleeping Beauty (SB) transposon. She presented the proof of principle for their strategy in a myelogenous human

ECTS Fellowship Award

The ECTS Fellowship Awards are one-year awards to support research in the bone field by New Investigators. The **ECTS Fellowship Pre-clinical Award** was received by **Dr Ciro Menale**, who is working at IRGB-CNR, located at Istituto Clinico Humanitas, Milan. His research is investigating RANKL signalling in mesenchymal stem cells, identifying candidate genes to modulate osteogenic potential without affecting RANKL production, with the eventual application being a new cell-based therapy for RANKL-deficient ARO.

cell line, in which she achieved a good and stable expression of the CLCN7 cDNA by a SB transposon approach. Moreover, in order to demonstrate the therapeutic effect of the CLCN7 expression from the SB transposon vector, she established an efficient protocol to differentiate induced pluripotent stem cells (iPSCs) into osteoclasts, derived from an ARO patient harbouring a CLCN7 mutation. As expected, patient-derived osteoclasts were incapable of generating resorption pits on dentine slices. Thus, she applied the SB transposon strategy on ARO iPSCs, obtaining a good rate of stable integration in target cells.

ALSO PRESENTED AT ECTS 2018

FGFR3-gain-of-function mutation modifies lumbar vertebrae structure and cranial synchondroses in a hypochondroplasia mouse model

Lea Loisay and colleagues from the Imagine Institute, Paris, have generated the first mouse model of hypochondroplasia. This is a rare disease resulting from a gain-of-function mutation in FGFR3, associated with short stature, craniofacial malformations and other developmental defects. The mice with the most common missense mutation pAsn540Lys have been characterised from 1 week to 6 months of age. By 14 days they show reduced development in the long bones and spine, and anomalies in skull bone fusion, while defective chondrocyte differentiation was observed in the cartilage growth plate in L4-L6 lumbar vertebrae. Deformations of the intervertebral disc (IVD) were accompanied by an increase in collagen II content of the annulus fibrosus within the disc. The results underline the importance of FGFR3 for both cranial synchondroses and IVD. As the mouse model effectively replicates human hypochondroplasia it will be useful for therapeutic studies.

Perivascular fibrosis and upregulation of the TGFβ pathway in CLCN7-dependent Autosomal Dominant Osteopetrosis type 2 (ADO2)

ADO2 is a rare disease characterised by dense but fragile bones resulting from defective osteoclast function, with most cases caused by mutations in the CLCN7 gene. This gene is also expressed in other organs. **Antonio Maurizi** (L'Aquila) presented the latest analysis of the effects of ADO2 in organs outside the skeleton, in a mouse model carrying the heterozygous, ADO2-causing mutation, *Clcn7*^{G213R}. A higher amount of perivascular collagen was found in the lungs, kidneys and muscles of the ADO2 mouse compared to the wild type, along with an increased number of F4/80+/ClC7+ macrophages associated with fibrosis. Further analysis of ADO2 lungs, muscle and kidney by RNA deep sequencing showed enrichment in the macrophage gene signature an upregulation of the profibrotic TGFβ pathway. In particular, the TGFβ-activated factors, α-Sma and Grem1, were upregulated in ADO2 lungs. In vitro, the ADO2 macrophages had a higher lysosomal pH, LC3bII accumulation and a higher p62 expression, suggesting altered autophagy, also known to be induced by TGFβ. These results suggest that tissue macrophages and activation of the TGFβ could be involved in the induction of the perivascular fibrosis observed in the ADO2 mouse model, with potential implications for human ADO2 as a more systemic disease.

ECTS 2018 supports Camurati-Engelmann patients' association

Through the volleyball tournament and at the networking dinner, ECTS 2018 raised over €1400 for the Spanish association for Camurati Engelmann disease (AAECE - Asociación de efectados por la enfermedad Camurati Engelman). Their representative Javier Gomez Melgar was happy to answer some questions for us.

Please tell us about Camurati-Engelmann disease?
Camurati-Engelmann disease is a rare disease of overgrown bone, particularly the long bones and the skull. There can also be muscular and neurological symptoms. Patients can develop megacephaly with prominent forehead, exophthalmos, thin extremities with thick bones and sparse muscle mass. They usually present a graceful appearance, marfanoid habit, an abnormal gait, reduced muscle strength with extenuated osteotendinous reflexes, arched legs, flat feet and valgus, lumbar lordosis and scoliosis.
I wanted to show a photograph of myself at the swimming pool to show how the disease progresses. Here, it is very visible in legs and arms and trunk. Otherwise, Camurati-Engelmann can be a silent disease, not immediately obvious to others unless you are in a swimsuit and have the disease somewhat developed.

At what age are people most often diagnosed?
When young children start to walk, you can then see the characteristic, cautious duck-like walk, muscle weakness, lack of psychomotor skills.

Is the cause of the disease understood yet? Does it run in families?
It's a genetic disorder that has a 50% chance of being inherited. The gene mutation is identified in TGF□1 but the mechanism is not yet completely understood.

What practical difficulties do people with Camurati-Engelmann disease experience in daily life?
Mobility and tiredness, every year it gets worse and without exercise the muscles decline. Pain in the bones is another major problem, and it is also possible to develop deafness or blindness.

How many people are diagnosed to have the condition?
There are only around 200 people diagnosed in the world, but we think there should be more cases. The disease is estimated to affect one in a million people. The disease is often confused with muscular atrophy, but a new genetic test should help identify patients. In Spain, there are 10 patients.

Are there particular specialists or experts in the condition?
As far as I know, there is one in the USA (Dr Wilcox) and one in Barcelona (Dr Guañabens).

Is there a treatment for the condition?
There are anti-inflammatory drugs available (glucocorticoids), but these only help with the pain and some other symptoms. Physical exercises or low impact sports such as swimming or aquatherapy are highly recommended as they help to improve muscle tone to support the skeleton. As a result, pain and limitation of movement can be reduced because the impact energy is distributed better throughout the body rather than localized in the bone. The Club Natació

Atlètic-Barceloneta offers aquatherapy classes that help to maintain physical fitness, and have been adapted to help with Camurati-Engelmann syndrome — strengthening and stretching of the muscles so that they do not contract with the growth of the bone, and can cope with the extra effort required to support the skeleton. People with Camurati-Engelmann disease try to be aware of our physical limitations, and develop our intellectual potential. Having a disease does not mean being a sick person.

How long has the patients' association been running, and what sort of aims and activities does it have?
We started in 2012. We started by giving talks to medical students as it is important that they understand about the disease. We also realised it is important to talk to schoolchildren so they understand more about rare diseases in general. The most important thing for us now is looking for more patients, make a register of those affected, and collect information on their symptoms, as these can be different for each person. We also have an annual meeting about the disease in Barcelona.

And what is your own role in the association?
I am the president. I work on organising events and building collaboration with doctors and other associations to spread knowledge about Camurati-Engelmann disease.

What did you think about the ECTS congress overall?
The congress was a very important opportunity to communicate about our disease to more doctors and to know more about how it is working in the research world. The money that was collected in the congress will be used to help publicize ourselves further and to maintain the association, as well as to invest in research and contact with other doctors.

ECTS perspectives on clinical hot topics

ECTS 2018 26-29 MAY 2018

In a new feature, position papers developed by ECTS Policies and Consensus Committee on clinical hot topics over the past year were presented during the Opening Ceremony.

Discontinuation of Denosumab therapy for osteoporosis- a systematic review and position statement by ECTS

Unlike bisphosphonates, denosumab is not incorporated into bone and its effects on bone turnover stop after the ending of treatment. **Elena Tsourdi** (Dresden) summarised the conclusions of a systematic literature review on the effects of stopping denosumab, including data from randomised phase II and phase III clinical trials, case studies and abstracts from major congresses. Most reports identify a reduction of BMD and an increase in bone turnover markers within 6 months of cessation of treatment. Several reports suggest an increased risk of vertebral fractures though this is not yet conclusive. ECTS has developed management guidelines suggesting an evaluation of patients after 5 years on denosumab, with those at high risk of fractures being considered for continuation of densoumab or an alternative treatment for up to 10 years. Moving to bisphosphonates has been shown to reduce bone loss after denosumab cessation, and this may be an option for patients at low risk of fracture at the 5-year point, but more study is needed to be certain of the optimal bisphosphonates treatment regimen. Published in *Bone*, December 2017.

Fractures in patients with CKD- diagnosis, treatment, and prevention: a review by members of ERA-EDTA and ECTS

In collaboration with the European Renal Association of Nephrology Dialysis and Transplantation, ECTS has reviewed the diagnosis, treatment, and prevention of fragility fractures in patients with chronic kidney disease (CKD), and current strategies for the management of these patients. In her presentation of this review, **Martine Cohen-Salal** (Paris) explained that there is little consensus on the most suitable biomarkers and strategies for prevention of fractures in these patients. Normal fracture assessment tools based on BMD may underestimate fracture risk for CKD patients, due to vascular calcifications, scoliosis and other manifestations of the disease. PTH has been identified as a surrogate marker for bone histology and additional serum markers are being evaluated.

To prevent fractures in CKD patients with eGFR >30 ml/min, normalisation of 25-hydroxyvitamin D and control of PTH are recommended. Most anti-osteoporotic medications can be used according to the recommendations for non-CKD patients, with a specific caution given for raloxifene because of its association with thromboembolism.

For CKD patients with eGFR <30 ml/min, most anti-osteoporosis treatments are contraindicated due to the reduced renal clearance, an issue especially for bisphosphonates. However, denosumab as a treatment that does not accumulate in bone has been tested in CKD patients as part of a larger phase III trial. These patients experienced similar gains in BMD compared to the other study participants, but further studies are required to determine long term safety and effects on fracture risk. The review has developed a decision tree for the management of fragility fractures in CKD patients. As these are complex cases, the review recommends the involvement of multidisciplinary teams combining renal and bone experts in any decision to initiate an anti-fracture treatment. Published in *Kidney International*, December 2017

Recommendations for the screening of adherence to oral bisphosphonates

Richard Eastell (Sheffield) presented the recommendations for the screening of adherence to oral bisphosphonates developed by the International Osteoporosis Foundation and European Calcified Tissue Society Working Group. Adherence to oral bisphosphonates after 12 months is estimated at 50% or less. The working group used data from the TRIO study to determine if bone turnover markers could be used to detect a lack of adherence in patients. They found that measured levels of PINP and CTX at baseline and 3 months, compared to the least significant change normally observed (reductions of 38% and 56% respectively), can provide effective detection of non-adherence. A detection level of 94.5% was determined when both measurements are used. The re-assessment of treatment should then consider reasons for non-adherence, including whether the treatment is actually suitable for the patient and whether other factors are involved. Published in *Osteoporosis International*, March 2017

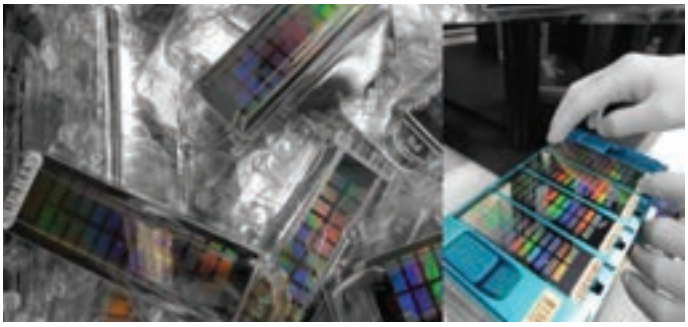
Omics moving forward to practical translation

ECTS 2018
26-29 MAY 2018

Academia met with industry in this ECTS 2018 symposium to discuss how discoveries in "omics" are now more likely to lead to clinical applications.

Fernando Rivadeneira (Rotterdam) opened the session with an overview of how genomics is giving us new insights into bone biology. The original promise of genomics reaching the clinical setting has made significant progress through large scale collaborative initiatives, such as the GEFOS genome-wide association (GWAS) consortium and the ENCODE project, providing insights into the regulatory landscape of the genome. In the area of genetic prediction, allelic scores for bone mineral density work at the population level to identify extreme groups of individuals at very high and low risk of osteoporosis and fracture; where clinical risk factors are still more useful for individual patient management. In the area of biological insight, major progress has been made by studying these genetic variations in knockout mouse models helping to characterise their effects on bone and non-skeletal tissues, and "paving the road" for the identification of new drug targets.

Since the second GEFOS GWAS (Estrada, *et al*. 2012) identifying 56 loci for BMD, major biological insight has been obtained from functional follow-up studies, for example Wnt16 has been confirmed by KO models as a crucial regulator of cortical bone thickness in mice, where further work demonstrated that over-expression regulates trabecular bone independent of oestrogen regulation. Given its action independent of estrogen, targeting WNT16 holds great potential as a drug compound for postmenopausal osteoporosis and provides a "proof of concept" of the efficiency of GWAS to prioritise a drug target within 5 years after discovery.



Microarray technology allows affordable and simultaneous high-throughput genome-wide genotyping of up to 800 thousand markers in 24 individuals in one chip. This "technological push" in the setting of large biobank networks (consortia) has boosted the identification of loci influencing variation of complex traits at a population level and the understanding of the biological pathways underlying disease susceptibility in individuals. (A. Verkerk)

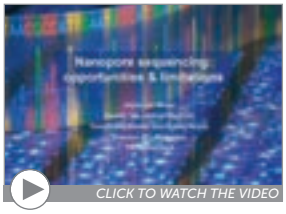
Similarly, FAM210A identified by Estrada, *et al*. and which was associated more strongly with fracture risk than with BMD, has been recently studied in tissue-specific inducible knockout mice. In these mice, they found Fam210a to be expressed in muscle mitochondria and cytoplasm of other tissues, but not in bone, and that grip strength, lean mass and bone strength and quality were all negatively affected; providing biological insight on how skeletal fragility can emerge through muscle dysfunction.

More recent studies to identify genetic markers for bone mineral density have involved analysis of non-invasive heel BMD in the extremely large sample of UKBiobank yielding hundreds of identified loci with biological implication on bone metabolism (see also below). Finally, using Mendelian randomisation the GEFOS consortium has leveraged the findings of the largest fracture risk GWAS to date (publication accepted by BMJ 2018) to assess the causal effects of the most relevant risk factors of fracture. This study shows that only low BMD, early menopause and decreased hand grip strength share common genetic factors (heritability) with fracture risk and are causally mediating fracture occurrence. Strikingly, there is no indication from this work that vitamin D and calcium levels exert a causal effect on fracture risk, suggesting that indiscriminate supplementation is not indicated. Instead, interventions targeting an increase in BMD are more likely to be successful in decreasing fracture risk.

Matthew Nelson (GlaxoSmithKline) then explained how genomics can help the pharmaceutical industry. The success rate from drug discovery through to the clinic is currently around 0.5% and takes an average 14 years. Moreover, the number of drugs approved per billion US dollars of R&D spend by the industry has halved every 9 years since 1950 ("Eroom's law"). This could be due to most of the low-hanging fruit being already discovered, and that its increasingly harder to improve on existing therapies. Many new drugs fail during phase II or III clinical trials due to lack of efficacy, or being no more effective than existing treatments, and because of safety concerns.

Genetic data may help to better match drugs to indications, and a study involving GSK estimated that selecting targets with supporting human genetic data can double the success rate in clinical development. The pharmaceutical industry has now focused much more on obtaining this data. GWAS approaches have progressed dramatically since 2007, with technology, lower costs and quality allowing studies in much larger populations. Just as important, major cultural changes have occurred, so that international collaboration to generate large sample sizes is now the norm, with open sharing of data and meta-analysis. However, according to Matthew Nelson, there is still much to discover and different kinds of questions about drug efficacy will be asked as the available data becomes more detailed and complex, for example as longitudinal data. GSK have developed the STOPGAP database (Systematic Target OPportunity assessment by Genetic Association Predictions) drawing on data from GWAS catologue, such as eQTLs, modelling data and other functional genomic data to map human genetic associations to effector gene candidates. The OPEN TARGETS public-private consortium in the UK has developed this approach into the more industrialised process POSTGAP, allowing a user to obtain a gene hypothesis from a genetic association. Its code will be publicly available soon.

As an example of how the field has progressed in the past few years, a study published in 2016 investigated variants in 6 genes encoding drug targets for obesity or type 2 diabetes through exome sequencing and targeted genotyping in a total of ~50,000 individuals, particularly to identify any association with cardiovascular risk. After two years of work, this identified a low frequency variant in GLP1R that was associated with protection against heart disease – evidence that GLP1R agonists in development for type 2 diabetes treatment were not likely to be associated with cardiovascular risk. However, with the availability now of the UK Biobank data from 500,000 people combining multiple types of genetic and other health-related data, it is now possible to recapitulate this result within hours or even minutes. Other biobanks and large data sources are developing. However, to really answer questions about genetic subtypes of disease we need sample sizes in the millions or tens of millions to carry out well-powered studies. This will be possible through integration of biobanks and resources, for example through the Genomics Resources Consortium.



Nanopore sequencing

In the ECTS 2018 Basic Science Update on Technology, **Joyce van Meurs** (Erasmus MC) explained how Nanopore sequencing, a third generation technology, can achieve direct sequencing of single molecules, including long reads and complex regions that Next Generation Sequencing finds challenging.

A nanopore is constructed from proteins that first convert DNA or RNA into single strands and then detect individual G/A/T/C bases through changes in an electrical current running across the pore. Each nanopore can process 450 bases per second and a flow cell has 2048 nanopores. Commercial systems are available from PacBio and Oxford Nanopore Technologies, with the latter company specialising in miniaturised, portable systems suitable even for use in the field. Initially the accuracy of nanopore systems was <80% but they are now >95% accurate, especially if multiple readings are performed to obtain a consensus result.

Other issues to consider:

- Nanopore sequencing generates very large data files, too large for a laptop to practically manage
- Proteins can block nanopores so extra care is required in sample purification
- The analysis pipeline still requires further development

There are already many publications using Nanopore sequencing, for example to analyse telomeres, the human centromere (which is otherwise difficult to sequence), and RNA sequencing without the bias introduced by a PCR step. Erasmus MC is utilising Nanopore sequencing for a range of projects including complete 16S sequencing for microbiome studies, and rapid diagnosis of infections useful for infants and epidemic crises.

ALSO PRESENTED AT ECTS 2018

A high resolution Capture-C promoter 'interactome' implicates causal genes at BMD GWAS loci

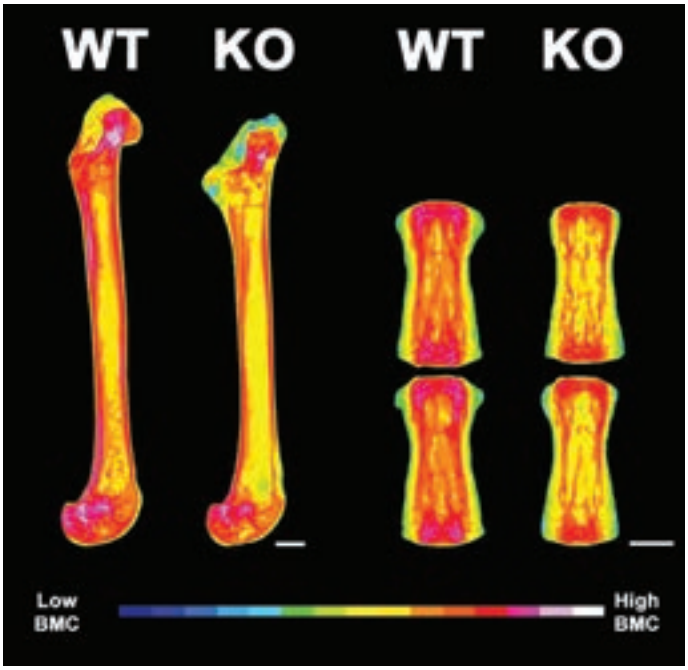
While GWAS studies have identified genomic signals associated with low BMD, these studies do not identify the genes responsible – a required step if the research is to lead to new therapeutic approaches for osteoporosis. Alessandra Chesi (Philadelphia) reported on a study performed in a model of human MSC-derived osteoblast cells, using a massively parallel, high-resolution Capture-C approach (SPATIaL-seq) to characterise the 3D interactome of all human promoters. Using ATAQ-Seq, open chromatin maps were generated to identify the most likely functional proxy SNPs for each of 110 BMD GWAS loci, and by intersecting the two sets of data, at least 30% of loci were found to have strong interactions with target genes. These include both genes within close proximity to the GWAS sentinel SNP and genes distant in the genome sequence (e.g. *EPDR1* at the '*STARD3NL*' locus). Functional studies are being performed with RNAi knockdown to observe effects on osteoblast differentiation.

Genome-wide association of bone mineral density in the UK Biobank full release identifies 301 novel loci and implicates DAAM2 in osteoporosis

BMD estimated by quantitative ultrasound of the heel (eBMD) could be a less invasive and cheaper method than DXA to identify people at risk of osteoporosis. A previous GWAS study of eBMD in the UK Biobank (first release of 150k samples) identified 203 loci explaining 12% of the trait variance for developing osteoporosis, including 153 previously unreported loci. John Morris (Montreal) described a new GWAS study in 426,824 participants from the UK Biobank (full release), prioritizing genes strongly expressed in osteoblasts and osteocytes, and other genes for osteoporosis and bone disorders. Overall, the number of associated loci was increased, explaining 20% of trait variance. As causal coding SNPs were identified for DAMM2, knockout mice for this gene were analysed for their skeletal phenotype. While *Damm2*$^{-/-}$ mice had normal bone structural parameters, maximum load in biomechanical testing was greatly reduced in their femurs and vertebrae, indicating reduced bone quality. DAMM2 is therefore identified as a novel determinant of bone quality, complementing other therapeutic targets for osteoporosis.

Tram2 is a novel genetic determinant of bone mass and strength

The OBCD consortium is performing high throughput phenotyping of knockout mice to identify novel susceptibility alleles for bone and mineral disorders. Victoria Leitch reported that *Tram2* knockout mice studied in this screen exhibit deafness, reduced body weight and spontaneous fractures, despite having normal bone serum markers. Further detailed investigation has revealed a substantially reduced cortical and trabecular bone mass, decreased bone strength and stiffness, reduced osteoblast differentiation and increased osteoclast nuclei and resorption. *Tram2* is known to support the folding of collagen type 1, but there are no collagen abnormalities in these mice. Its likely that *Tram2* deficiency affects bone development during growth, and then impairs bone turnover during adulthood. As *TRAM2* was also identified in a previous GWAS study it represents a potential novel therapeutic target for osteoporosis and further studies in the knockout mice will seek to define the cellular and molecular mechanisms involved.



Representative pseudo-coloured x-ray microradiographs of femur and vertebrae from p112 female WT and Tram2-/- (KO) mice. Scale bar is 1mm. (V. Leitch)

ECTS Steven Boonen Award

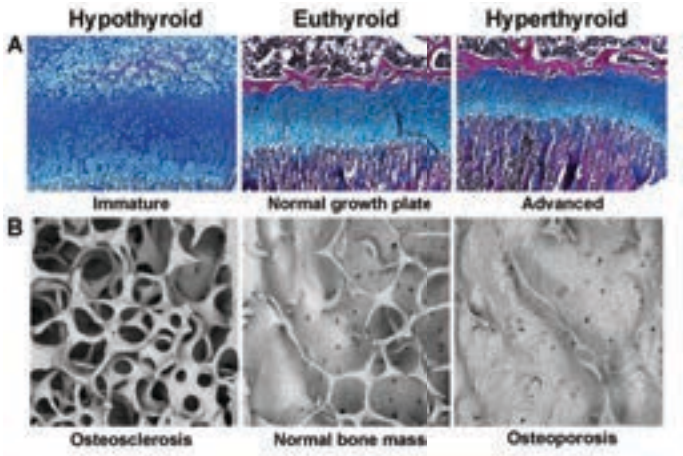
SUPPORTED BY AMGEN *EUROPE*

ECTS 2018
26-29 MAY 2018

The Steven Boonen Award for excellence in clinical and translational medicine was given this year to Professor Graham Williams, who has been Professor of Endocrinology at Imperial College London since 2005 and heads the Molecular Endocrinology Laboratory at the Hammersmith Campus.

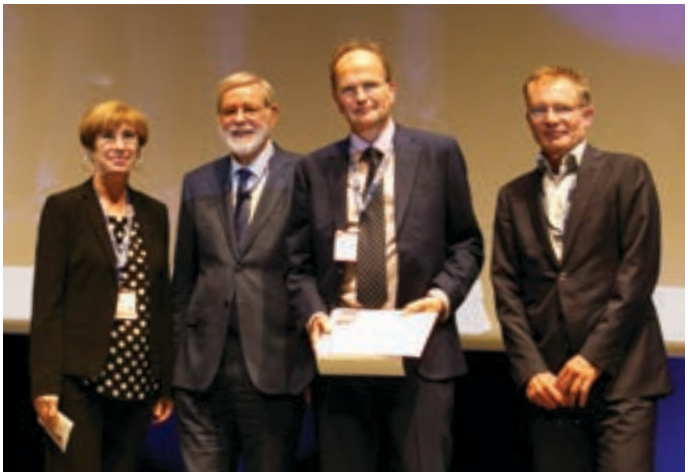
Prof Williams has made major contributions to our understanding of thyroid hormone effects on the skeleton at both clinical and molecular levels, and he also works in collaboration with the International Knockout Mouse Consortium to identify genes that determine bone and cartilage development and maintenance, and which are involved in the pathogenesis of osteoporosis and osteoarthritis. His work is generously funded by the Wellcome Trust, Medical Research Council and European Union. Professor Williams received a Wellcome Trust Investigator Award in 2016 and has published more than 180 articles and chapters.

Graham Williams's lecture gave us a fascinating overview of thyroid hormones and their role in skeletal development and the regulation of bone mass. He discussed the consequences of congenital hypothyroidism and juvenile thyrotoxicosis on the immature developing skeleton and outlined the effects of thyroid hormone excess on bone turnover and fracture susceptibility. Professor Williams went on to outline the skeletal dysplasia recently discovered in patients with mutations affecting thyroid hormone receptor alpha and discussed how this condition, together with insights from genetically modified mice, has advanced understanding of thyroid hormone action in the skeleton. These clinical and mechanistic studies have now been examined in large populations, conclusively showing that even a mild degree of thyroid hormone excess is associated with bone loss, low BMD and an increased risk of fracture. Overall, Graham Williams has made a huge contribution to the field, establishing our fundamental understanding of the role of thyroid hormones in bone and cartilage and the translation of these findings to clinical practice.



Regulation of skeletal development and bone maintenance by thyroid hormones. A. During childhood hypothyroidism delays endochondral ossification causing disorganisation of the growth plate and short stature, whereas hyperthyroidism accelerates skeletal development leading to advanced bone age. B. In adults hypothyroidism inhibits bone turnover leading to retention of bone and osteosclerosis, whereas hyperthyroidism causes high bone turnover, accelerated bone loss and osteoporosis. (G. Williams)

Management guidelines for the long term follow up of thyroid cancer have already been amended to limit the use of excessive thyroid hormone treatment in the majority of long-term survivors, and it is envisaged that more detailed mechanistic understanding of thyroid hormone effects in bone will lead eventually to novel therapeutic approaches for osteoporosis, osteoarthritis and fracture repair.



We asked Graham a few questions after the congress.

How did you become interested in the effects of thyroid hormones on bone?

I was coming to the end of a two-year period of laboratory training in Professor Reed Larsen's laboratory at Brigham & Women's Hospital, Harvard Medical School in Boston. I was working on mechanisms of thyroid hormone receptor action in the regulation of target gene expression. I was due to return to the University of Birmingham, UK in early 1992, and Reed encouraged me to seek out my own area of research to pursue independently. As a clinical scientist I was keen to develop a physiological research of clinical importance that focused on an unexplored thyroid hormone responsive target tissue. I considered several options and spoke to many people before deciding on starting out to investigate the role of thyroid hormones in the skeleton. It turned out to be a great choice that continues to be endlessly fascinating and one that has grown and fueled considerable scientific and clinical interest.

Your work combines clinical and basic research. How do the two areas of work interact?

They are mutually complementary. Understanding how thyroid hormones control skeletal development and linear growth provides novel insight into the pathogenesis and new treatment approaches for new skeletal dysplasias such as the recently identified resistance to thyroid hormone alpha, which results from mutations of the THRA gene encoding thyroid hormone receptor alpha. Determining how thyroid hormones regulate the bone remodelling cycle and bone turnover in adults helps our understanding of the mechanisms involved in the pathogenesis of osteoporosis. We are developing our basic studies to characterise the downstream signalling pathways that are responsive to thyroid hormones in bone and cartilage with the aim of uncovering novel drug targets that should also have broader applications in tissue repair following injury. In the clinical research arena, I have been fortunate to be able to collaborate within the worldwide Thyroid Studies Collaboration, which undertakes the largest population-based epidemiological studies of the role of thyroid dysfunction in many chronic diseases, but especially in bone loss and fracture susceptibility. These studies have helped to inform clinical guidelines for the long-term management of thyroid cancer patients with suppressive doses of thyroid hormones that can result in increased susceptibility to fracture. The most recent guidelines now recommend careful use and restriction of suppressive doses of thyroxine to the few patients who are at higher risk of cancer recurrence thereby minimising exposure to thyroid hormone excess in the vast majority of survivors.

Your lecture highlighted the use of genetic mouse models to understand disease mechanisms. What difference has this approach made for progress in your area of research?

This approach has enabled important advances to be made and it will result in further important discoveries I am sure. For example, we analysed mice with thyroid hormone receptor alpha mutations several years before patients with THRA mutations were identified. The mouse phenotype faithfully predicted the skeletal dysplasia subsequently found in patients. These disease models allow for investigation of underlying mechanisms of disease and facilitate investigation of new potential treatments. A huge advantage of using mouse models is that studies can be performed using animals that have an identical genetic makeup apart from the mutation of interest. This allows for causative effects of the mutation to be identified much more confidently. Using animal models of disease also ensures availability of tissue samples that are not easily obtained from patients. Finally, the ability to perform bespoke genetic manipulation of signalling pathways, such as thyroid hormone signalling in a cell specific way, allows us to focus on hormone actions in individual bone cell types without worrying about disruption of circulating hormones that can complication our understanding quite considerably.

Euthyroid status is necessary to achieve peak bone mass. Is this something that should be checked in the general population?

I don't think it is feasible to do this for several reasons. First, on an individual basis it is not possible to say when bone mass has peaked so it would be hard to measure. In the population it is estimated to be reached in the 3rd decade; there are numerous contributing factors of which thyroid hormones are just one. Much easier is to ensure that patients with thyroid dysfunction are treated promptly and efficiently to ensure normal thyroid status is restored as soon as possible. In this way detrimental effects of abnormalities of thyroid function can be prevented.

What advice would you give to a young person interested to develop a career as a clinician scientist?

A good question. Most importantly you need to have a love of science and a real passion for wanting to embark on a research career; it is hard work but immensely rewarding and most of all it is great fun. I think research takes over your life so it is critical to work on something that excites you and in a field that constantly challenges and asks questions. Seeking good advice and mentorship is critical at an early stage. The choice of department and laboratory in which to embark on research training is

very important and requires a lot of thought and advice. Fundamentally, a young trainee needs to find an environment suitable for them personally – some people like to work within a small "family" unit whereas other like to be within a large team. The choice of supervisor is important as you will need to ensure she or he has a strong training background and record of productivity. There also need to be opportunities for trainees to develop independence. In summary, do your homework, work hard, have a strong idea of how you wish your career to develop and most importantly choose a stimulating subject and environment. Most of all have fun – a career as a clinician scientist is tough but it is definitely worth the effort and you will meet great and inspiring colleagues, travel and have constant variety throughout your career.

What did you find interesting at ECTS 2018?

ECTS is a great society and ECTS2018 was a wonderful meeting – my favourite moments were of friendship, discussions with colleagues and new collaborators from all over the world in diverse fields (human genetics, clinical geneticists, rare diseases, mouse biologists etc), and opportunities to hear about new science. I particularly found the emerging technology around nanosequencing really impressive, I enjoyed the rare diseases workshop and was fascinated by the work linking skeletal biology to metabolism. Perhaps the most inspiring aspect of the meeting is the opportunity it provides for the next generation of clinicians and scientists through the ECTS Academy – it is fabulous to see such extraordinary and diverse talent coming though and long may it continue – our Society is destined to thrive with their future excellence and leadership!

Screening for fracture risk in osteoporosis

ECTS 2018
26-29 MAY 2018

We are now moving away from a single marker of bone mineral density in deciding when to treat patients for prevention of osteoporotic fractures.

In the ECTS Clinical Update, Eugene McCloskey explained how fracture risk models have been developed in Sheffield over the past 20 years, resulting in the free FRAX prediction tool designed mainly for use in primary care to estimate 10-year risk of fracture. This incorporates risk factors that are independent of BMD, identified in population-based cohorts from around the world. A recent comparison of FRAX with the other available fracture prediction tools Garvan and QFracture has showed that all three tools produce similar results, when compared in the same population. However, FRAX is the most widely validated tool and is recommended by NICE in the UK for targeting more detailed BMD assessments. As intervention thresholds are set at a national or regional level, FRAX provides direct access to the guidelines specific for each user's location. A single threshold does not accurately reflect real life, and age-dependent thresholds are defined in many countries.

The Sheffield group have recently completed the SCOOP study to quantify the benefit of using FRAX in screening for fracture risk, and the results were presented at ECTS. This has found a possible benefit of screening in relation to hip fracture, as patients who underwent active management as a result of risk assessment by FRAX had a 28% reduction in hip fractures over 5 years, with the effect most significant for those identified by FRAX at being highest risk who were particularly targeted for treatment. Nevertheless, for imminent fracture risk, a first fracture is still the best predictor of further fractures.

The ROSE trial conducted in Denmark evaluated a two-step population screening programme using FRAX. While there was no overall effect found on reduction of fracture incidence (intent-to-treat analysis), stronger evidence of fracture reduction was found in those returning the risk factor questionnaire, a similar self-selected population to that in SCOOP.

Also at ECTS 2018, Thomas Merlijn (Amsterdam) reported on how use of the FRAX screening tool has been evaluated in a prospective randomised trial in the Netherlands, but in this case found no significant association with reduced fracture risk. The SALT Osteoporosis Study recruited 11,131 women with at least one clinical risk factor for osteoporosis. The intervention group had bone densitometry and vertebral fracture assessment and their 10-year risk of fracture was calculated by FRAX, with those over an age-dependent threshold of fracture risk being recommended osteoporosis treatment. Follow-up data was completed for around 99% of participants. Time to a first fracture was the primary end point, and fractures occurred in 634 out of 5673 participants in the intervention group, and 639 out of 5542 in the usual care group. In contrast to SCOOP and ROSE, the study concluded no significant reduction in fracture risk as a result of screening. Further comparison between these studies awaits full publication of the SALT study.

Closing the treatment gap for osteoporosis

In the ECTS Big Clinical Session, Cyrus Cooper (Southampton, Oxford), President of the International Osteoporosis Federation, spoke about how we can close the treatment gap for osteoporosis.

Around the world, too many people who are at high risk of fracture and who could benefit from anti-osteoporosis therapies are not receiving treatment, despite their proven effectiveness. In the EU, it is estimated that 58% of patients eligible for treatment are not receiving it. The cost to healthcare systems of osteoporotic fractures has been estimated at over €37 billion per year (2010). Given also the major impact on quality of life and excess mortality associated with fracture, we have to do better in identifying and treating people with osteoporosis.

The World Health Organisation in 1994 defined osteoporosis as having a BMD with T-score <-2.5 SD from the young normal mean score, but this does not take into account absolute risk or prevalent fractures. In the UK, the NOGG guidelines for the treatment of osteoporosis have been updated to recommend a fracture risk assessment using FRAX, which in the UK includes age-dependent intervention thresholds. However, this still leads to an inequality in access to treatment for the over-70s, not taking into account prior fractures. FRAX in itself has been recently validated in the SCOOP study as an intervention to reduce risk of hip

fractures (see above) and was found cost-effective in a health economics analysis based on NHS costs. The impact of screening is highest for those at greater risk of fractures. Another current challenge is the poor adherence to the full range of anti-osteoporotic medications, with pharmaco-epidemiological research showing that many patients discontinue treatment within one year, even though there is evidence for the benefits of longer term treatment, as shown by a recent large scale study of the incidence of clinical vertebral fractures in postmenopausal women.

The IOF has developed the Capture the Fracture programme to spread best practice for secondary fracture prevention, primarily through implementation of effective Fracture Liaison Services (FLS). The regions of England that have the best outcomes for mortality after secondary fractures were found to be those where hospitals have set up or expanded FLS. The SCOPE project at IOF developed a scorecard for osteoporosis care in Europe that identified many gaps in provision and inequalities in access to diagnosis and treatment. Closing the treatment gap for osteoporosis is high on the agenda of the IOF and among ECTS members, and also within governments and health systems. Collaboration across these organisations and raising public understanding of osteoporosis are vital to improve treatment rates and outcomes for patients.

East meets West - Concepts for case finding in osteoporosis

ECTS congress has regular East meets West sessions organised with our partner associations from China, Japan and South Korea. In one East meets West session during the ECTS 2018 pre-congress day, approaches to case finding in osteoporosis were compared between Eastern and Western countries.

Presentations were given by **Ling Xu** (China), **Yoshiya Tanaka** (Japan), **Ha Yong-Chan** (South Korea), **Steve Cummings** (USA), **Eugene McCloskey** (UK) and **Claus Glüer** (Germany). All the countries represented have ageing populations and case finding is an important strategy for prevention of osteoporotic fractures. There are some similarities and differences in how case finding is performed in each country, depending on how the national health system operates and differences in national guidelines for diagnosis. For example, South Korea has a publicly funded programme of population screening for low BMD in selected age-groups. In 2018 everyone aged 54 or 66 years will be tested. In contrast, private health insurers in the USA don't provide full reimbursement of DXA measurements and as a result, these are not commonly performed.

The FRAX tool developed in the UK for risk assessment of osteoporotic fractures is already used in most other European countries, and around the world including in China. In the UK, FRAX is the first line assessment for women over 65 and men over 75, where a hip fracture has not already occurred. The approach for risk assessment in Germany developed by the DVO has some differences, with a wider range of clinical risk factors and focusing on hip and vertebral fractures as end point.

In China, lack of access to a GP is a challenge for case finding. The Chinese Win Over Osteoporosis strategy is developing a tiered medical service to direct those at risk of fractures to specialist care centres. Fracture Liaison Services (FLS) are developing in most of the countries represented. In the USA, individual hospitals organise their own FLS and it is not easy to know how many are operating, or how successful these are.

The round table discussion highlighted how the type of health system and reimbursements are one of the main factors for the success of a case finding strategy. Education of both GPs and the wider population is also essential for successful implementation. In this context, **Bente Langdahl** (Denmark) suggested that joining forces with other societies that deal with ageing related health issues may be advantageous. Instead of individual societies approaching governments, authorities and patients with suggestions about how to improve one aspect of ageing, they could join forces and promote general healthy ageing. The panellists largely agreed that this may make it easier for governments to understand the concept and provide funding for it. It would probably also be more meaningful for patients. Instead of going for DXA and diabetes screening etc. at separate clinics on different days, people could turn up at one clinic that could screen for several threats to healthy ageing.

The session finished with agreement that healthy ageing, with the important aspects of physical as well as mental mobility, is an important goal for society. Promoting healthy ageing will help to strengthen efforts to fight osteoporosis and other bone disorders in the coming years.

ECTS and ASBMR debate screening for fracture risk

ECTS 2018 26-29 MAY 2018

This year's ECTS/ASBMR debate was on the motion "This house believes that population-based screening to detect high fracture risk should be offered to older postmenopausal women".

The ECTS congress cast its vote before the debate: FOR: **60%** AGAINST: **23%** UNDECIDED: **17%**

FOR THE MOTION – Carolyn Crandall

Speaking in favour of the motion and representing ASBMR in the debate was Carolyn Crandall (San Francisco), a specialist in internal medicine whose research focuses on fracture risk in osteoporosis and osteoporosis treatment. Here is a summary of her main points:

- It is assumed for this argument that we should talk about screening older people, over the age of 65
- What is high fracture risk? Population-based screening could involve a clinical decision tool, and/or measurement of low BMD by DXA.
- We now have the SCOOP and ROSE randomized trials establishing the proof-of-concept of using the clinical decision tool FRAX for reducing fracture risk.
- While the UK SCOOP study did not find an association between the use of the FRAX-based screening intervention and risk of all fractures, the risk specifically of hip fracture was found reduced by ~30% where FRAX was used with its associated management changes. The FRAX-based screening intervention was also found to be a highly cost effective intervention that did not cause anxiety for the participants.
- In the ROSE study conducted in Denmark, evaluating a two-stage screening inviting participants to a DXA scan based on FRAX scores, the intent-to-treat analysis showed no significant difference between the intervention and control groups. However, the per-protocol analysis comparing the data on high risk participants that underwent DXA with those in the control group found a clinically statistically significant risk reduction for all major osteoporotic fractures, and particularly hip fractures.
- Detecting high fracture risk based on low BMD determined by DXA is more established in the USA. A recent systemic review shows that total hip and femoral neck BMD measured by DXA have AUC values that discriminate risk of hip fracture in women. There is now agreement among professional organisations to perform routine BMD testing in women aged 65 years and older. The rationale is that half of postmenopausal women will have osteoporosis-related fractures, and that the overall net benefit of screening is at least moderate, taking into account the impacts of fractures and the proven efficacy and low risks of bisphosphonates.
- We don't advocate putting osteoporosis medications into drinking water! There is no evidence of anti-fracture efficacy of drug treatments in people with BMD T-scores > -2.5. But ROSE and SCOOP are very promising trials. The costs of hip fractures are astronomical and we have effective drugs to treat those most at risk. We should therefore introduce population-based screening in older women, whether FRAX- or BMD- based.

To see video clips of the debate click on the links below

ECTS/ASBMR Debate	ECTS/ASBMR Debate	ECTS/ASBMR Debate	ECTS/ASBMR Debate	ECTS/ASBMR Debate	ECTS/ASBMR Debate	ECTS/ASBMR Debate
Introduction by the Chairs	Vote for the motion	Vote against the motion	Audience comments and questions	Rebuttal 1	Rebuttal 2	Final vote
▶	▶	▶	▶	▶	▶	▶

AGAINST THE MOTION- Eugene McCloskey

Speaking against the motion on behalf of ECTS was Eugene McCloskey (Sheffield) who has been involved in the development and validation of the FRAX tool for fracture risk prediction. Here is a summary of his main points:

- We need to look at what we mean by "screening". This is defined by UK National Screening Committee as "the process of identifying people who may be at increased risk of a disease or condition", to be followed by information, further tests and treatment to reduced associated risks and complications.
- A screening programme should have several key criteria in place as established by published literature since the 1960s, scored for this debate as green, amber or red for screening to detect high fracture risk. The condition should be an important health problem, which is clear in the case of osteoporotic fractures, and there should be robust evidence linking the measured risk or disease marker and the disease – this is "green". The test should be simple, safe, precise and validated, with suitable cut-off levels for the population – this is "amber". The intervention leads to better outcomes – this is "green". The screening programme must be shown effective in randomised clinical trials, good value for money and clinical, socially and ethically acceptable for patients and clinicians –this is "green"
- Implementation – you need have all "ducks in a row" so that the screening programme can be delivered and then patient management can be adjusted and resourced – at present this is "red" (see below)
- There are already several groups of patients that are already identified as being high risk, as the "low hanging fruit" – those already with a hip fracture receiving hospital care, fragility fracture patients (usually self-reporting) or those identified through case finding and served by Fracture Liaison Services.
- Resources and patient adherence to treatment are the real issues for implementation, we know that bisphosphonate therapy works to reduce non-vertebral fractures but the probability of patients receiving therapy 12 months after hip fracture has declined in the USA. Fracture Liaison Services have been shown to have a positive impact on the treatment of high risk patients but many areas of the world have no access to good FLS (see the East meets West session)
- In the ROSE trial, the problem was that the offer of DXA had a very low uptake, indicating that many people are simply not interested to engage. Non-participants tend to be older, physically impaired or have other issues affecting access or motivation. Eugene McCloskey emphasised here that the intention-to-treat analysis in ROSE showed no impact on fractures. While the SCOOP study is positive, the result is in a self-selected portion of the UK population and did not include people who were not interested to respond to the initial questionnaire.
- While universal screening of women over 65 years old has been recommended in the USA since 2002, the actual screening uptake is low, with less than 1 in 4 privately insured women being screened.
- So real-world implementation has to involve putting in place the necessary mechanisms, leadership and budget. The same factors affect how FLS and hip fracture care are performing less well than they should, and we must address those issues before introducing a new initiative. So this means the overall answer to the debate question is NO.

In rebuttal, Carolyn Crandall emphasised that the evidence obtained for effectiveness of screening is good enough. So we should aim to set up both the screening programme and obtain the necessary resources for implementation. Parallel efforts should ensure that clinicians can educate patients about the actual level of risk of osteoporosis treatment so that the uptake of treatment can be improved.

Eugene McCloskey concluded that the idea of screening is a good one, but we have to do it effectively to finally benefit patients. We also need to remember the interest of healthcare payers to ensure that funds are spent in the most cost-effective way and the criteria are not in place to implement a new population-based screening programme.

At the end of the debate, the final vote of ECTS congress:

FOR: **39%** AGAINST: **57%** UNDECIDED: **4%**

So Eugene McCloskey won the debate on behalf of ECTS with a dramatic swing of **34%**, and received the Golden Femur Award!

Bone and ageing

ECTS 2018
26-29 MAY 2018

ECTS 2018 featured sessions on how we should adjust osteoporosis care for older patients, especially important as the population ages, and the latest basic research exploring how ageing itself affects bone health.

Treating older osteoporosis patients

In the Clinical Update, Mattias Lorentzon (Gothenberg) reviewed the options for treatment of osteoporosis in the oldest patients, usually defined as those 80 years and older. Falls and frailty are major contributors to fractures in older patients, and exercise can help prevent falls. A meta-analysis of randomised controlled trials of fall prevention exercise, covering 4305 community-dwelling participants, found a significant 60% reduction in the risk of falls resulting in fractures, and also a reduction in risk of all injurious falls, though with substantial heterogeneity in the results from the 17 studies included. The largest trial so far to evaluate the benefits of exercise, the LIFE study, randomised adults aged 70-89 either to a programme of aerobic, strength, flexibility and balance training, or to a group participating in a health education programme. However, there were no significant differences found in serious fall injuries or fracture risk between the two groups over the mean follow-up of 2.6 years. Exercise is still believed to be beneficial, but this is not yet proven in the studies performed so far.

There is evidence from meta-analysis that calcium and vitamin D supplementation modestly reduces fracture risk in postmenopausal women (see here). In older people, low vitamin D levels and calcium intake can increase PTH and bone resorption. A study performed in 1992 in 3270 women living in nursing homes found that vitamin D3 and calcium supplementation reduced the risk of hip and non-vertebral fractures, continuing over 3 years.

While anti-osteoporosis treatments have been extensively studied in postmenopausal women, there are relatively few randomised controlled trials in those over 80 years old. As zoledronic acid can be given intravenously once a year, it is suggested as a suitable treatment for older people where compliance with more regular medication may be poor. A post-hoc analysis of fractures in over 1900 postmenopausal women aged over 75, treated annually with zoledronic acid, found significant reductions in clinical vertebral and non-vertebral fractures, compared to placebo groups, over 3 years. The risk reduction in hip fracture did not reach significance but this could be due to a low number of hip fractures. Similarly for denosumab, a post-hoc analysis of data from the FREEDOM trial of women aged over 75 years (as one of the high risk groups), found their reduction of risk for hip fracture was 62% compared to 40% the whole trial population. For alendronate, the most common osteoporosis treatment, randomised controlled trials have not included many patients over 80. However, in a cohort study recently performed in Gothenberg in patients over 80 years and who had a prior fracture, use of alendronate was associated with a 34-38% lower risk of hip fracture, compared to untreated individuals. Certainly, the absolute reduction in risk of fracture is greater in older versus younger patients, and older patients have a potentially greater benefit from treatment.

Cellular senescence

Cell senescence is a fundamental biological process, improving the efficiency of cell growth and proliferation during early development and wound healing. However, chronically persisting senescent cells are now thought to be a key contributor to many age-related diseases including musculoskeletal disorders, organ dysfunction, diabetes and Parkinson's disease. Cells enter a senescent state as a result of accumulated damage, but sometimes also as a response to telomere shortening or other modifications that can induce a damage response, such as oncogenic mutations. While the senescent cell no longer proliferates, it can secrete soluble factors that can negatively influence the local microenvironment.

In the ECTS 2018 Basic Science Update on cellular senescence, Peter de Keizer (Utrecht) discussed how cell senescence processes may be targeted in the future as a therapeutic strategy, effectively addressing the ageing process directly. As a proof-of-concept, Baker *et al* demonstrated the elimination of senescent cells in a mouse model using a suicide gene to induce their apoptosis, resulting

in an extension of lifespan by around 25%. A similar study (see below) has shown beneficial effects in reducing bone loss in a rapidly ageing mouse model. To develop therapeutic strategies further, it is important to understand the mechanism that stops senescent cells undergoing apoptosis, and if this can be switched with a new class of drugs ("senolytics") as the basis of an anti-ageing therapy. This hypothesis is being investigated in Peter de Keizer's group. They recently identified FOXO4, activated in senescent cells recruited to sites of chronic DNA damage, as a pivot for tipping them towards apoptosis via blocking its interaction with P53. A potential therapeutic strategy was demonstrated with a specially-designed peptide to interfere with the FOXO4-P53 interaction, and improve renal function and other signs of ageing in fast-ageing and naturally aged mouse models. As the peptide selectivity is still too broad for clinical translation, with the danger of affecting non-senescent cells, work continues to develop further peptides within a spin-out company.

Joshua Farr (Mayo Clinic) then discussed his research to target senescent cells, or their secreted factors (including in particular IL-6, IL-8 and PAI-1), to improve bone density during ageing. Investigations performed in mouse models of ageing have identified senescent cells with $p16^{Ink4a}$ expression in the bone microenvironment, especially in mice over 18 months of age. While several cell types were involved, myeloid cells and osteocytes were prominent in their contributions to the senescence-associated secretory phenotype (SASP). Further quantification of senescence-associated distension of satellite DNA by confocal microscopy showed higher numbers of senescent osteocytes in aged versus young cortical bone. Similar results were observed in bone biopsies from older as compared to younger women, indicating parallel findings in humans.

Several studies have been performed investigating small molecules to target senescence. The Farr group has published a study in a mouse model of bone loss during ageing, demonstrating the use of a suicide gene transposon or senolytic compounds to directly eliminate senescent cells, and the alternative approach of using a JAK inhibitor to target their proinflammatory secretome. In already aged mice aged 20-22 months, higher bone strength and bone mass, and improved bone microarchitecture were found in the treated mice compared to those treated with vehicle. Additional benefits were found for cardiovascular function, insulin sensitivity and reduced bone frailty. This study also examined the effects of SASP factors *in vitro*, finding that they increase osteoclastogenesis. These therapeutic approaches could therefore complement antiresorptive therapy for osteoporosis. The group is also currently investigating whether estrogen has a role in cellular senescence in bone.

ALSO PRESENTED AT ECTS 2018

Ageing-related bone loss is attenuated through Tgif1-ERK1/2 signalling in osteoclasts

Miki Maeda from UKE Hamburg presented results identifying Tgif1 as a novel regulator of bone resorption with an important role in ageing-related bone loss. Tgif1 knockout mice have a low bone turnover phenotype, and Tgif1 expression was previously associated to osteoclast differentiation. In this study, mice with Tgif1-deficiency specifically in osteoclasts were found to be protected from ageing-related bone loss at 8 months, due to reduced osteoclast number and bone resorption. Further experiments have suggested a mechanism where Tgif1 deficiency affects osteoclast differentiation and function, through a reduction of ERK1/2 phosphorylation, specifically via an increase in PP2A expression.

Androgen receptor in neurons is protective against age-related loss of cortical bone in male mice

Androgens and androgen receptor (AR) play an important role in male bone health, especially in cortical bone growth. In female mice, it has been shown that neural estrogen receptor affects cortical bone mass, so a team at KU Leuven investigated whether neural AR exerts a similar bone-regulation mechanism in males. They generated male mice with inducible AR knockout in the nervous system, with significant AR deletion in the brain stem and cerebral cortex, though not in the hypothalamus. Results presented by **Ferran Jardi** showed that young mice had no significant differences in cortical and trabecular bone parameters. However, at 36 and 46 weeks, significant age-related losses of cortical bone were observed, similar to the levels of bone loss in castrated mice with controlled testosterone levels. Both groups of mice at 46 weeks had increased levels of uncoupling protein 1 in brown fat, suggestive of an increase in sympathetic tone. Overall, the results confirm that androgen action in the nervous system is necessary to preserve male cortical bone during ageing.

Neutralizing FGF-23 restores the altered bone phenotype and delays the onset of anaemia in myelodysplastic mice

Myelodysplastic syndromes (MDS) are hematopoietic disorders affect mainly the over-70s and are thought to be caused by a dysfunction in the osteo-hematopoietic niche. MDS been recently shown by researchers at TU Dresden to be associated with a two fold increase in osteoporosis risk compared to age-matched controls. **Heike Weidner** presented their latest results investigating the possible cellular and molecular mechanisms of bone loss in a mouse model of MDS, testing mice aged 2 and 6 months, and in data obtained from MDS patients. A higher osteoid volume was measured in the mouse model and raised serum levels of FGF-23 at 6 months. Higher FGF-23 levels and osteoid volumes (x-2-4) were also measured in the MDS patients. Treatment of the MDS mice with a FGF-23 antibody improved bone homeostasis and erythropoiesis. Targeting of FGF-23 is therefore a promising new therapeutic option for MDS.

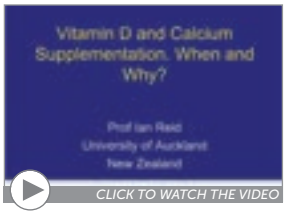
Heike Weidner's video was voted the winner of our Science Sketch competition.

Risk factors for bone disease

ECTS 2018
26-29 MAY 2018

The ECTS 2018 Clinical Updates described the latest understanding of risk factors for bone diseases and perspectives on diagnosis.

Vitamin D and calcium



In the Clinical Update, Ian Reid (Auckland, New Zealand) discussed the use of vitamin D and calcium supplementation for osteoporosis patients.

While there have been many studies performed on this question, two studies in particular give us clear evidence on who to treat, and when. The first clinical study was performed by Chapuy, *et al*. in elderly women in a residential setting. These women were severely deficient in vitamin D and a clear effect was found on risk of non-vertebral fractures. This finding is uncontested, that supplementation has a clear benefit in those with vitamin D deficiency. In contrast, a 2006 study in a much larger trial did not find evidence of an effect of vitamin D or calcium on fracture risk, but this was performed in generally healthy, community-dwelling women, so there is no actual conflict with the Chapuy study.

Recent IOF clinical guidelines now accept a differing approach for vitamin D and calcium supplementation for community dwelling individuals and those in residential care. Professor Reid discussed how further studies have made sense of these different outcomes. The beneficial effect of calcium supplements appears to be transient and quite small. In fact, a systemic review did not find an association between calcium supplementation and a reduced risk of fracture, except again in frail elderly women in residential care with low dietary calcium intake. The assessment of risks and benefits of calcium need to take into account also non-bone effects. Calcium supplements are associated with a risk of hypercalcuria and renal stones, and gastro-intestinal symptoms, which could interfere with other oral medications. A controversial meta-analysis by Prof Reid's group suggested a higher risk of myocardial infarction, at least in those who were not already taking calcium at the time they were randomized. Low vitamin D levels are certainly associated with osteomalacia, being also effective in osteomalacia treatment, and are now known to raise PTH levels. A recent UK study has determined what supplementation is required to normalise 25(OH)D and PTH levels in postmenopausal women. This does not usually require very high vitamin D supplementation. However, a further study has confirmed beneficial effects on BMD for vitamin D deficient women (< 30 nmol/L), but again not in the wider community-dwelling population.

Overall, for the general population the advice is to aim for sufficient dietary calcium intake (>500 mg/day) and maintain serum 25(OH)D levels. For frail elderly people especially in a residential setting, specific calcium and vitamin D supplementations may be appropriate, in order to reduce fracture risk.

In other news... A new meta-analysis of 33 randomized clinical trials of calcium and vitamin D supplementation in community-dwelling adults has again found no significant association between supplementation and a reduced risk of fracture. However, the authors did not rule out a possible benefit for people in the residential setting who have additional risk factors.

Diagnosis of secondary osteoporosis

Best practice for the diagnosis of secondary osteoporosis was presented in the Clinical Updates by Lorenz Hofbauer (Dresden).

Established causes of secondary osteoporosis include endogenous glucocorticoid excess or systemic treatment with glucocorticoids. This results in rapid bone loss that is only partially rescued if the cause is removed. In cases of a Fracture of Unknown Origin, some detective work is required to identify possible causes of osteoporosis. A rational approach will check personal and family histories and perform a full physical examination and lab workup including some specific tests related to known endocrine and hematologic diseases. Typical disorders that may be uncovered during this process include MGUS, undiagnosed cancers causing osteolytic bone metastases, or celiac disease. The German EOOP-DIMEOs consortium is performing Next Generation Sequencing in people under 50 years of age with unexplained early onset osteoporosis to identify the genetic factors and pathways involved.

Paget's disease of bone

The Clinical Update on Paget's disease of bone was given by Luigi Gennari (Siena, Italy).

The disease involves disordered bone turnover resulting from altered osteoclast differentiation and function, and has monostotic and polyostotic forms. The disease results in reduced bone strength, deformity, pain, osteoarthritis and a higher risk of fractures. It is not actually a rare disease, with around 1% of the population affected, and it is particularly prevalent in Great Britain. The pathogenesis of PDB is thought to involve a combination of genetic determinants and environmental factors, with potential environmental triggers including paramyxovirus infection, mechanical loading, exposure to environmental toxins, and contact with animals that could explain some local clustering. Interestingly, the prevalence of PDB symptoms in the hip has decreased since the 1940s, perhaps due to a change in lifestyles related to mechanical loading. The SQSTM1 gene mutation, observed in around 10% of sporadic cases and 40% of cases of the rarer familial PDB, has been identified as contributing to some other disorders of the cardiovascular system, brain and energy metabolism in humans and in mouse models, so is an interesting target for further research.

Many PDB diagnoses are made through incidental findings, e.g. from a skeletal x-ray, but over 40% of cases are diagnosed as a result of self-reported pain. Other patients are identified as a result of complications of the disease, which in addition to bone symptoms can include hyperparathyroidism, vascular calcifications, and PDB-associated giant cell tumour. Treatment with zoledronic acid has been shown to maintain bone turnover within the reference range for several years with a single dose. However, a long term comparison of intensive versus symptomatic treatment with bisphosphonates found that there is no difference in clinical benefits, and concluded that treatment should focus on controlling bone pain symptoms when they occur.

In other news... An integrated analysis of DNA-methylation and gene expression in a cohort of 80 postmenopausal women identified 22 methylation sites associated with variations in BMD. Combining the two sets of data improved their predictive performance.

Primary hyperparathyroidism

Richard Eastell (Sheffield) discussed the latest developments in the diagnosis and treatment of primary hyperparathyroidism.

The incidence of primary hyperparathyroidism peaked around 1973 as a result of improved diagnosis, however a new higher rate of diagnosis started with a peak occurring in 1998. This is suggested to result from the introduction at that time of new guidelines for osteoporosis management with more extensive screening, revealing also new cases of primary hyperparathyroidism. The condition presents in three "generations" of symptomatic, asymptomatic and normocalcaemic primary hyperparathyroidism. Symptomatic disease is now rare in Western countries, but is still common in developing countries such as India, where symptoms are often more severe due to vitamin D deficiency. As asymptomatic disease is more common in the West, and is under-diagnosed, a recent study has proposed use of machine learning to identify people with a mild level of disease, without human input.

A higher risk of vertebral fractures in patients with primary hyperparathyroidism has become apparent, without necessarily being accompanied by low BMD. Bone microarchitecture changes identified in primary hyperparathyroidism, including trabecular abnormalities, have been found to be partly reversible after treatment. There is also some evidence suggesting a reduction in fracture risk in treated patients, though not yet conclusive. However, long term improvement of BMD after parathyroidectomy has now been observed. It is currently debated whether surgery should be offered to all patients or taking a more selective approach should be taken. Potential new medications could include thiazides to safety reduce hypercalciuria in primary hyperparathyroidism, while denosumab has now been tested for the first time specifically in patients, actually with a greater increase in BMD compared with other osteoporosis patients.

ALSO PRESENTED AT ECTS 2018

Research into potential benefits of vitamin K2 in postmenopausal women with osteopenia

Vitamin K2 has been suggested to prevent bone loss, for example the higher vitamin K2 content in the traditional fermented food of northern Japan has been linked to the bone health of the population there. Vitamin K is certainly involved in bone biology as a co-factor in osteocalcin carboxylation which promotes bone mineralisation. Vitamin K2 has several sub-types with different chain lengths in the molecules and the MK-7 type found in fermented foods has been the focus of several clinical studies.

Sofie Hertz Rønn and colleagues (Aarhus) performed a 3 year double-blind study in 142 postmenopausal women who received daily MK-7 or placebo supplements, as well as calcium and vitamin D3. After one year, undercarboxylated osteocalcin was found to decrease by 65% in the MK-7 group, and some improvement in bone microstructure was found. However, at 3 years no significant differences in BMD were found between the MK-7 and placebo groups for any of the sites measured (total hip, femoral neck, lumbar spine. Changes in bone microstructure after 3 years were also similar between the two groups. The team are still completing analysis of bone biomarkers, so it will be seen if the previously observed effect on osteocalcin carboxylation was maintained for 3 years. The study has not found any long term benefit for BMD from MK-7 supplementation in postmenopausal women, but there may still be benefits for bone health in other groups, for example younger people.

How do high levels of exercise affect the risk of fracture?

Karl Stattin (Uppsala) presented an interesting epidemiological study on fracture risk involving data on participants in Vasaloppet, the world's largest cross country skiing race, to determine if very high levels of exercise affect fracture risk. Race participants are extremely active and also tend to participate in other endurance sports the rest of the year. Data on fracture incidence was obtained for almost 190,000 male and female race participants from national registers and Vasaloppet race participation during 1991-2009, finishing times and other race parameters were used as proxies for exercise. Registry data was compared with over 500,000 non-race participants from the general population. The overall analysis showed that race participants had lower rates of hip, proximal humerus and lower leg fractures, higher rates of forearm fractures and other less severe fractures, and no significant difference in vertebra fractures, compared to the general population. As the forearm fractures were incident throughout the year, the higher risk is not considered associated to the race itself. This study has now been published.

Organic matrix quality at actively forming trabecular surfaces is strongly associated with fragility fracture incidence independent of BMD and clinical diagnosis

Eleftherios Paschalis (Vienna) and colleagues have used Raman microspectroscopy to analyse bone biopsies from female patients with osteoporosis and children with rare bone disorders that had a history of fragility fractures, compared to healthy controls and women with a low BMD but no history of fragility fractures. They found that several organic matrix parameters were altered between the three groups, and identified the collagen cross-linking compound pyridinoline as a potential new marker for the risk of fragility fractures.

Structural geometry of bones is prominently associated with risk of fracture in children

Low BMD is an established risk factor for fractures in healthy children. Olja Grgic (Rotterdam) presented some new findings from the Generation R study performed in 1839 children. Whole body and hip DXA scans were obtained at the age of 6 years and 7.5% of the children experienced an incident fracture during the study. While BMD measurements were found associated with the risk of fracture, a stress index related to the quantity and distribution of bone in the femoral region was found a better predictor of fracture compared to BMD measurements.

Genetic basis of falling risk susceptibility

A higher risk of falling contributes to fracture risk, especially in the elderly. GWAS performed at Erasmus MC has indicated a genetic component in falling risk. Katerina Trajanoska presented results from an analysis of genetic variation in a large, population based cohort between 89,076 cases who had experienced a fall in the previous year, and 362,103 controls. This has identified two novel loci associated to fall risk, with only modest heritability. These were correlated to fracture risk, grip strength and other characteristics. The gene expressions are most highly enriched in brain cerebellum tissue, suggesting a connection to falls risk via postural control.


AUDITORIUM 1
AUDITORIUM 1
Bruker microCT
BRUKER

Clinical weight loss and bone

ECTS 2018
26-29 MAY 2018

The ECTS 2018 workshop on clinical weight loss and bone discussed how weight loss due to anorexia nervosa and bariatric surgery can have a negative effect on bone health, and the treatments being developed for affected patients.

Karen Miller (Boston) discussed some of the latest research to understand the mechanisms by which bone health is affected in anorexia nervosa. Around 85% of people diagnosed with anorexia nervosa experience osteopenia with some developing osteoporosis. Their risk of fracture is seven times that of normal women. In addition, female adolescents with anorexia nervosa are likely to reach a lower than average peak bone mass due to nutritional deficiencies and the effects of the disease on growth hormone and estrogen axes. The revised DSM-5 criteria for diagnosis of anorexia nervosa, published in 2013, included removing amenorrhea as being necessary for diagnosis and introducing a new definition of atypical anorexia nervosa for those with psychological symptoms who are not low weight.

A cross-sectional study performed by Dr Miller's group investigated bone loss in diagnosed patients under these new criteria. This has shown that a majority of women with atypical anorexia nervosa also have low BMD (Z□score <–1.0), with a higher proportion affected among patients with low weight. A small study in women with anorexia nervosa using quantitative CT imaging and DXA has identified an abnormal trabecular bone structure associated with endogenous IGF-I, leptin, and androgen levels, independently of body mass index. Also, mean lumbar and femoral bone marrow fat has been found to be higher in females with anorexia nervosa, inversely correlated to BMD. The possible influence of neuroendocrine factors on bone mass has also been investigated. While no direct relationships have been found between leptin and ghrelin levels and BMD, levels of Peptide YY, a satiating ligand expressed in the intestine and elevated in women and adolescent girls with anorexia nervosa, was found to be strongly associated with low BMD.

There are currently few treatment options to improve bone health in female adolescents with anorexia nervosa. Administering high estrogen doses as obtained by prescribing oral contraceptives does not improve BMD, possibly because of suppression of IGF-1. However, a regimen of low dose hormone replacement that mimics the levels that rise during puberty has been found to improve spine and hip BMD in adolescents. Also, girls with anorexia nervosa need to catch up in BMD with their age group, and hormone replacement therapy on its own is not sufficient to achieve this. As a result, various bone therapies have been investigated, including testosterone (often suppressed in both female and male patients), recombinant IGF-1 and teriparatide, and also bisphosphonates. While some positive results have been obtained in clinical studies, as yet no treatment guidelines have been developed. It's clear that a lot more research is required to fully understand bone loss in anorexia nervosa and develop effective therapies.

In the second talk, Elaine Wu (Boston) discussed the impact of bariatric surgery on bone health. Bariatric surgery is now established as a treatment for severe obesity, with dramatic weight loss and improvement of co-morbid conditions such as diabetes. However, less than 1% of eligible patients elect to undergo treatment, and there are several concerns including the long term effects on bone fragility. The three kinds of surgery are Roux-en-Y gastric bypass, gastric banding and sleeve gastrectomy, and these procedures have different effects on bone.

There are practical challenges in DXA imaging of severely obese patients as a result of soft tissue artefacts, but QCTand HR-pQCT have proved more effective in providing clearer image quality and useful data on bone microstructural parameters. Analysis performed in patients who had received a Roux-en-Y gastric bypass two years previously found a loss of BMD by 5-7% at the spine and 6-10% at the hip and deterioration in bone microarchitecture, with a 65% increase in cortical porosity and an elevation of bone turnover markers. A recently published, slightly larger study found significantly worse effects in postmenopausal women. Other recent studies have now analysed bone turnover markers up to 5 years after gastric bypass surgery. A study in type 2 diabetes patients treated by a gastric bypass found a significantly higher bone turnover in these patients compared to those that did not receive surgery, which was correlated to increases in PTH levels. Elaine Yu has recently completed a longitudinal study of 21 adults with a gastric bypass, and has measured progressive loss of BMD and declining bone microarchitecture at multiple skeletal sites throughout

the five year period, though the majority of bone loss does occur in the first two years. Changes in fasting peptide YY have been correlated to increases in CTX in gastric bypass patients, suggesting one possible gut/bone connection. Concerning adjustable gastric banding, there are fewer studies with mixed results. Certainly the effects on bone remodelling are minimal compared to gastric bypass, and as the extent of weight loss is reduced, its difficult to determine an effect independent of weight loss when comparing the two treatments. Sleeve gastrectomy has not been much studied for its effect on bone, but a randomised trial performed in type 2 diabetes patients who received sleeve gastrectomy showed comparable weight loss and declines in hip bone density to gastric bypass, over 2 years. Decreased mechanical loading from the weight loss itself could explain some of the bone loss in the first two years after surgery, but the effects of surgery on the systemic hormone and metabolic profile are likely to be more important in the longer term, even a potential effect via changes to the gut microbiome.

As obese people tend to have higher than average bone density, there remains a question whether the bone loss observed in bariatric surgery patients would actually raise the risk of fracture. The various studies so far performed do not give a clear answer, but they did not discriminate between the different kinds of surgery. Elaine Yu and colleagues therefore investigated bariatric surgery and fracture incidence data in a US health insurance claims database, and found a 43% higher risk of clinically important, non-vertebral fractures among gastric bypass patients compared to those who had received an adjustable gastric band, as analysed more than two years after surgery.

Further studies with longer follow-up are definitely required, including in sleeve gastrectomy recipients. Possible interventions to reduce bone less in bariatric surgery patients could include exercise and multiple nutritional supplements, which have been found to prevent some, but not all bone loss. Certainly, adequate vitamin D and calcium supplementation (calcium citrate) is essential for bariatric surgery patients. There have been no trials to date of pharmacological interventions in bariatric surgery, and clinical guidelines are based on expert opinion. In fact Dr Yu advises against the use of oral bisphosphonates in these patients due to concerns about their absorption and erosions at the anastomotic site.

Overall, an open discussion with patients about the benefits and risks of surgery is essential, so they can make an informed decision about treatment. Bone density scanning is recommended for those at higher risk of osteoporosis before treatment goes ahead, and in all patients following treatment.

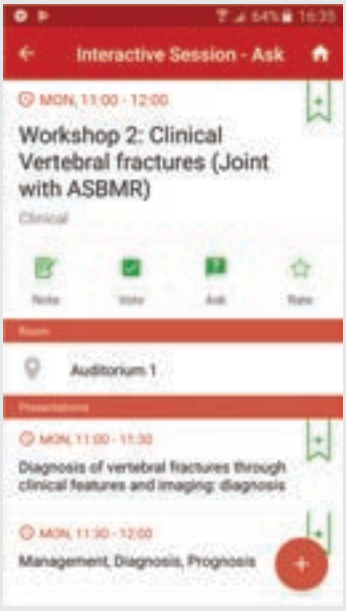
Management of clinical vertebral fractures

ECTS 2018
26-29 MAY 2018

The joint ECTS/ASBMR workshop on clinical vertebral fractures piloted use of the ECTS congress app for questions to be registered during the talk, with those "liked" the most being selected for answering by the speakers.

Emma Clark (Bristol) discussed diagnosis of vertebral fractures through clinical features and imaging. Well-known risk factors for osteoporosis, such as age, smoking and steroid use, are even more prevalent in patients who have experienced vertebral fractures, but these patients are clinically under-diagnosed by as much as two thirds. While many vertebral fractures are asymptomatic and not clinically recognised, the incidence of vertebral fractures has been associated with increased back pain. Meanwhile, lateral back pain has been found to identify postmenopausal women at higher risk of prevalent vertebral fractures. Negative attitudes towards pain among health professionals and patients themselves, making them less likely to identify the need for an X-ray, may contribute towards this under-diagnosis.

Dr Clark has set up the VFRAC Study to better classify the type of back pain experienced as a result of vertebral fractures, working with study participants to hear how patients themselves describe their pain, as well as collecting other clinical data. The eventual aim of the study is to develop a new predictive tool and a checklist for general practitioners. Height loss is also a predictor of vertebral fracture but the Bristol team has concluded that other risk factors are probably more important, such as age or experiencing a fall.

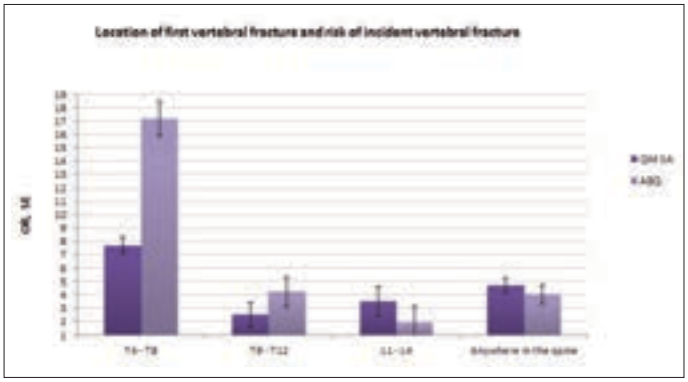


For radiologists, interpretation of images can be made more complicated by normal variants of spine anatomy or non-osteoporotic conditions that can be easily mistaken for vertebral fractures. Even a simple rotation of the spine can appear in some cases to suggest a vertebral fracture. Emma Clark finished her presentation by emphasising that radiologists should understand the importance of reporting all vertebral fractures they observe, so that we can identify moderate and severe fractures for possible treatment, and that communicating this clearly in reports by specific use of the term "fracture" will ensure that the primary care professionals will take appropriate action.

John Schousboe (Minneapolis, Minnesota) then discussed further the clinical consequences, management, diagnosis and prognosis of vertebral fractures. Studies in postmenopausal women and also older men show that sub-clinical vertebral fractures identified only through radiography are nevertheless associated with a higher risk of new back pain that sometimes severely affects activity. Lumbar spine pain can have an impact as severe as hip fracture, for example requiring extended bed rest.

The impact of vertebral fractures increases with the number of fractures experienced, and data from clinical trials shows that a cascade of fractures can often develop at all levels of BMD. John Schousboe explained, "Vertebral fractures can increase kyphosis, and limit mobility leading to further loss of bone mass, muscle strength, and impairment of balance; these in turn can increase the risk of additional vertebral fractures." We don't have specific studies yet on this hypothesis, but it is generally accepted that limitations of physical activity affect muscle strength and other physical and mental function, and bone strength is known to be lost in immobilised non-weight bearing legs comparing to the weight bearing limb of the same person. Kyphosis progression has been described as a risk factor for subsequent vertebral fractures. Biomechanical issues leading to increased vertebral compressive load are likely to be behind this association.

Anti-osteoporotic treatments are now well established to reduce the risk of further vertebral fractures, with denosumab and intravenous zoledronic acid being particularly effective and fast-acting. While a short rest period is helpful for the worst clinical vertebral fractures,



Location of first vertebral fracture and risk of incident vertebral fracture (F. Koromani)

we need to get all patients mobile as quickly as possible and into a rehabilitation regimen combining physiotherapy and education. Many designs of brace are available. There is limited quality data on their clinical effectiveness but considering the urgent need for patients to regain their mobility, Dr Schousboe does make use of soft and then hard braces if required. The further treatment option of vertebroplasty was recently evaluated by a meta-analysis of clinical trials. Here, no significant effect of vertebroplasty on pain reduction was found when compared to sham treatments in blinded trials, however in open label trials there appeared a significant benefit compared to usual care. Dr Schousboe still considers this a useful treatment option for a small proportion of patients. Exercise recommendations tend to combine strength and balance training and avoiding extremes of flexing and rotation — postural alignment and ergonomic advice is an important part of rehabilitation.

Vertebral Fracture Assessment

In her contribution to the ECTS Clinical Update, Emma Clark discussed further the approach taken at Bristol for Vertebral Fracture Assessment with lateral DXA scanning, when evaluating for osteoporosis and fracture risk.

Emma Clark highlighted how DXA scans of the spine can be hard to interpret. Mild vertebral fractures are unlikely to be a result of osteoporosis, whereas moderate or severe vertebral fractures can be predictive of hip fracture. Considering the extra time required from the radiologists for Vertebral Fracture Assessment, it is not considered appropriate to include it with every DXA scan, when the majority of the vertebral fractures are mild and this time could be used more beneficially for other patients. At Bristol, selection criteria for Vertebral Fracture Assessment target the most at-risk patients - those already diagnosed with osteoporosis, females over 70 and males over 80 years of age. As a result the proportion of patients under active management in the Fracture Liaison Service has increased from 3 to 10%, and the rate of vertebral fractures has been found to be reduced by a third in these patients.

ALSO PRESENTED AT ECTS 2018

Location of first spinal fracture as determinant of future vertebral fracture risk

Fjorda Koromani (Rotterdam) presented new analysis of vertebral fracture risk in the Rotterdam study. Vertebral fractures were scored with two different scoring methods, quantitative morphometry assisted by SpineAnalyzer (QM SA) and algorithm based qualitative (ABQ) method. The analysis showed, as already established, that people with prevalent vertebral fractures had elevated risk of further vertebral fractures, but that the location of the first fracture made a difference to the risk of incident vertebral fracture. In fact, a fracture located at the mid-thoracic region (T4-T8) was strongly associated to risk of incident vertebral fractures. The lower the first vertebral fracture was located in the spine, the less strongly it was associated with incident vertebral fracture. T8 fractures were significantly and strongly associated to risk of further incident vertebral fractures, independent of BMD, in both sexes, and with both scoring methods, but more strongly with ABQ. This suggests that special attention should be given to fractures at the mid thoracic level.

ECTS Phillipe Bordier Award

The **ECTS Phillipe Bordier Award** for a significant clinical contribution to the field of bone and calcified tissue was given to Eugene McCloskey, who is Professor in Adult Bone Disease and Honorary Consultant at the University of Sheffield. He has focused in his research on the diagnosis, epidemiology and prediction of fracture risk in osteoporosis, and is an expert in vertebral fracture definition. He has been a key person behind the development of the FRAX tool that is now coming into use worldwide for the prediction of 10-year fracture risk based on clinical risk fractures and BMD measurement.


ECTS 2018
AUDITORIUM 2
& Speakers' Preview Center
AUDITORIUM 1
International Conference on Children's Bone Health (ICCBH)

Exploring the human microbiome

ECTS 2018
26-29 MAY 2018

In the ECTS 2018 educational symposium on microbiome research, **Jan Knol**, R&D director at Danone Nutrient Research and Professor at Wageningen University introduced us to the role that gut health and the microbiome are now known to play in our overall health.

It has long been known that gut bacteria assist our digestion, and additional functions beneficial to health are now being discovered. The typical human gut contains around 100 million bacteria, with 5 main phyla represented among around 1000 bacteria species. Many of these microorganisms are unculturable, so there has been a strong focus on developing metagenomics to describe the functions of the microbiome. As a result, around 3.3 million unique genes are identified in the human gut, many more than in the human genome itself.

The proportion of the different phyla of the microbiome have been found distinctive for different stages of life, for example in newborns, older children and adults, with bifidobacteria more dominant during childhood, and there are typical distributions also observed relating to diet and antibiotics use. There are differences observed in the microbiomes of infants that are delivered by caesarean section compared to vaginal delivery, and also between breastfed and formula fed infants. Though these differences even out later in childhood, there is interest in the health benefits of a more natural gut microbiome profile during the early years. A trial of synbiotic bacteria in infants delivered by caesarean section has been recently shown to increase bifidobacteria levels, emulating more the gut microbiome of vaginally born infants.

A big question is whether microbiome changes are a causative factor in some diseases, or whether instead the gut microbiome population is affected by a disease state. So far, studies have focused on gut bacteria, but the contribution of resident fungal microorganisms is hardly investigated, and metagenomic analysis revealed the presence of completely new viruses, so there is much more to be investigated and understood over the coming years.

Klara Sjögren (Gothenburg) then presented her exciting work on the role of the gut microbiome in regulating bone mass.

Her work in this area started in 2009 with a hypothesis; as the gut microbiome was known to affect the immune system, there could be therefore a systemic effect via the immune system on bone health. Experiments in germ free mice found reduced numbers of osteoclasts and increased bone mass, reduced frequency of CD4(+) T cells and osteoclast precursor cells in bone marrow, which was normalised with gut colonization. The germ free mice also had decreased expression of inflammatory cytokines in their bone tissue.

More recent studies by Dr Sjogren's group in germ free and colonized knockout mice, with targeted inactivation of signalling pathways involved in the innate immune system, identified the effect of gut microbiota on bone mass to be dependent on NOD1 and NOD2 signalling. However, another study in germ free mice from a different genetic background found a quite different effect of decreased bone growth and reduced cortical thickness, while a further study identified an early increase in bone resorption in the first month after recolonization, that was eventually compensated by increased skeletal growth and eventually higher bone mass at 9 months. It is likely that several factors related to genetic background, sex, diet and colonization time may explain the variability of results from these studies.

It is now more interesting to use physiologically relevant mouse models with intact gut microbiomes to investigate the effects of various diets, pro- or prebiotics, and antibiotics, on bone mass via this mechanism. Studies in Gothenberg and elsewhere show that probiotics protect mice from ovariectomy-induced cortical bone loss. While a greater intestine permeability as a result of estrogen deficiency should increase exposure to antigens and activating the immune system, probiotics appeared to reverse this effect. However, there is evidence (unpublished results) of some types of probiotic bacteria having negative effects on bone, even if they have other beneficial effects. At Clinical.trial.gov there are three randomized clinical trials of probiotics for their effects on bone health registered: one trial in older women with osteopenia and two in early postmenopausal women, with press releases so far being positive. **The results from the study in older women have just been published** and showed that in the group supplemented by Lactobacillus reuteri the loss in bone density was halved compared to placebo. This work is pioneering the new field of "osteo-microbiology", which is highly multidisciplinary, and may yield completely new therapeutic approaches for the prevention of osteoporosis.

ECTS Fellowship Award

The ECTS Fellowship Awards are one-year awards to support research in the bone field by New Investigators. The ECTS Fellowship clinical award was received by Dr Manuela Schoeb, who has started a new position at Leiden University Medical Centre. She is developing a prospective study on the bone material strength index obtained the "Osteoprobe" impact microindentation device, as a surrogate marker for determining fracture risk and investigating the response to therapy for osteoporosis and hyperparathyroidism.

ALSO PRESENTED AT ECTS 2018

Gut microbiome composition is associated with bone mineral density levels in healthy children of school age

Carolina Medina-Gomez (Rotterdam) presented results from the prospective Generation R Study, to identify associations between gut microbiome composition and BMD in healthy children. In 2111 Generation R children, total body BMD was obtained by DXA and 16S rRNA microbiome profiles determined in stool samples at age 10. As expected, Firmicutes were the most abundant group in the analysed microbiomes. In general in this pediatric cohort, relative abundances of 6 taxonomic units belonging to *Enterococcae*, *Eryspelotrichaceae* and *Lachnospiraceae* families were associated with higher BMD, whereas *Hungatella* was associated with low BMD. As 16S sequencing does not have strain level resolution, it is not possible from this data to identify the specific species that are involved in the association. However, various strains of *Enterococcus faecium* are found in commercial probiotic products, and these have been shown to increase bone strength in animal models. Replication and extension of these results will help increase our understanding of the gut microbiome as an essential contributor to bone health.

Meet the Expert - Microbiome

During this session Carolina Medina-Gomez gave us an insight into measuring and interpreting the microbiome from a statistical point of view.

Dr Medina-Gomez highlighted the two most used methodologies to profile the microbiome: through metagenomics or 16S rRNA. Metagenomics is expensive but provides high resolution at the taxonomical level and allows the detection of non-bacterial microorganisms (such as fungi and viruses) and importantly allows a direct assessment of the genes present in the microbiome. Alternatively, 16SrRNA is cheaper but can only detect bacteria and archaea, has limited taxonomical resolution and the functional potential assessment will depend on the known information for the identified bacteria.

In this fast developing field there is still not a 'golden' method for statistically analysing the microbiome, although different alternatives have been postulated and were presented during the session. The complexities in microbiome analysis are defined by its dynamic nature alongside the high dimensionality, non-normality and phylogenetic structure of the zero-inflated data. Dr Medina-Gomez explained that the most important thing is to understand the assumptions and possible bias introduced by the different methods. This will enable you to justify the choices you made for normalization and analysis of your data, and thus, provide reliable foundations upon which the final output can be built.

After the congress we asked Dr Medina-Gomez to explain more about the techniques involved in studying the microbiome.

How do the latest techniques of metagenomic analysis improve on what available previously for study of the gut microbiome?

At the wet-lab level, there is a huge worldwide effort to try to standardize the methods to ensure that you can combine your data with other labs and the results should be comparable. This seems simple but is not, there are many variables such as how the samples are collected, which reactives are used to extract the DNA, at what temperature the samples are stored. And as the data is so dynamic, all these variables can affect your microbiome profiling.

Regarding the analysis, in order to normalize your microbiome data you need to analyse in terms of "relative abundances" that each bacteria proportion is dependent from the proportion of the other bacteria present, and at the statistical level this can generate several issues.

Let me explain it with an ordinary example. I am investigating if doing exercise has an effect in health, and I have data on when people are active, when they are sedentary (like sitting in the office working, watching TV...) and the time they sleep. So if I see an association with health and the hours that someone do sports... is it actually the association with the time that that person exercise, or that that means that they are spending less time sitting, or that they sleep less? As these components are not independent, it is hard to unequivocally define which factor is the most important. New methods allow you to apply transformations to make these components independent from each other, but these have other types of assumptions.
Other things that are appearing more and more in literature are machine learning approaches to find the association of microbiome profiles and health outcomes.

What are the steps involved in the metagenomics analysis of human microbiome samples. Is there anything technically challenging about it?
First step is the collection of the stool. In large studies, the logistics for this collection and transport to the research centers is quite cumbersome. Then you need to extract the DNA, following by the sequencing (in the case of 16S you have different standard options for the various regions of the genome you want to sequence, and that choice can alter greatly the bacteria you will find). Then you need to do lots of bioinformatics to try to exclude all possible sequencing artifacts, cluster the data and possibly use a reference to do a taxonomical assignment. Here, depending on the reference you use also you can get different results.
Finally comes the data analysis, as I explained in the previous question. I think right now that harmonization of the methods is what is more challenging. We need to agree in methods internationally and harmonize the data to be able to use it efficiently. Since the beginning the microbiome research community has been really open, so almost every publication has made the data available, unfortunately all the issues mentioned above make its comparability difficult and is almost unthinkable to merge all these data to get larger statistical power.
Also, I think in the future the analysis will focus in the whole system, and not analysing one bacteria at a time, as done until now. Although this model is simple and easy to assess and interpret, it overlooks how these bacteria communicate, cross-feed, recombine, and coevolve. I was in a conference last week and was amazed by the amount of studies already talking about "consortia" in probiotics, instead of probiotics using just one specific strain.

How do you communicate about the study to the Generation R participants, e.g. about what you are doing in the analysis and about the results?
Unfortunately, we do not have rights to contact the participants of the study, although there are information meetings in which they can inform themselves (their parents) of what we will do with the samples and why this might be important. All analysis is performed using sample IDs which cannot be connected to any personal information of participants by any of the researchers.

New data on efficacy of osteoporosis therapies

ECTS 2018
26-29 MAY 2018

Results on the efficacy and mode of action of both antiresporptive and bone anabolic osteoporosis therapies were presented at ECTS 2018.

Antiresorptive therapies

Zoledronate every 18 months for 6 years in osteopenic postmenopausal women reduces non-vertebral fractures and height loss

Around 80% of fractures in postmenopausal women occur in those with osteopenia, but the efficacy of bisphosphonates for this group is not established. **Ian Reid** (Auckland, New Zealand) presented results from a double-blind trial involving 2000 post-menopausal women aged > 65 with osteopenia, randomly assigned to treatment with zoledronate or normal saline at 18 month intervals and followed over 6 years. Monthly vitamin D supplementation was maintained. In the zoledronate group, the rate of non-vertebral fractures was a third less, there was a significant reduction in height loss as a proxy for vertebral fractures, and overall mortality was also reduced. No reports were received of osteonecrosis of the jaw or atypical femoral fractures as potential adverse events previously associated to bisphosphonates use. The study suggests that treatment of women with osteopenia with bisphosphonates is effective and the study team are keen for their findings to be replicated.

Richard Eastell (Sheffield), a clinician not connected to the study, told us "This is a first for zoledronic acid and gives us a rational basis for using the drug in patients with osteopenia. The trial did not use supplemental calcium, so this might justify just giving supplements of vitamin D alone. Also, the zoledronic acid can be given every 18 months, rather than the current regimen of every 12 months, which would be more convenient for the patient, and possibly safer. Finally, the trend to reduced mortality is interesting, as a similar finding was reported also by Lyles, et al. in 2007."

In other news... Many other new studies have been published on denosumab in the past year. Data on the extension of the FREEDOM trial to ten years has shown continued lower fracture risk with denosumab and continued steady increases in BMD. It has been observed that most of the gains in BMD can be reversed within 12 months after cessation of denosumab therapy. Horne, et al. have published results from a study of postmenopausal women offered oral or intravenous bisphosphonate therapy after denosumab. They found that bisphosphonates helped to protect against further BMD loss to some extent, with alendronate being most effective, especially if given after a short delay following cessation of denosumab. Denosumab has also been investigated in comparison to zoledronate in a phase 3 study for treatment of multiple myeloma. The results determined denosumab to be non-inferior to zoledronate in terms of the time until a first skeletal-related event (bone lesions or metastases), providing a new treatment option for multiple myeloma patients.

Denosumab treatment improves bones hardness accompanied by lower osteocyte viability persisting during drug-holiday

Interesting effects of antiresorptive denosumab treatment on osteocyte viability and bone hardness have been found by researchers in Germany. **Katharina Jähn** (Hamburg) reported on an analysis of iliac crest biopsies collected from 25 postmenopausal women receiving denosumab, 10 on drug holiday from denosumab, and 11 age-matched osteoporotic controls. The denosumab-treated bone samples showed significant higher bone hardness compared to age-matched controls, while trabecular BV/TV was reduced in the drug holiday group compared to those still receiving denosumab. However, the number of empty osteocyte lacunae was significantly higher in the denosumab and drug holiday groups compared to the osteoporotic controls, suggesting a reduction in osteocyte viability.

Bone matrix mineralization after denosumab treatment discontinuation

Georges Boivin (Lyon) presented results from a cohort study of 15 postmenopausal female subjects who received 12 months of denosumab in one of two parent clinical trials and agreed to have bone biopsies ≥12 and ≤36 months off-treatment. The aim of the study was to determine changes in bone matrix mineralisation in various bone compartments after discontinuation of treatment. Bone biopsies from participants in the FREEDOM trial were used as controls. The results showed a similar degree of bone mineralisation as during denosumab treatment throughout the period of discontinued therapy (mean of 25 months), both for total bone and in all bone compartments. Heterogeneity index

was higher compared to the controls still receiving treatment at 2 and 3 years, suggesting a transient increase in bone remodelling after the end of treatment.

Denosumab reduced bone remodeling, eroded surface, and erosion depth in cortical bone of iliac crest biopsies from postmenopausal women

Further results presented from the FREEDOM trial by **Pascale Chavassieux** (Lyon) focused on the effects of denosumab treatment on cortical bone histomorphometry. Iliac crest bone biopsies obtained at months 24 and 36 in the trial were analysed to characterise the cortical eroded surface, including an automated-interactive image analysis of erosion depth developed previously in Lyon. The analysis found that eroded surface and osteoclast number per bone surface area, and mean and maximal erosion depth were all lower in the denosumab group versus placebo. However, no significant differences were found for endocortical wall thickness, cortical porosity or cortical thickness. These differences in cortical bone could contribute to the reduction in fracture risk associated with denosumab compared to placebo in the FREEDOM trial.

Denosumab compared with risedronate in glucocorticoid-treated subjects

Kenneth Saag (Birmingham, Alabama) presented results from a head-to-head multi-centre study of denosumab versus the bisphosphonate risedronate, to determine their effects on BMD in patients receiving glucocorticoid (GC) medication. GC use is the second most common cause of osteoporosis as a result of the pharmacological side effects on bone metabolism, which are still not completely understood. The randomised study has tested both treatments in patients receiving GCs who at enrolment were either continuing long term GC treatment (> 3 months) or who had recently initiated treatment (< 3 months). The primary endpoint results at month 12, already published and presented at ECTS 2017 indicated that denosumab was both superior and non-inferior to risendronate in its effects on BMD in the lumbar spine, in both groups. Now at 24 months, 590 out of 795 participants have completed the study. Measurements of hip and spine BMD showed that denosumab was superior to risendronate in both groups, particularly for hip BMD where risedronate remained close to baseline. Adverse events were similar between both groups. The study was not sufficiently powered to analyse fractures as a primary endpoint and so larger studies will be useful, as well as other head-to-head comparisons of denosumab with other antiresorptive drugs for GC-receiving patients.

In other news... Several studies in the past year have investigated how to improve muscle functioning, an important factor for fracture risk in elderly people alongside bone health. Bimabrumab, a new treatment for muscle loss and weakness, is being evaluated in 16 people with insulin resistance. By week 10, lean mass was increased while fat mass and insulin sensitivity were reduced. Villareal *et al* have conducted a study in 160 obese older adults comparing the effects on physical performance of different exercise regimes and participation in a weight management program, also measuring body composition and BMD. A combined aerobic and resistance exercise with weight loss was found most effective in improving functional status.

Denosumab improves muscle function and glucose homeostasis

Denosumab is available as an antiresorptive treatment for osteoporosis but is also being evaluated for improvement of muscle function in Duchenne Muscular Dystrophy. As RANKL is expressed also in the muscle and in the liver, **Nicolas Bonnet** described how his Geneva group has investigated denosumab treatment for its effects on muscle strength and glucose homeostasis. They have tested treatment versus placebo in mice overexpressing human RANKL with severe osteoporosis and reduced muscle strength, and in a group of women from the GERICHO cohort - 18 postmenopausal women around 65 years of age receiving denosumab, matched to 49 controls. In the mouse model, denosumab increased limb force and normalized ITT and liver glucose production. In the human subjects, treatment maintained appendicular lean mass and handgrip, while these decreased in the control group. These results suggest that denosumab may contribute to reduced fracture risk, not only via antiresorptive activity, but also through maintaining muscle strength.

Anabolic and sequential therapies

In the ECTS 2018 Big Clinical Session, **Lorenz Hofbauer** (Dresden) discussed the latest developments in anabolic (or bone-forming) and sequential therapies.

While there are many approved anti-resorptive therapies, only one anabolic therapy teriparatide has been approved so far, hence there is a major unmet need for additional bone anabolic agents. Two new bone anabolic therapies have completed phase 3 clinical trials and are undergoing regulatory approval.

Based on research into inhibitors of Wnt signalling pathway, including in studies of rare diseases with activation of osteoblast differentiation, sclerostin was identified as a target for bone anabolic therapy. Romosozumab as an anti-sclerostin therapy has been shown in the FRAME trial in postmenopausal women to produce a rapid increase in BMD at the lumbar spine and hip within the first six months, followed by a more gradual increase. Following up with an anti-resorptive agent is generally agreed to help consolidate the gain of BMD, and in the FRAME trial denosumab was administered in the second year.

The more recent ARCH trial in postmenopausal women investigated 12 months treatment with romosozumab followed by 12 months on alendronate, in comparison with continuous treatment using alendronate. It showed a greater gain in BMD and a significant reduction in incidence of new vertebral fractures in those receiving romozozumab. Notably, romosozumab exerts a dual mode of action that includes an antiresorptive effect. Some cardiovascular adverse effects observed in the trial are currently being investigated.

Peptide fragments of parathyroid hormone-related peptide (PTHrP) are being developed to obtain a bone anabolic effect without the hypercalcaemia associated with excess PTH. Abaloparatide, a synthetic analogue of PTHrP has been studied in the phase 3 ACTIVE trial against placebo and teriparatide over 18 months. In postmenopausal women with a prior osteoporotic fracture, abaloparatide therapy resulted in a highly significant reduction in incidence of vertebral fractures (-86%) compared to placebo and was associated with slightly better outcomes for major osteoporotic fractures than teriparatide, also with less cases of hypercalcemia. An extension of this trial with alendronate given to all participants again showed maintenance of benefits of bone formation. However, PTH receptor agonists are contraindicated for cancer patients.

A further study of sequential therapy, the DATA-Switch randomised controlled trial investigated different combinations of teriparatide and denosumab. It was shown that prior treatment with denosumab blunts the bone-forming effect of teriparatide. Few current cases are treatment-naïve and many will have already received anti-resorptive agents, but its now generally agreed that bone anabolic treatment should ideally be taken first, when considering sequential therapy.

ALSO PRESENTED AT ECTS 2018

Effects of romosozumab in postmenopausal women with osteoporosis on iliac crest bone biopsies after 2 and 12 months

New data from the FRAME trial in women with postmenopausal osteoporosis was presented by **Pascale Chavassieux** (Lyon), showing how romosozumab acts on bone formation in different bone compartments. Iliac crest bone biopsies were analysed by microCT and histomorphometry after 2 and 12 months of treatment by romosozumab or placebo. By month 2, the ratio of bone formation rate/bone surface with romosozumab was increased by > 3-fold in the cancellous bone and >2-fold in the endocortical bone, and trabecular separation was decreased. Over 12 months treatment, a sustained decrease in bone resorption with increases in both trabecular and cortical bone thickness, bone mass and improvements to bone microarchitecture were observed.

Early increases in N-terminal propeptide of type 1 procollagen (P1NP) with romosozumab therapy as an indicator for BMD response

Cesar Libanati (UCB, Brussels) reported a sub-study performed within the STRUCTURE trial (see also below) to determine if measurement of early changes in P1NP levels could be a useful predictive marker of BMD response to treatment with romosozumab or teriparatide. A previous study provided evidence of P1NP being a predictive marker for BMD response to teriparatide in treatment-naïve patients. The STRUCTURE trial enrolled 436 women who had taken bisphosphonates for at least 3 years. Measured at month 1 after transition to one of the bone anabolic therapies, levels of P1NP were found to increase by at least 10 ⊠g/L in nearly all participants (95% of those on romosozumab and 91% of those on teriparatide). There were differences in outcomes for the

two groups - hip and lumbar spine BMD were generally found to improve more over 12 months in those receiving romosozumab compared to teriparatide. However, the association of outcomes with the difference in early P1NP levels was quite weak, and so this study does not provide enough evidence for using P1NP as a predictive marker in this setting.

Effects of teriparatide on hip and upper limb fractures in patients with osteoporosis

Randomized clinical trials of teriparatide in osteoporosis patients have so far been small trials in which there have been few incident fractures, so insufficient to identify any benefit for fracture risk at specific sites from the treatment. **Adolfo Diez-Perez** (Barcelona) and colleagues have therefore performed a systematic review and meta-analysis of RCTs of teriparatide, selecting eventually 23 trials of at least 6 months duration, that had been peer-reviewed and between them provided data on 8,644 participants (3,893 treated with teriparatide), including data on non-spine fractures. Among all the trials combined, a total of 166 fractures of various kinds were reported. The analysis using established techniques showed that overall risk for hip fracture was reduced by 56% with teriparatide treatment (p=0.019), while differences in the overall risk of humerus, forearm and wrist fractures were not statistically significant.

The antidiabetic drug metformin improves the skeletal effects of plyometric exercise in ovariectomized rats

Metformin used to treat type 2 diabetes is suggested to have beneficial effects on bone. **Mats Mosti** and colleagues (Trondheim) were interested to test whether metformin combined with exercise could counteract the bone loss experienced with estrogen deficiency. Ovariectomised Sprague Dawley rats were subjected to either exercise, metformin, exercise combined with metformin, or no treatment, and an additional sham ovariectomised control group was studied. The exercise involved a plyometric jumping test measuring the height achieved, and bone phenotype was analysed at the end of 8 weeks. All groups improved their jumping performance during the experiment. However, the combined metformin and exercise group performed significantly better than the exercise-only group. They gained more femoral BMD than the metformin-only group and their trabecular number, separation and bone density were restored to the levels measured in the sham ovariectomised group. Lower bone turnover was measured in both exercise groups, but these results suggest that metformin can further improve the skeletal effects of plyometric exercise.

In other news... Anabolic osteoporosis treatment Kendler *et al* reported results from the VERO trial on teriparatide versus risedronate in postmenopausal women, with analysis of fractures as the primary outcome. This showed a significant reduction in the risk of new vertebral and clinical fractures with teriparatide compared to risedronate. There have also been several papers on romosozumab, an anti-sclerostin therapy, for osteoporosis and rare diseases. Langdahl *et al* reported on the results of the STRUCTURE trial to compare romosozumab and teriparatide bone anabolic therapies on BMD over 12 months in postmenopausal osteoporosis transitioning from bisphosphonate therapy. They identified gains in hip BMD in the romosozumab group, while no BMD gain was observed in those on teriparatide.

Advances in regenerative medicine

ECTS 2018
26-29 MAY 2018

Johannes Grillari (Vienna) kicked off the Basic Science Update on Technology with news of the latest developments in induced pluripotent stem cell (iPSC) technology, particularly in the context of regenerative medicine and the development of disease models.

The processing of donated human cells to create stem cells still has scope for further development, even though key transcription factors such as Oct4 are well established. Current research is exploring new factors for inducing stem cells including as mRNAs and proteins. Skin fibroblasts are commonly used as the donated cells, however a skin biopsy is still quite invasive and can be a particular risk for patients with certain rare skin conditions. Other less invasive sources of human cells include hair follicles and blood samples. Prof Grillari reported on a promising new approach to derive iPSCs from cells isolated in urine samples, as a completely non-invasive and low cost approach.

It was already known that kidney epithelial cells are shed in urine in some disease states, as a result of shear stresses in the renal tubular network. Prof Grillari's group and others have successfully isolated mesenchymal stem cells from the urine of patients and healthy volunteers, and also from small urine samples, with 5-25 CFU isolated from 100 ml urine. Differentiation protocols have been developed over several years to many cell lineages including chondrocytes, osteoblasts and adipocytes, though not yet hepatocytes, and the cells are fully pluripotent when tested in mouse models. By manipulating the epithelial-to-mesenchymal transition it has been found easier to differentiate the urine cells, in comparison to skin fibroblasts. The approach was used in the EU-funded *SYBIL project* to develop iPSC from a patient with autosomal dominant osteopetrosis type 2, and other researchers are working on differentiation to osteoclasts for regenerative stem cell therapy (poster 187).

Clinical application of iPSCs is still at an early stage, with various safety and stability concerns. Where the mechanisms of iPSC therapies are related to exosome secretion, a cell-free therapy based on extracellular vesicles isolated from iPSC may be feasible as a safer and more effective approach, as recently reported for cardiac repair. The most advanced treatment is for age-related macular degeneration where retina sheets composed of iPSC have been developed. While iPSC technology is currently quite expensive, it is becoming an essential approach especially for research and therapy of rare diseases, and we expect many exciting developments over the coming years.

In other thematic sections we include reports on the other presentations given in the Basic Science Update on Technology on Intravital Microscopy and Nanopore Sequencing.

Working Group on bone bioengineering, regeneration and implants

This Working Group session highlighted recent advances in the field of tissue engineering, bone repair and biomechanics.

Maria Pau Ginebra (Barcelona) gave a short introduction about the influence of surface topography and nanostructure on the osteogenic differentiation of mesenchymal stroma cells. She presented recent data from her group that calcium phosphate scaffolds, which display a nano-needle-like structure, showed increased osteoinduction compared to plate-like structured implants. This demonstrated the potential of biomimetic 3D-printed scaffolds for bone regeneration.

Melanie Haffner-Luntzer (Ulm) highlighted the potential influence of the immune system on fracture healing. She demonstrated the negative effects of enhanced systemic and local inflammation on bone regeneration in a murine combined trauma model and in osteoporotic mice and human fracture patients. Furthermore, she showed that a balanced activation of immune cells like neutrophils and mast cells is crucial for successful initiation of repair after fracture.

Ralph Müller (ETH Zurich) structured his talk according to his recently granted ERC application. He gave an introduction to the term "mechanomics" and highlighted the importance of local strain distribution in tissue and cellular response. He demonstrated his research approach to discover novel genes and proteins which are regulated in osteocytes dependent on the biomechanical environment. At the end of his talk, he gave an outlook to apply the concept of mechanomics also to bone regeneration.

Silvia Lopa (Milan) reviewed several interesting papers about recent applications in bone tissue engineering. Furthermore, she presented a translational application from her group using a hydrogel-based microfluidics system for repair of injured tissues. They generated a novel hydrogel with small channels inside, and investigated the transmigration and flow of granulocytes and other cells within their system.

ECTS Mike Horton Basic/Translational Award

The **ECTS Mike Horton Basic/Translational Award** was given to Prof. Dr. Ralph Müller (ETH Zurich), one of the fathers of micro-computed tomography (microCT). In the mid-1990s in Boston, he developed the first prototype of what has now become an essential tool for bone research. More recent work at the Laboratory for Bone Biomechanics, ETH Zurich has developed methods for longitudinal measurements using time-lapse microCT and other novel bioimaging and visualization strategies. He has published over 600 papers with more than 27,000 citations and an h-index of 85. He has also made an impact as an educator, supervising 41 doctorate theses and 77 graduate theses to date.

Fish as a model of skeletal diseases

ECTS 2018
26-29 MAY 2018

The fundamentals of skeletal biology evolved in the common aquatic ancestors of humans and modern fish, and so fish species are useful experimental models for studies of bone disease. In this workshop, Matthew Harris (Boston) and Paul Eckhard Witten (Ghent) invited us to connect with our inner fish.

Zebrafish (*Danio rerio*) in particular is now established as an important in vivo model of the vertebrate skeleton. Thousands of mutants are already obtained for functional studies in embryo or adult fish, either through mutagenesis, or more recently by gene editing.

Matthew Harris explained how zebrafish have become particularly useful in studies of rare diseases. Examples include osteogenesis imperfecta, where zebrafish mutations have been identified as models of the different types of this disease, and hypohidrotic ectodermal dysplasia where the phenotype of the fish scales is analogous to the hypotrichosis found in that condition. Zebrafish are also being used to investigate fundamental questions about the control of organ position and size during growth, especially as zebrafish have fin regeneration capabilities. Systematic genetic screens have identified mutants with varying fin size and regenerative capacity. The Harris lab has previously shown that fin growth is positively regulated by potassium channel signalling, but that inhibition of calcineurin increases the growth rate and final fin size. Their most recent results now show that bioelectric signaling through the potassium channel Kcnk5b is required for the growth effect of calcineurin, a really novel finding that the group are now investigating in long bone analogues.

An adult zebrafish stained with alizarin red and alcian blue to detail the form and pattern of the skeleton. Scales of the dermal skeleton, normally formed across the flank, are removed to aid in visualization of the internal skeleton. (M. Harris)

Paul Eckhard Witten then gave us a broader view of how the skeleton has evolved in various fish. Teleosts like zebrafish and medaka are among the more advanced fishes, having continued to evolve since the Triassic period just as much as the mammals. Fish skeletal anatomy is in many ways more complex than that of humans, for example they have more than twice the number of components in their skull. All vertebral organisms have two skeletal systems, the dermal skeleton and the endoskeleton, which have some overlapping functions and biology and some differences. In mammals and humans the main surviving component of dermal skeleton is in the dentition. However, while fish and many mammals can regenerate teeth, it is not clear why this is not possible for humans. In fact, results from the Ghent lab question the involvement of epithelial stem cells in the dental epithelium of Medaka and African bichir, both of which are able to continuously replace their teeth. However, there is evidence that de-differentiation and re-generation of scleroblasts is involved in fin regeneration, rather than stem cells, and a similar mechanism may occur in teeth replacement in medaka fish.

The differences in biology of the dermal skeleton and endoskeleton become clear when considering calcium and phosphorus metabolism. As the aquatic environment is rich in calcium, fish never have calcium deficiency – they can exchange calcium through their gills rather than involving their skeleton in calcium metabolism. However, phosphorus has low aquatic concentrations. As a result fish are far more dependent on phosphorus in their diet, as shown by Witten, *et al*. where salmon on a P-deficient diet were found to have continued bone formation, but greatly reduced bone mineralization.

As in humans, teleost fish feature a wide range of connective and skeletal tissues, which response to biomechanical and epigenetic stimuli. Their small genome size corresponds also to small anatomical features, e.g. bone trabeculae are just 3-5 μm wide, creating some complications for imaging and histology. There are other differences in skeletal biology in the smaller teleost fish species thought to have developed according to their small size. Larger teleost fish such as carp have typical endochondral bone formation, whereas

zebrafish have bone gaps filled with cartilage. Also, zebrafish have bone that contains osteocytes, while medaka evolved bone without osteocytes. Despite these differences to mammalian biology, zebrafish and medaka are now established as useful models for skeletal and other human disorders. They have many practical advantages, not least the large numbers that can be obtained for study. But "the best model we have is the model we understand best". A lot of work has been done now to characterise teleost fish models, but their phenotyping in experiments should be as precise as a medical diagnosis to get the best results.

New insights into bone and cartilage biology

ECTS 2018
26-29 MAY 2018

In the Basic Science Update on bone biology, Thorsten Schinke (Hamburg) presented what has been discovered over the past 25 years about osteoblasts and osteocytes, and Nadia Rucci (L'Aquila) discussed osteoclast research.

Thorsten Schinke described firstly how research in the 1990s in knock-out mouse models and in rare bone disease genetics identified Runx2 as the key gene involved in osteoblast differentiation. This is not a good drug target, being a nuclear protein not regulated by ligand binding, but later research on the mutations causing rare human osteosclerosis disorders identified sclerostin and Lrp5 within the Wnt signalling pathway as potential drug targets related to osteoblast function and bone formation. More recent data suggests that sclerostin blocks bone formation through Lrp5 interaction. There still remain many questions about how the complex Wnt signalling pathways regulate bone formation in osteoblasts, and the role of individual Wnt ligands. WNT1 mutations have been found in some cases of osteogenesis imperfecta and early-onset osteoporosis, and Wnt1 is being investigated further in the Schinke lab in mouse models to explore its role as an agonist of osteoblast activity.

The role of osteocytes in various physiological and pathological processes has received much more attention in the past few years. Regulation of bone remodelling by osteocytes has been linked to mechanosensation, mediated for example by -catenin signalling. They are also involved in the release of calcium through osteolysis, control of bone matrix mineralization, and phosphate homeostasis through release of FGF-23 — the latter showing the endocrine role of osteocytes in systemic human diseases involving disrupted phosphate homeostasis. Osteocytes are also controlled by coupling with osteoclasts. Prof Schinke and others have identified the lipid sphingosine-1-phosphate as a potential coupling factor, and this has now been demonstrated through inducible deletion in adult mice as a potential new target for bone anabolic therapy for osteoporotic diseases.

Nadia Rucci started her talk by explaining how RANKL was first identified in 1998 as the cytokine primarily responsible for osteoclast differentiation and function. Twenty years later, we have now a complete picture of the mechanism of RANKL-initiated osteoclast differentiation, with a key inhibitor of differentiation OPG, and several other pro- and anti-osteoclastogenic cytokines also identified, and interactions with the immune system. The team at L'Aquila also discovered the role of bone matrix proteins PRELP and CHAD in regulating osteoclastogenesis and osteoclast motility respectively. The cell structures enabling adhesion of osteoclasts to the bone matrix, and the cell machinery involved in bone resorption have also been characterised in detail. Genetic defects in osteoclast bone resorption are involved in rare bone diseases including the several forms of osteopetrosis and pycnodysostosis.

However, osteoclasts perform biological functions beyond "eating bone". They contribute to the regulation of haematopoiesis by mobilizing hematopoietic progenitor cells and calcium from the endosteum, and contribute to the hematopoietic stem cell niche in the bone marrow. Recent research also suggests that osteoclasts can regulate angiogenesis through release of TGF from the bone matrix, or directly producing pro-angiogenic cytokines including VEGF, BMP7 and EGF. Its also been established that osteoclasts help regulate bone formation, by production of "clastokines" such as TRAcP, Wnt10b and Sema4D, that either promote or inhibit osteoblast differentiation, while ephrins have also been identified as important bi-directional mediators of interaction between osteoclasts and osteoblasts. Concerning energy metabolism, it is currently being investigated to what extent osteoclasts employ glycolysis versus oxidative metabolism. For example the MYC/ERRα pathway has been reported to be involved in osteoclast metabolic reprogramming during their differentiation. Certainly, osteoclasts are very complex, multi-functional cells and there is still much research to be done to fully understand them.

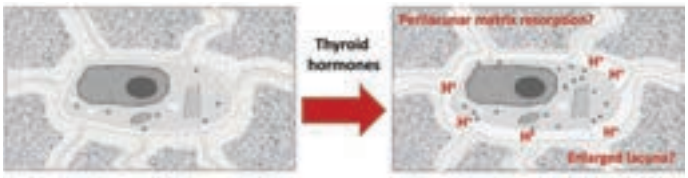
ALSO PRESENTED AT ECTS 2018

PGC1a deficiency negatively regulates bone mass and strength

PGC1a is upregulated in brown fat in response to cold, and is a master regulator of energy metabolism, thermogenesis and skeletal muscle metabolism. **Graziana Colaianni** and colleagues wanted to know if PGC1a deletion had an effect on bone. Phenotyping by micro-CT showed that, compared to wild type mice, PGC1a-heterozygous and PGC1a-deficient mice both had decreased cortical bone thickness, their trabecular bone had a higher degree of anisotropy, and bending strength was reduced by ~48%. Ex vivo analysis of bone tissues from PGC1a-deficient mice found lower mRNA for osteocalcin in cortical bone and collagen 1 in bone marrow stromal cells. Substantial changes in white adipose tissue were also found in these mice. Further studies are to examine mice with osteoclast and osteoblast-specific deletions of PGC1a to further understand its role in bone metabolism.

Exogenous hyperthyroidism induces osteocytic osteolysis in male mice

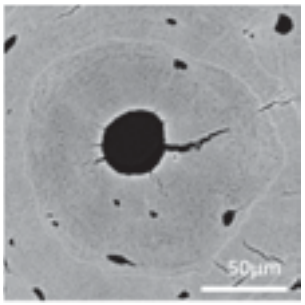
Osteocyte osteolysis has been demonstrated to occur during lactation as a means by which calcium is liberated by remodelling of the perilacunar bone matrix. **Elena Tsourdi** (Dresden) and colleagues had previously observed higher bone turnover and a higher proportion of low mineralized bone in mice with hyperthyroidism. They have now investigated whether, in addition to osteoclast-driven bone resorption, osteocyte osteolysis could also be involved in these changes. L-thyroxine in drinking water was used to induce hyperthyroidism in male mice, and after 4 weeks, the mouse bone tissues were analysed. A larger osteocyte lacunar area was found in tibial cortical and trabecular bone of hyperthyroid mice, and osteocytes had a higher TRAP activity compared to the euthyroid controls. Further in vitro experiments on a murine osteocyte cell line treated with thyroid hormone revealed an increase in typical osteoclast markers related to bone resorption activity. Together, these results suggest that hyperthyroidism-induced osteocytic osteolysis may contribute to low bone mineralization in hyperthyroid mice.



Thyroid hormones as putative inducers of osteocytic osteolysis (E. Tsourdi)

Cement lines mineralization is a bone tissue-age related phenomenon: a study in human femoral cortical bone

The peripheral boundaries of osteons in cortical bone are known as cement lines, due to their appearance as thin lines in tissue sections, though in three dimensions these are more like a sheath around the osteon cylinder. Its been disputed for some time whether cement lines are mineral-rich or mineral-deficient, and it is not known if and how their mineralization changes during ageing. **Petar Milovanovic** reported on an investigation in Hamburg that used quantitative backscattered-electron imaging and micro-Raman spectroscopy to analyse the calcium content of osteons and their cement lines in femoral cortex samples from ten postmenopausal women with untreated or bisphosphonate-treated osteoporosis, with samples representing various tissue ages. The analysis showed a consistently higher calcium content in the cement lines compared to their osteons, and a significantly higher v1 phosphate/amide I ratio. Positive correlations were found between the mineralization of cement lines and the osteonal mineralization level, but these decreased with increased osteonal mineralization, probably relating to tissue-age. Using these methods, analysis of cement lines in different healthy or pathological states should be helpful to better understand crack propagation and fracture resistance mechanisms in bone.



The mineralization of the osteons and corresponding cement lines. A backscattered electron image of an osteon shows clearly the hypermineralized cement line as the osteon's outer boundary. Calcium content is reflected in gray values: low Ca=dark, high Ca=light pixels. (P. Milovanovic)

Role of lipocalin 2 in energy metabolism-bone crosstalk

Lipocalin 2 (Lcn2) is a circulating protein linked in recent years to bone and energy metabolism, including interactions with the central nervous system. **Mattia Capulli** presented the latest published findings from the team in L'Aquila who previously identified Lcn2 as a novel regulator of bone mass in unloading conditions, with potential implications for osteoporosis in the ageing population. While Lcn2-/- mice have reduced bone mass, *in vitro* silencing of Lcn2 in osteoblasts does not affect the expression of mRNA related to osteoblast function, ALP activity or matrix nodule mineralization, suggesting a non-autonomous mechanism linking osteoblast activity to other biological processes in vivo. The knockout mice also have higher body weight and food intake, so the team investigated if energy

metabolism was altered. Hyperinsulinemia was detected, but this was not found associated to high bone formation. It was then found that expression of glucose transporter Glut1 which is necessary for osteogenesis was reduced by around 80% in the mouse bone. As other groups have obtained contradictory results on Lcn2, further studies are necessary to fully understand its complex regulation of bone mass, likely involving multiple systemic in vivo interactions.

OPG-Fc attenuates insulin-resistance and muscle weakness in a mouse model of diabetoporosis

Researchers in Geneva are investigating osteoporosis and type 2 diabetes in *Pparβ* -/- mice that replicate this increasingly common co-morbidity (termed "diabetoporosis"). **Lucie Bourgoin** presented on their recent investigation on the impact of OPG treatment (a RANKL inhibitor) in this model. Recombinant OPG-Fc was previously shown to limit muscle damage in dystrophin deficient mdx mice, so the group investigated the hypothesis that OPG-Fc could simultaneously act on bone loss, muscle weakness and insulin sensitivity in *Pparβ*-/- mice. Mice were treated with OPG-Fc or placebo for 4 weeks, and tested for muscle function by treadmill and handgrip tests, followed by standard tests for insulin sensitivity, histological analysis and gene expression. *Pparβ*-/- mice displayed peripheral insulin resistance, a lower trabecular and cortical femur bone volume associated with a lower volume, maximal speed and force of muscles. Treatment with OPG-Fc rescued most of these changes, especially those related to bone loss, muscle function and insulin sensitivity, demonstrating the likely central role of RANKL in osteoporosis, sarcopenia and diabetes.

Lin28a overexpression protects chondrocyte from osteoarthritis phenotype

Cartilage damage in osteoarthritis is promoted by an imbalance of chondrocyte activity and chondrocyte apoptosis. As cartilage has poor regeneration capability, damage is irreversible once it has occurred. **Yohan Jouan** (Paris) presented results of a study to see whether overexpression of RNA binding protein Lin28a could regenerate damaged cartilage through dedifferentiation of osteoarthritic chondrocytes. Transgenic mice with tamoxifen-inducible Lin28a expression were used in both in vitro and in vivo studies. In vitro studies using organotypic femoral head culture generated from 10 day old female mice. After induction of Lin28a expression, analysis of chondrocytes showing reduced MMPs expression and apoptosis, and increased anabolic gene expression and cell proliferation. In vivo, osteoarthritis was induced surgically in tamoxifen-treated male transgenic mice, and littermates used as controls had sham operations. After 8 weeks Lin28a overexpression was found to protect cartilage against degradation, with increased proliferation of chondrocytes and reduced MMP13 expression. Bone microarchitecture showed no difference in subcondral bone structure but a 3-fold increase in osteophyte size in the transgenic mice. These results demonstrate the potential of Lin28 overexpression as a novel therapeutic strategy for cartilage regeneration in osteoarthritis.

Working Group on Cartilage pathophysiology, common and rare disorders of cartilage

The Cartilage Pathophysiology Working Group was chaired by **Antonio Rossi** (Pavia) and began with **Eric Hay** (Paris) speaking about the role of Wnt signalling in osteoarthritis. We learnt that both canonical and non-canonical activation and inhibition of the Wnt pathway leads to osteoarthritis. The protective role of hypoxia during osteoarthritis was also discussed.

Laurence Legeai-Mallet (also from Paris) outlined her lab's work on FGFR3 signalling during ciliogenesis. She provided a fascinating insight into the orientation of primary cilia in the growth plate and the disorganisation that occurs in a mouse model of achondrodysplasia due to FGFR3 gain of function.

Ray Boot-Handford (Manchester) then spoke about the role of ER stress due to protein misfolding in Metaphyseal Chondrodysplasia Type-Schmid. Aggregated and misfolded collagen X could be removed by proteolysis, reducing the severity of this disease in animal models. **Michael Briggs** (Newcastle) spoke about new therapeutic targets for genetic skeletal disease. This was an interesting overview of how missense mutations in Matrillin-3 cause Multiple Epiphyseal Dysplasia through ER retention and decreased proliferation. Finally, **Yuuki Imai** (Japan) provided a thoughtful summary of his work on epigenetic control of transcriptional regulation of chondrocyte differentiation and skeletal maturation.

In other news… Transdifferentiation has been discussed since the 1980s but there appears now renewed interest especially in the bone field. Hu *et al* have identified the transdifferentiation of chondrocytes to bone cells during fracture healing, contrary to the established model of osteoprogenitors infiltrating cartilage during endochondral ossification.

ECTS Mike Horton Basic/Translational Award

The ECTS Mike Horton Basic/Translational Award was given to Prof. Dr. Ralph Müller (ETH Zurich), one of the fathers of micro-computed tomography (microCT). In the mid-1990s in Boston, he developed the first prototype of what has now become an essential tool for bone research. More recent work at the Laboratory for Bone Biomechanics, ETH Zurich has developed methods for longitudinal measurements using time-lapse microCT and other novel bioimaging and visualization strategies. He has published over 600 papers with more than 27,000 citations and an h-index of 85. He has also made an impact as an educator, supervising 41 doctorate theses and 77 graduate theses to date.

ECTS Excellence in Research Award

The ECTS Excellence in Research Award recognising scientists who have contributed significant advances in the field of musculoskeletal research was given to Professor Hans van Leeuwen. His research conducted at the Erasmus Medical Centre, increasing our understanding of vitamin D and calcium regulation of bone metabolism, especially describing the role of epithelial calcium channel TRPV5, and the genetics of osteoporosis. He has founded two start-up companies and has had roles in both ECTS and ASBMR.

ECTS Iain T Boyle Award

The ECTS Iain T Boyle award, for young scientists who have made significant progress and contribution to the field of bone and calcified tissue, was received by Dr Frank Oury. After a PhD in neurobiology obtained in Strasbourg he worked with Gerard Karsenty at Columbia University, making significant discoveries on the endocrine activities of bone. He returned to France in 2014 to become a group leader at Institut Necker Enfants-Malades (INSERM) working on hormonal regulation of brain development, including memory and ageing, using multidisciplinary approaches. He has already 16 publications and several other awards.

Cancer and bone

Advances in molecular imaging applied to studies of cancer and bone

In the Basic Science Update on Technology, **Delfim Duarte** (Porto) explained how research on the role of the bone marrow microenvironment in leukemia can benefit from the latest advances in intravital imaging techniques.

Cells often behave very differently *in vivo*, compared to *in vitro* culture. The observation of disease processes *in vivo* in real time has been made more feasible by the introduction of multiphoton laser confocal microscopy. This allows tissues at depths of 150-500 µm to be imaged at much lower levels of illumination compared to conventional laser confocal microscopy, reducing the risk of phototoxicity. A combination of both techniques, pioneered by Lo Celso et al, enables intravital imaging of the bone marrow niche within mouse calvaria to observe the movement of individual haematopoietic stem cells and their interactions with osteoblasts and bone vasculature. In general, imaging can be performed anywhere from 3 up to 14 hours, allowing the video imaging of cell migration, proliferation and cell death (using FRET). The approach can also be combined with other approaches such as immunofluorescence.

In his later talk during the symposium on Molecular Imaging, Delfim Duarte explained further how intravital imaging can now be used to monitor the longitudinal action and efficacy of therapeutic drugs *in vivo*. His work focuses on acute myeloid leukemia (AML), mostly diagnosed in the elderly, for which an effective therapy is not yet available. The remodelling of the bone marrow vascular niche by AML cells contributes to AML progression, but the mechanism of this process is still not fully understood, and anti-angiogenic therapies have not proved effective for treatment of patients.

Using intravital imaging of a mouse model of aggressive AML, Dr Duarte and collaborators observed that endosteal blood vessels were more severely affected and were eventually eliminated, while central bone marrow vasculature was much less affected, and survived. As well as providing more detail on how hematopoietic stem cell niches are affected in AML, this could be one explanation for chemotherapy resistance — that AML cells in endosteal regions with reduced vasculature are much less accessible for drug delivery. Later in the study, protection of the endosteal epithelium either with a genetic approach to induce Notch2 signalling, or with the drug doxorubicin, was found to increase chemotherapeutic efficiency and promote survival.

Intravital imaging was also applied to the study of T-cell acute lymphoblastic leukemia (T-ALL), which is quite rare in adults, but represents around 15% of cases of childhood acute lymphoblastic leukemia. Here again, microenvironment remodelling in the bone marrow by cancer cells is thought to contribute to disease progression and chemotherapy resistance. Observations of the disease progression were made over time from cells seeded into mouse calvarial bone marrow. The experiments used a tile mapping approach to provide simultaneous high resolution and tissue-wide imaging, specific reporter systems to visualize AML cells and osteoblasts, and injected Cy5 dextran dye to stain the vasculature. After treatment with dexamethasome, surviving chemoresistant cancer cells could be observed, and these showed more motility than the cells that had been destroyed. In this case, there was no preferential distribution of T-ALL cells to specific niches, rather a stochastic distribution through the bone marrow. But a dramatic remodelling of the microenvironment was observed with progression of the disease, ultimately depleting osteoblasts completely from the endosteal spaces.

Using intravital imaging, Dr Duarte's group has now performed further investigations of the motility of both AML and T-ALL cancer cells before and after chemotherapy, also evaluating inhibition of CXCR4, a regulator of cell migration, to overcome chemotherapy resistance. Experiments in the respective mouse models used standard chemotherapies and injections of CXCR4 inhibitor AMD3100, while time-lapse imaging and mathematical analyses (mean square displacements) were used to quantify effects on cell motility. The results confirmed again that both AML and T-ALL cells were motile at baseline, with AML migration being significantly faster. Cell migration was observed higher in chemoresistant T-ALL cells but lower for AML cells. CXCR4 antagonism markedly reduced migration of T-ALL cells in the bone marrow at both the seeding and post-chemotherapy stages, suggesting a promising therapeutic strategy for T-ALL. That similar results were not found for AML suggests possible redundancy mechanisms.

The second talk in the Molecular Imaging symposium by Professor Gary Cook (King's College London) discussed molecular imaging of bone metastases in the clinic.

Bone metastases are common, with around 70% of breast and prostate cancer patients being affected. Compared to other types of cancer, survival is quite long at 2-3 years but there is associated excess morbidity. Hybrid imaging techniques such as SPECT/CT, PET/CT and whole body MRI have improved detection of bone metastases compared to traditional bone scintigraphy, but we are still lacking effective ways to assess treatment responses, especially as it is challenging to measure changes in lesion volume. These limitations complicate the analysis of treatments for bone metastases in clinical trials. However, molecular imaging provides a possible solution by specifically detecting cancer cells, abnormal osteoblast or osteoclast activity or other molecular changes in bone metastases. The high resolution of modern imaging (e.g. in ^{18}F-FDG PET/CT or ^{18}F-choline PET/MRI) has really transformed our ability to detect abnormal metabolism and determine the precise position of bone metastatic lesions. Hybrid SPECT/CT bone scintigraphy was found to reduce the equivocal reports of bone metastases in prostate cancer patients down to 6%, compared to 61% with planar scintigraphy and SPECT.

The use of ^{18}F-FDG PET/CT to image tumour cell metabolism is now becoming established in the clinic for following treatment responses in metastatic disease. Alternatively, using ^{18}F-fluoride as a bone-specific PET imaging agent may show greater uptake of fluoride in progressing breast cancer patients, but the flare phenomenon that can occur following successful therapy can complicate the analysis. For prostate cancer, ^{68}Ga-PMSA has now joined ^{18}F-choline as a potentially useful tracer for detection of metastatic disease.

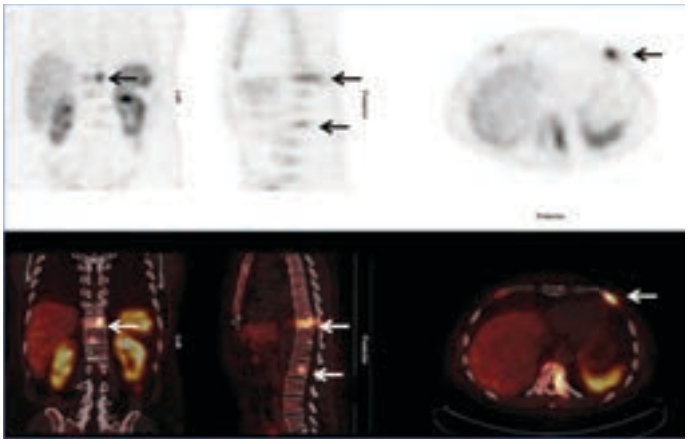
Using MRI, diffusion-weighted imaging involves the measurement of restricted Brownian motion of water molecules in tumour tissue due to its higher cellularity, and seems to be effective for detection of small metastases using whole body MRI, but requires further validation.

Other work has targetedαVβ3 integrin as a marker of osteoclast activity in osteolytic bone metastases, for example with ^{99}mTc-maraciclatide single-photon emission computed tomography, where a significant correlation was found between therapy response and the change in tracer uptake.

According to Professor Cook "we are in the era of trying to cure oligo-metastatic disease and oncologists are wanting to detect small volume disease with curative intent – the more sensitive we can be in detecting lesions, the more patients will be treated appropriately".

Also available online are the following presentations on cancer and bone from the ECTS 2018 Basic Science and Clinical Updates:

Management of SRE in patients with bone malignancies - Georg Pfeiler (Vienna)
Molecular insights into bone tumors and predisposing disorders - Fernando Gianfrancesco (Naples)
RANKL and oncogenesis - Dominique Heymann (Sheffield)



Increased αVβ3 integrin expression in prostate cancer bone metastases demonstrated on 99mTc-maraciclatide SPECT/CT imaging
(GJH Cook, et al. (2018)

Working Group on Cancer and Bone: Breast and prostate bone metastases

The Cancer and Bone working group featured 4 presentations covering basic and clinical aspects of breast and prostate cancer bone metastases.

The first talk by **Aymen Idris** (Sheffield) provided a comprehensive introduction to the broader topic of metastatic bone disease and introduced the 'vicious cycle' driving this incurable disease. Furthermore, Dr. Idris discussed the critical role of NFκB signalling pathway in breast cancer bone metastasis and demonstrated that targeting of several components of the pathway reduces tumor burden and bone destruction, providing a novel strategy to alleviate metastatic bone disease.

The second talk by **Gabri van der Pluijm** (Leiden) focused on molecular mechanisms driving prostate cancer-induced bone metastasis. Various *in vitro*, ex vivo and *in vivo* models were discussed as well as the importance of cancer stem cells and contribution genetic alterations in the complex process of metastasis.

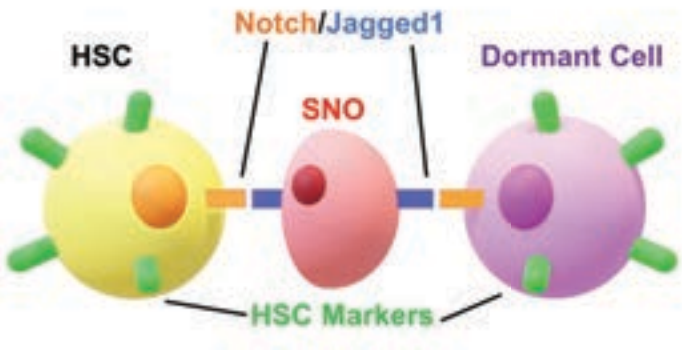
After the two presentations on basic/translational research, **Robert Coleman** (Sheffield) and **Peyman Hadji** (Frankfurt/Main) discussed clinical aspects and strengths and limitation of current treatment options of prostate and breast cancer bone metastases. Dr. Coleman presented studies showing that anti-resorptive therapies (bisphosphonates and denosumab) reduce skeletal-

related events (SREs) in metastatic castration-resistant prostate cancer without increasing survival while α-emitting agent radium-223 improves survival in addition to decreasing SREs. As shown by Dr. Hadji, in breast cancer patients bisphosphonates reduce the risk of developing SREs and increase survival in post-menopausal women and show clear benefits when used in adjuvant setting. Denosumab effectively reduces the risk of SREs, and ongoing clinical studies will tell in the near future whether women with high risk of early breast cancer benefit from adjuvant denosumab therapy. In addition, several important clinical guidelines were discussed by Drs. Coleman and Hadji. All excellent presentations stimulated lively discussion, which would have lasted longer if time had permitted.

ALSO PRESENTED AT ECTS 2018

The key role of the Notch2 signal in breast cancer dormancy and tumour-initiation

Kashmala Carys (L'Aquila) presented new insights into the role of Notch2 in the switching between a state of dormancy and tumour-initiation in breast cancer cells, focusing on the development of bone metastases from individual cancer cells disseminated in the bone tissues. Experiments with MDA-MB-231 cells (MDA) showed that they could be induced into a dormant state when co-cultured *in vitro* with Spindle shaped N-cadherin osteoblasts. This impairment of proliferation could be rescued by silencing Notch2 *in vitro*. *In vivo*, treating mice harbouring 'presumably dormant cells' with an inhibitor of γ-secretase (a Notch2 signal-activating enzyme) led to a 3-fold increase in liver metastasis. Conversely, mice injected with Notch2^{HIGH} expressing MDA cells showed both 70% less in-bone tumour growth and 67% less distant organ metastasis than mice injected with Notch2^{LOW} expressing MDA cells. Interestingly, this Notch2^{HIGH} population made up 1-5% of cells and were enriched in the classic markers for hematopoietic stem cells. To test this sub-population's stemness, mammosphere formation assays were performed showing Notch2^{HIGH} expressing MDA-MB-231 cells generated more, and larger mammospheres compared with the rest of the cell population. This research indicates that Notch2 is able to maintain breast cancer cells dormant at the endosteal niche in the bone, but under the right environmental pressures the same Notch2^{HIGH} population has a strong tumour-imitating ability, possibly leading to the relapse seen in patients.



Spindle shaped N-Cadherin osteoblasts (SNOs) induce a state of dormancy in breast cancer cells through the Notch/Jagged1 signalling pathway (K. Carys)

Increased cancer mortality in older men with higher serum concentrations of FGF-23

Raised serum FGF-23 is associated with higher mortality among chronic kidney disease patients, but its association to mortality in the general population is poorly studied. **Pawel Szulc** (Lyon) investigated this topic in a cohort of 814 men aged 60-87 (STRAMBO) followed up prospectively for 8 years. FGF-23 serum levels were measured at baseline. During 8 years, 167 participants died. After adjusting for potential confounders including smoking and co-morbidities, a higher all-cause mortality was found in men with higher FGF-23 levels. An even stronger association of FGF-23 was found for cancer mortality. This link persisted after excluding men self-reporting cancer at baseline, and those who died of cancer within the first two years. In contrast, no association was found with cardiovascular mortality.

In other news... Communication in the bone C Englom, *et al*. reported a two-way interaction between lung tumours and bone, in the absence of bone micrometastases. Here, osteocalcin-expressing osteoblasts resident in an altered bone stroma were found to promote the lung tumour growth via SiglecF neutrophils. This study highlights how cancer is a systemic disease, with bone metabolism often playing a role.

In other news... Precision medicine in cancer treatment Bisphosphonates have been associated with increased survival when given to postmenopausal women. The AZURE trial has determined that amplification of MAF, a transcription factor for several genes associated to bone metastases, could be a clinically useful biomarker to predict the response of breast cancer patients to bisphosphonate treatment. A novel approach for CXCR4-directed endoradiotherapy was also highlighted as an interesting concept in targeted therapy. This was found promising for the treatment of relapsed multiple myeloma patients with extramedullary disease.


ECTS
ECTS
learn
sha
ECTS
European Calcified Tissue Society
Apply for Membership
Information on our activities
Enter the Scavenger Hunt
Sign up for Fundraising Beach Volley

Bone and vascular alterations in kidney disease

ECTS 2018
26-29 MAY 2018

Update on guidelines for renal osteodystropy

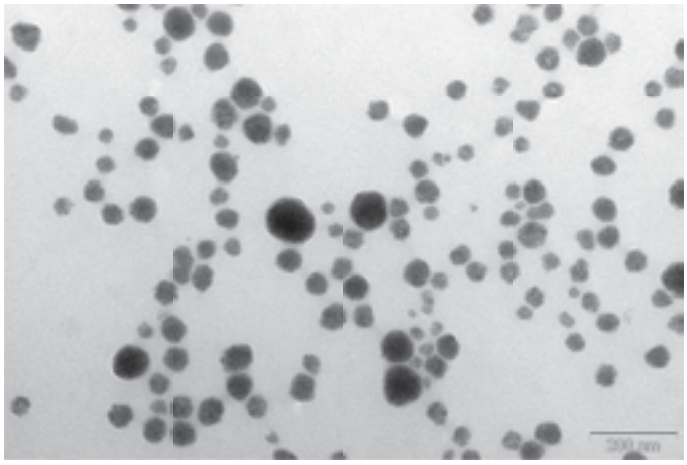
Chronic kidney disease (CKD) patients have a higher risk of hip fracture, and for those patients on hemodialysis, this risk rises even further compared to the general population. In the Clinical Update, **Roland Chapurlat** (Lyon) discussed renal osteodystrophy, the skeletal component of the systemic mineral and bone metabolism disorder observed in many CKD patients.

The four traditional types of osteodystrophy are all found in renal patients, with adynamic bone (low bone turnover) being increasingly common in those on dialysis. The classification of renal osteodystrophy is now simplified by KDIGO in terms of bone turnover, mineralization and bone volume. Risk factors for adynamic bone include older age, presence of diabetes and use of glucocorticoids. The stage of kidney disease also affects the level of bone turnover, rising in the later stages in most patients. Overall, CKD patients have a higher rate of BMD loss during ageing, compared to those with healthy kidneys. While kidney transplant partly resolves renal osteodystrophy in most patients, around one third still have symptoms after a transplant.

Prof Chapurlat then reviewed the diagnostic approaches available for renal osteodystrophy. Areal BMD had previously not been found to be effective for prediction of fracture risk in CKD patients, but there is now enough evidence that BMD obtained with DXA can predict incident fractures in patients with CKD stages G3a-G5d. Biochemical markers are important to determine bone turnover, as some anti-resorptive agents could make the low bone turnover in adynamic bone much worse. As the impaired kidney function affects the correlation to bone turnover, multiple measurements of serum BAP and PTH are recommended to observe any trends, and markedly high or low values can be used to estimate the level of bone turnover. The new guidelines also give advice concerning use of bone biopsies in CKD patients. A new approach not yet translated to the clinic is to analyse bone microstructure with HR-pQCT. Patients on dialysis have been observed to develop alterations in cortical and trabecular bone microstructure and a recent small study demonstrated that the use of HR-pQCT imaging alongside bone turnover markers improved the detection of low versus non-low bone turnover in renal osteodystrophy.

Calcification propensity in kidney disease

Ectopic calcifications are likely to occur in renal patients at locations such as the heart valves, arteries, and cornea, and are associated with increased mortality. Calcifications occur in the same places, but with less severity, in the general population. **Andreas Pasch** (Bern) explained how hydroxyapatite forms in bone and how this interacts with calcium and phosphate levels across the body in healthy and pathological states. While calcium and phosphate are chemically close to supersaturation in the body fluids, human serum has buffering proteins, primarily fetuin-A, that prevent the precipitation of hydroxyapatite



Primary calciprotein particles (left) contain amorphous calcium phosphate, whereas secondary calciprotein particles (right) contain crystalline calcium phosphate (hydroxyapatite). This solid-solid phase transformation occurs spontaneously, accelerated by higher levels of promoters (calcium, phosphate), lower levels of inhibitors (e.g. fetuin-A, magnesium) and higher temperature. Reciprocal parameters delay the transformation. (A. Pasch)

throughout the body under physiological conditions. Each fetuin-A monomer is able to bind 90-108 calcium ions and 60-72 phosphate ions, and these assemble into primary calciprotein particles of around 100 fetuin-A monomers, holding around 10,000 calcium ions and 7,000 phosphate ions.

Under certain conditions primary calciprotein particles spontaneously transform to secondary calciprotein particles (see Figure opposite). Primary and secondary calciprotein particles are involved in the biologically-controlled crystallization cascade forming hydroxyapatite in bone, and ectopic calcifications. Secondary calciprotein particles have also been used for reproducible induction of vascular calcifications in *in vitro* experiments in vascular smooth muscle cells. Dr Pasch and his team have developed a blood test for calcification propensity based on detecting the maturation time from primary to secondary calciprotein particles.

Clinical studies to evaluate this test have so far been performed in 6000 hemodialysis, kidney transplant and chronic kidney disease patients. These trials found that the degree of calcification propensity is also associated with all-cause mortality, and graft failure and cardiovascular mortality in transplant recipients. Running the test in a sample of the general population in Switzerland has found a normal distribution of calcification propensity, as in patient populations, and 10% of the population was identified with a gene locus indicating a hereditary-determined calcification propensity. A multivariate analysis has identified several serum markers as determinants of calcification test measurement, including lower monomer fetuin-A, and higher phosphate and calcium levels. Dialysate magnesium is a possible treatment for calcification in renal patients and a randomised controlled trial in 59 hemodialysis patients has shown reduced calcification propensity during magnesium treatment as measured by the blood test. While current treatment focuses on PTH, calcium and phosphate levels, the results of this research could lead to a therapeutic strategy focused on the new blood test and crystal formation time, addressing several contributors including also magnesium and fetuin-A. By reducing this "Mineral Stress" found within the buffering system in pathological conditions, we can achieve a better prognosis for affected patients.

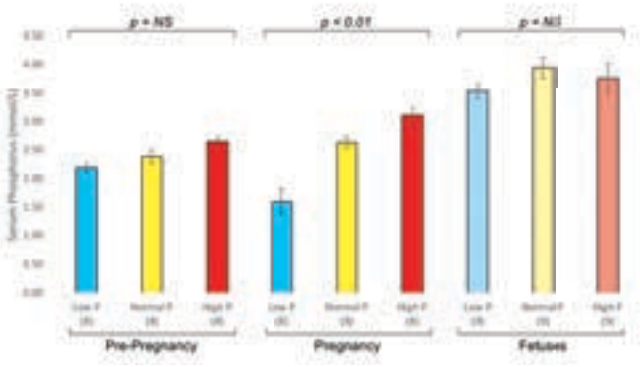
ALSO PRESENTED AT ECTS 2018

Dissecting the alterations of bone remodeling activity in cystinosis

Cystinosis is a rare condition of accumulation of cystine into cell lysosomes, resulting from a loss-of-function mutation in the CTNS gene encoding for lysosomal transporter cystinosin. Renal dysfunction (Fanconi syndrome), bone deformity, bone fragility, osteomalacia and rickets are observed in cystinosis. It was presumed that the effects on bone were only a complication of the phosphate loss due to renal dysfunction. **Giulia Battafarano** and colleagues (Rome) wanted to know if there was also a direct effect of cystinosin deficiency on the skeleton. They are investigating Ctns knockout male mice that develop skeletal features of cystinosis, but not Fanconi syndrome, up to 9 months of age. In the 1 month old mice already analysed, cystine was found accumulated in the bone cells, accompanied by a reduction in trabecular bone volume and BMD in the femurs. A reduced number of osteoclasts and defective osteoblast differentiation was also observed, showing that a loss of cystinosin is a direct contributor to osteopenia in *Ctns* knockout mice.

Fetal serum phosphorus is set independently of maternal serum phosphorus and phosphorus intake, including the extremes of maternal hyperphosphatemia and hypophosphatemia

K. Berit Sellars and colleagues (Newfoundland) performed experiments on 30 wild type female mice placed on either a low, medium or high phosphate diet. These mice were mated and analysed along with their fetal offspring for FGF-23 levels and phosphate metabolism parameters. While the mothers' serum phosphate, FGF-23, and PTH levels were affected as expected based on dietary phosphate intake, the fetal serum phosphate levels were maintained independently of maternal levels. Also, there were no changes in the expression of genes related to phosphate and calcium transport in the placentas. This demonstrates that some mechanism independent of maternal phosphorus status is responsible for maintaining fetal phosphate levels and placental phosphorus transport.



Fetal serum phosphorus is set independent of maternal values. (K. Berit Sellars)

Evidence supporting a vascular disease-muscle function relationship

A study performed in a cohort of elderly women from the Australian Calcium Intake Fracture Outcomes Study has found evidence of a link between vascular disease and muscle strength. **Alexander Rodriguez** (Melbourne) explained how participants in the study were tested for bone density at baseline, and 193 were identified as having severe abdominal aortic calcification (AACsev) visible in their scans, scored semi-quantitatively according to established criteria. These study participants were followed up after 5 years with standard tests for grip strength and mobility (Timed-up-and-go-test), as well as appendicular lean mass determination. Grip strength was found to decline significantly more in those classified with AACsev compared to the other participants, and this association was independent of age, treatment allocation, diabetes, smoking, renal function, prevalent vascular disease, and BMI. The effect of AAC may be evident in discrete measures of muscle strength such as grip strength, rather than complex movements such as the Timed-up-and-go-test which involves multiple systems.

Bone marrow adipose tissue – complex questions

ECTS 2018
26-29 MAY 2018

An expansion of bone marrow adipose tissue (BMAT) has been associated with the progression of osteoporosis and other diseases. The reasons for this, and indeed the fundamental biological role of BMAT, remain unclear. The ECTS symposium on bone marrow fat featured presentations from two scientists working in this new field of research.

Erica Scheller (Saint Louis) **explained how research into BMAT is revealing its positive contribution to health and energy metabolism**. She started her presentation by describing the natural evolution of bone marrow adipose tissue (BMAT), which appeared after the development of white adipose tissue in fish, and before the appearance of brown adipose tissue in mammals.

In contrast to white adipose tissue, BMAT is normally preserved during caloric restriction, and Dr Scheller's group have identified a distinct mechanism by which BMAT resists the β-adrenergic stimulation which is a major regulator of adipose tissue lipolysis. As UCP1 is the gene responsible for thermogenesis in brown adipose tissue, Dr Scheller has been investigating its expression in BMAT, and presented unpublished results from this work. Her group also investigated the bone marrow adipocyte microenvironment by 3D electron microscopy, confirming that bone marrow adipocytes feature multiple lipid droplets and a dense mitochondrial network, and identifying their significant interactions with endothelial and perivascular cells, and with active osteoblasts. They estimate that each bone marrow adipocyte will interact with over 100 hematopoietic cells, and have evidence through immuno-electron microscopy that a subset of bone marrow adipocytes are interfaced to the sympathetic nervous system.

Micro-CT scans of bones stained with osmium tetroxide, allowing visualisation of the BMAT expansion with caloric restriction (E. Scheller)

in vitro co-culture studies suggest that adipocytes may be induced by leukemia cells to undergo lipidolysis, thus providing the leukemia cells with "fuel" and promoting cancer progression. While BMAT has been identified as having some associations with metabolic and bone disease, it should not be seen intrinsically as a "bad" tissue. As is increasingly clear with other adipose tissues, BMAT is likely to have important physiological roles, both locally in the bone marrow and at a systemic level, and further research should reveal more about its positive properties.

William Cawthorn (Edinburgh) **discussed further the endocrine activities of BMAT and its impact on metabolic homeostasis.** BMAT gradually becomes established during childhood, and in adulthood up to 70% of the total bone marrow volume is BMAT; however, this tissue is still poorly understood. Further expansion of BMAT tissue is observed in many diseases such as osteoporosis and type 2 diabetes, in response to chemotherapy and other insults, and in people with anorexia nervosa and other conditions of caloric restriction. It's not yet clear why this occurs, or what is the contribution of excess BMAT in these disorders. Many groups are investigating possible effects on bone metabolism and haematopoiesis. Given the association of white adipose tissue and energy metabolism, Dr Cawthorn's research has investigated possible similar activities of BMAT, asking whether it contributes to production of adiponectin, a major adipose-derived hormone that is the third most abundant protein in the blood.

Circulating adiponectin concentrations decrease with obesity. Also, they increase with caloric restriction, which occurs without an increase in adiponectin production by white adipose tissue. Dr Cawthorn's previous research in transgenic mouse models and humans showed that BMAT produces adiponectin and may contribute to some systemic effects specific to skeletal muscle. To build on this, more recent experiments were performed in rabbits as a useful model for analysing bone marrow in larger quantities. Under caloric restriction,

unexpectedly it was observed that adiponectin did not increase, in contrast to the increases observed in rodents and humans. Further analysis found that BMAT expansion was not occurring in rabbits with caloric restriction. In fact with greater caloric restriction, BMAT decreased further. There was also a reduction in leptin, and bone loss found with both levels of caloric restriction. These findings support the concept that, during caloric restriction, BMAT expansion may be required for increases in circulating adiponectin. They also demonstrate that decreased leptin is not sufficient to cause BMAT expansion, and that increased BMAT is not necessary for bone loss during caloric restriction.

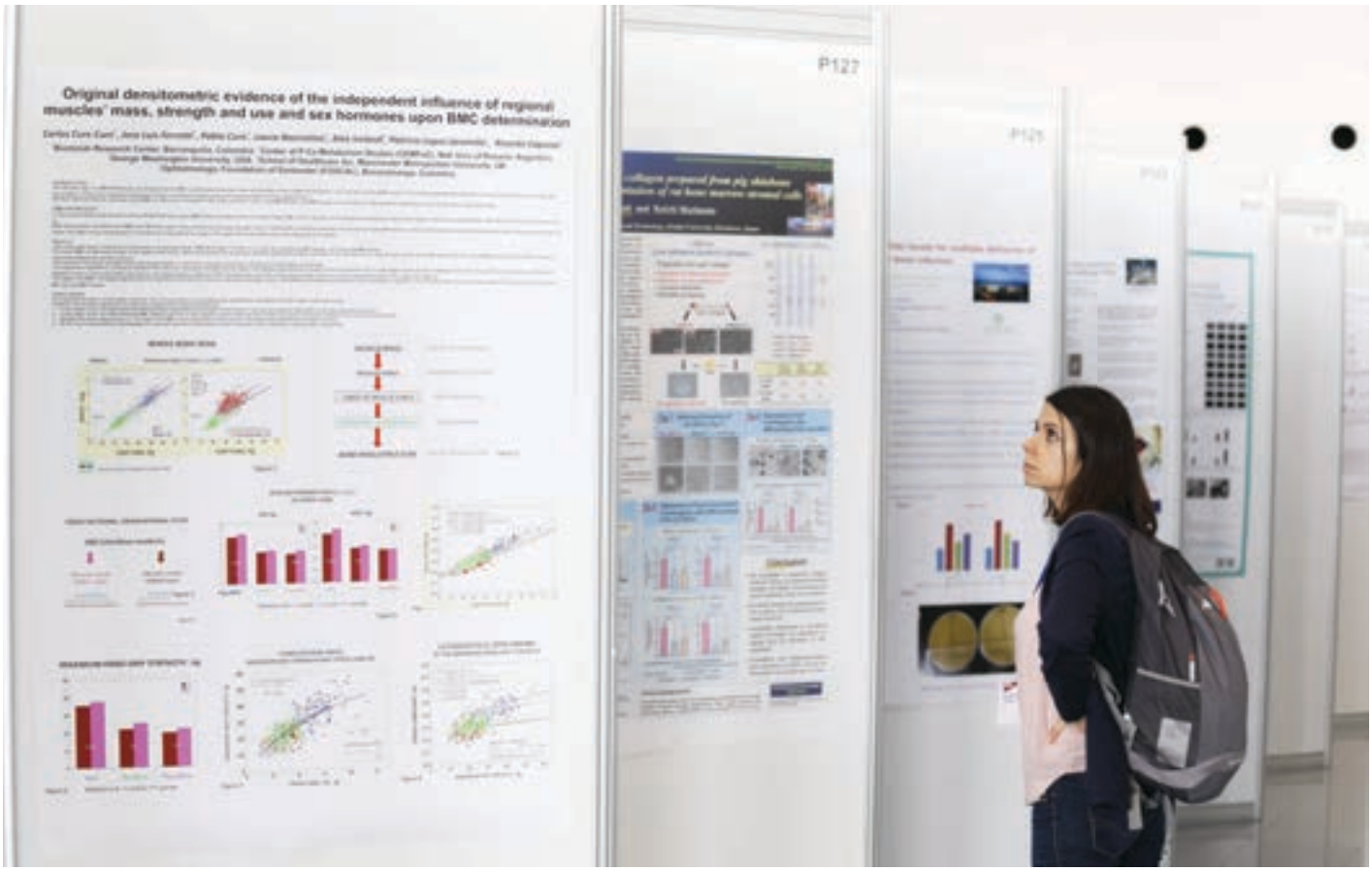
In order to understand these observations, and the systemic functions of adiponectin under caloric restriction, further studies are being performed in wild type and transgenic knockout mice. So far, circulating glucocorticoids were found to increase in the mice along with an increase in BMAT, and this was not found in the rabbit model, while some sexual dimorphism in the responses to caloric restriction in the knockout mice points to a more complex picture (unpublished data). The team at Edinburgh are also working to develop a mouse model that lacks BMAT completely, to help answer these questions about the function of BMAT.

Both speakers and several other delegates at the ECTS congress are involved in the new International Bone Marrow Adiposity Society, which is holding its next meeting in August 2018 in Lille.

ALSO PRESENTED AT ECTS 2018

FSH is positively associated with vertebral bone marrow adiposity in postmenopausal women from the AGES-Reykjavik cohort

Bone marrow adiposity increases with hormonal changes in postmenopausal women and is associated with osteoporosis and vertebral fracture, while follicle stimulating hormone levels reduce after menopause. **Annegreet Veldhuis-Vlug** and **Gina Woods** with collaborators from the USA and Iceland performed a cross-sectional, observational study in 237 women and 245 men from the AGES-Reykjavik cohort to investigate the association of FSH with bone marrow adiposity. In the women they found mean vertebral bone marrow adipose tissue was 0.65% greater for each 10 IU/L increment of FSH, while in men there was no association found. Longitudinal studies would be helpful to determine if FSH levels actually predict bone marrow adiposity in adults.



Inflammation and bone

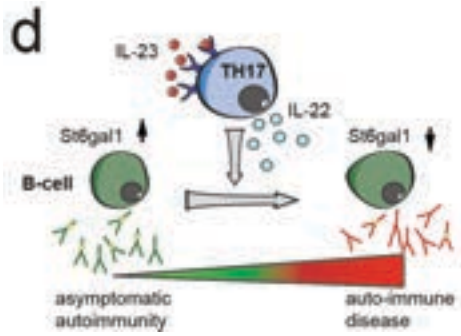
ECTS 2018
26-29 MAY 2018

The ECTS 2018 symposium on Inflammation and Bone discussed the latest progress in our understanding of the development of rheumatoid arthritis and how patients can be better assessed.

Gerhard Kronke (Erlangen) **started the session with his talk on the mechanisms underlying the onset of inflammation and bone loss in rheumatoid arthritis**. Around 1% of the adult population worldwide suffers from this disease, which is not yet fully understood and not yet curable. Bone erosions found in rheumatoid arthritis can develop quite early in the course of the disease, known to be linked to increased osteoclastogenesis and osteoclast activity.

However, rheumatoid arthritis is primarily driven by an autoimmune response, especially through anti-citrullinated protein antibodies (ACPA) produced by auto-reactive B cells, which also develops early in the disease pathogenesis. Many experts now think that rheumatoid arthritis starts not in the joints, but as a result of new antigen formation (triggered by citrullination) and inflammation in the mucosal tissues (oral cavity, gut) including the production of ACPAs, leading to a systemic disorder. ACPAs also directly induce osteoclast differentiation through direct binding to osteoclasts, which also express citrullinated antigens. There is also an observed correlation between a positive detection of ACPAs and accelerated bone loss in rheumatoid arthritis patients, and the detection of ACPA has been found to predate the onset of rheumatoid arthritis by as much as 10 years. So, auto-antibodies are not sufficient on their own to induce rheumatoid arthritis.

Studies by the Kronke group and others have shown that ACPA from patients that go on to develop RA have a differing glycosylation profile, as a result of a post-translational sialylation modification. The degree of IgG-sialylation has also been shown to control the capacity of ACPA to induce osteoclastogenesis and bone density in RA, including in patients, and in mouse models. Recently, studies have revealed an interaction of Th17 and B cells, dependent on IL-21 and IL-22, that switches B cells to a proinflammatory state and decrease sialylation of IgG. This modification in turn increases the proinflammatory activity of IgG and can account for the switch in rheumatoid arthritis from asymptomatic autoimmunity to inflammation and bone loss.



Regulation of Antibody activity by Th17 cells (G. Kronke)

Stephanie Finzel (Freiburg) **then discussed the available clinical approaches for assessment of bone loss in rheumatoid arthritis.** Radiography is still the gold standard, but it cannot detect bone erosions early enough for a successful treatment. High resolution peripheral quantitative computed tomography (HR-pQCT) has a very low irradiation and high contrast against soft tissues, so is suitable as a monitoring tool for hands or feet of RA patients. Dr Finzl's group has successfully used this tool to observe subchondral bone damage in rheumatoid arthritis patients in clinical remission, determine the contribution of ACPA and rheumatoid factor on bone erosions, and investigate bone changes in ACPA-positive asymptomatic patients. Here, early bone alterations were found, challenging the view that bone damage is only resulting from synovitis. HR-pQCT has also been used for monitoring of bone erosion repair in clinical studies, including for TNF inhibitors, and denosumab.

In view of "the reproducibility crisis", users of HR-pQCT have formed SPECTRA, a multidisciplinary consortium to collaborate on validating the technique as an outcome tool, especially to identify damage at an early stage. This has resulted in a more precise definition of bone erosion to discriminate them from vessel channels in HR-pQCT images, reliability exercises and work on an algorithm for the automated detection of cortical interruptions with HR-pQCT used alongside conventional radiography and MRI. In summarising, Dr Finzel highlighted the value of HR-pQCT as a highly sensitive tool for detection of bone changes as early as 12 weeks, especially useful in clinical trials, and is promising for clinical use alongside existing imaging techniques such as MRI and MSUS. However, more work is needed on validation, especially determining cut-off values to avoid false positives.

ALSO PRESENTED AT ECTS 2018

Characterisation of the role of the fractalkine receptor CX3CR1 in inflammatory osteoclasts

Maria-Bernadette Madel (Nice) presented new findings explaining how a sub-set of osteoclasts may exert an immune function through inducing TNFα-producing CD4+ T cells. Her group previously reported that deregulated immune responses are associated with osteoclastogenesis and severe bone destruction in inflammatory bowel disease. Now, the group have performed a RNA-Seq analysis comparing normal osteoclasts (that induce immunosuppressive CD4+ regulatory T cells) with inflammatory osteoclasts. Among 1484 genes that were differentially expressed, CX3CR1 (fractalkine receptor) was strongly upregulated in inflammatory osteoclasts, as well as 12 other CX3CR1-interacting genes. Further experiments in ovariectomised CX3CR1 knockout mice showed they had a significantly higher BV/TV and cortical bone thickness compared to ovariectomised wild type mice, and their bone marrow cells had reduced capacity for differentiating to osteoclasts. These results suggest a role for CX3CR1 in inflammatory bone destruction and work is continuing to determine the precise mechanism involved.

Mechanisms of action of the sphingosine 1-phosphate metabolic pathway in spondyloarthritis

Alaeddine El Jamal and colleagues (Lyon) had previously found elevated levels of sphingosine 1-phosphate in the serum of spondyloarthritis patients, and also obtained evidence from in vitro experiments that S1P metabolic pathway contributes to mineralisation of murine osteoblasts and chondrocytes. The group are now investigating possible mechanisms connecting elevated S1P levels to the ectopic calcification often found in enthuses of spondyloarthritis patients. The expression of S1P receptors have been found to increase 20 fold for osteoblasts and 60 fold for chondrocytes during their differentiation into mineralizing cells. Experiments with specific antagonists for S1P receptors S1P1 and S1P3 were used to investigate the activity of the pathway in these cells. Fingolimod, a FDA drug which blocks both receptors and sphingolipid metabolism in general had the strongest inhibitory effect on mineralisation and alkaline phosphatase activities. However, results from testing the specific inhibitors of each receptor suggest that S1P activity is restricted to the intracellular compartment of osteoblasts, while in chondrocytes both receptors may be involved in the mechanism. Overall, the study indicates that S1P metabolic pathway could be a potential therapeutic target in the treatment of spondyloarthritis.

Working Group on Rheumatology & Bone

Willem Lems (Amsterdam) chaired the session and gave the first talk, about osteoarthritis (OA) as a bone disease. He spoke about osteoporosis treatments and their impact on OA pathogenesis, noting that OA is characterised by increased bone turnover which can manifest as increased or decreased bone mass. Some emerging therapies for OA were touched on, including injection of recombinant FGF19 (spirifermin) to improve joint space width.

Kenneth Saag (Birmingham, Alabama) gave a tour of glucocorticoids in rheumatoid arthritis (RA) treatment. We learnt of increased fracture incidence in RA patients treated with glucocorticoids, which can be improved using bisphosphonates.

Piet Geusens (Maastricht) spoke about the use of Q-PCT (quantitative peripheral quantitative computer tomography) to detect signs of RA in the finger joint. Q-PCT could detect erosions and surface details associated with RA in finger joints, and it was noted that semi-automated Q-PCT works well in clinical applications.

From the accepted abstracts, **Hong Zhou** (Sydney) summarized her work on the role of glucocorticoids in OA pathogenesis in aged mice. Mice with decreased glucocorticoid signalling showed attenuated OA symptoms after surgical provocation compared to controls. The increase in glucocorticoid levels on ageing may play a role in OA pathogenesis.

Finally, **Janak Lai Pathak** (Guanzhou) discussed treatment of RA with the traditional Chinese medicine Bi-Qi. He noted that moderate doses of this widely-prescribed treatment alleviate symptoms of RA including inflammation and cartilage degeneration, and provided an interesting starting point for future studies on the effects of this drug.

ECTS supports New Investigators

ECTS 2018
26-29 MAY 2018

Kashmala Carys reports back to us on the interactive NI mentoring session, giving New Investigators the chance to discuss various career-related and technical topics with leaders in the field, and on the New Investigators seminar.

The NI Mentoring Session was a relaxed evening that started off with Spanish wine and networking.

The chairs of the session (**Katherine Staines** and **Bjorn Busse**) then directed us to an array of experienced academic and industrial professionals, ready to provide us with insight and direction to develop our career pathways. Set up akin to a 'speed-dating session', we were given the opportunity to sit and talk to these mentors in a group for around 10 minutes, after which we could move to another mentor.

The first mentor I had the pleasure of meeting was **André van Wijnen**. This meeting gave us, as mentees, the opportunity to understand how we take the next step to become the mentor. André explained the main difference between the thinking patterns of mentees and mentors, is that mentees need to think for themselves to direct their career to their career goal. In contrast, mentors need to consider and incorporate the personal goals of each team member/mentee in his/her laboratory. He likened this to having the ability to align arrows that naturally face in different directions, so they point in one parallel direction. This ultimately produces a motivated and productive laboratory for the long-term.

The last mentor I met was **Mathew Nelson** from GSK. This was another eye-opening meeting which allowed us, as young academics, to explore the other-side of the 'scientific career-coin'; working in industry. Matthew noted that even within industry, scientists can remain creative, helping to drive the research, and a good team within GSK will naturally do this. In response to our burning questions he added that a solid background in academia with published papers will set you in good stead to apply for a job role in industry, and the more experience you have, the better. Addressing a final misconception, he added that if you do decide to go into industry, the publications don't stop publications being one of the integral parts of working in industry.

A very well-managed and interactive session that will help to guide my life after a degree for years to come.

The New Investigators seminar was a laid back and fun scientific session arranged by the ECTS academy on the Sunday evening of the congress.

A range of projects was selected by the academy for oral presentations with the aim to win by an audience vote. From examining the strength of the fibula in footballers, to understanding why hypoglycemia in mothers leads to a decrease in foetal bone ossification, the range of topics managed to capture the importance in understanding the underlying relevancy of calcified tissue regulation.

After a great discussion and questions session, an anonymous vote was held. Deservedly, this was won by **Annika vom Scheidt** from Hamburg with her presentation "Mechanical characteristics of mineralized lacunae and surrounding bone tissue in osteoporotic and healthy humans". The next question on everyone's mind - who will win the volleyball?!

Insights from outside: the reproducibility crisis

ECTS 2018 26-29 MAY 2018

This year's Insights from Outside symposium posed some deep questions about how we do science.

John Ioannidis, Professor of Medicine and of Health Research and Policy at Stanford University, addressed the question "Can we save medicine?". His research shows that 96% of published literature during 1990-2015 claims to have obtained statistically significant results based on p-values, but this is simply too good to be true based on our real-life experience of obtaining negative results. Despite this apparent high level of success in discovery, translation of results is proceeding at a glacial pace.

How might we have created this situation? We incentivise certain kinds of research practices. A typical researcher's career aspiration to become a PI has usually involved working as a siloed, solo investigator, working on small projects and small sample sizes. Cherry-picking, or at least active mining of data in order to obtain results with a p-value < 0.05 considered "enough" for significance, is all too common. Data registration, sharing and replication of results are still not commonly practiced. In fact it has been estimated that at most, only 25% of preclinical research findings can be independently reproduced. Also, head-to-head non-inferiority comparisons appear to show a success rate of 96% in favour of the sponsor's treatment, when compared to non-industry sponsored trials. For preclinical studies in animal models, the use of blinding and randomisation is still very limited.

Even if a research result is credible, a further question is whether it is actually relevant and useful. For example is there a real problem to be solved, how does that fit with the priorities of patients, is a study large enough to be informative, and does it reflect real life, and is the benefit of the research worth money invested in it. A majority of clinical studies do not meet all the criteria of what may be defined as actually useful. This is even the case for studies published in major medical journals that are meant to publish only the highest quality research. An analysis of systematic reviews found that only 25 out of 1394 reviews published in the Cochrane database were based on high quality evidence, had a significant outcome and a favourable interpretation of the intervention.

All this points to a "medical misinformation mess" that is hard for clinicians and patients to interpret. Systematic reviews and meta-analysis might be thought helpful to consolidate findings, but Prof Ioannidis has identified major problems, including non-publication of negative results, redundant or unnecessary studies, and flawed analysis, with only around 3% being decent and clinically useful. Guidelines created by professional societies may also be susceptible to bias and self-preservation, for example expanding the number of people requiring treatment.

Marcus Munafò, Professor of Biological Psychology at the University of Bristol, explained more about the psychological issues affecting how we do science. His own field of psychology is similarly affected by the non-reproducibility of research results. Almost no-one intends to falsify data but as human beings we are affected by various cognitive biases and the pressure to publish results and get grants. Cognitively, human beings are designed to see patterns in the world around us, for example the famous "face on Mars", when there is really no real effect to observe. Most of us fall on a spectrum of behaviour somewhere between the perfectly objective disinterested scientist to the fraudster falsifying data, and can be susceptible to, for example post-hoc storytelling (the garden of forking paths), fishing for p-values just under 0.05, or overselling results.

Professor Munafò highlighted the contribution of small sample size to these problems, which can make it easier to obtain the larger effect sizes and small p-values to get a publication, but its been shown that larger studies are more likely to replicable. Financial rewards and salary structures, for example insecure non-tenured salaries and bonuses for publication in high-ranked journals, may also unconsciously or consciously affect the behaviour of scientists. The effect of citations has also been investigated. Positive, exciting results are more likely to be cited and it can be harder to find papers that contradict these results, and are less highly cited, affecting the visibility of studies in the literature and the presumed "self-correction" of scientific progress.

So what solutions could we apply to improve how we do science? John Ioannidis and Marcus Munafò described some helpful research practices that are

already starting to be introduced, which generally fall under the new paradigm of "Open Science". For example large scale collaboration research, as seen in genetic epidemiology, data sharing and data registration, more appropriate statistical methods ($p<0.005$?) and better handling of conflicts of interest. Conflicts of interest can include non-financial issues such as supporting a particular cause or lifestyle. Open access to data is appropriate in most cases, but there are also situations where restricted access is desirable, for example studies involving a few individuals with a particularly rare disease.

A renewed emphasis on publishing the methodology of studies is essential for the reproducibility of results. This should also include registration of the experimental protocol and analysis plan. Some journals have instigated "registration reports" with this information, pre-approved before the results are added, others have introduced "badges" to show where papers have employed Open Science practices. Research funders in the USA introduced an important reform for clinical trials in 2000, requiring all clinical trials to register their primary outcomes, addressing the practice of people changing their primary outcome during the trial. After this change there was a noticeable change in the number of trials reporting null or negative results.

Overall, if we can re-engineer the reward system and introduce quality control measures to incentivise the best science and researcher conduct, we can avoid incentivising sloppy or fraudulent research practices and get better and more useful research results in future. Understanding and aligning the interest of all stakeholders, including government, industry, researchers, medical professionals and the public is essential to achieve this goal.

Recipients of ECTS grants and awards at ECTS 2018

ECTS 2018
26-29 MAY 2018

ECTS Steven Boonen Clinical Research Award

Graeme Williams

The ECTS Steven Boonen Award is supported by AMGEN Europe

ECTS Iain T Boyle Award

Frank Oury

ECTS Excellence in Research Award

Hans van Leeuwen

ECTS Mike Horton Basic/Translational Award

Ralph Müller

ECTS Philippe Bordier Clinical Award

Eugene McCloskey

ECTS Fellowship Awards

Manuela Schoeb
Ciro Menale

ECTS New Investigator Awards

Simona Bolamperti
Lucie Bourgoin
Guilia Battfarano
Kashmala Carys
John Morris
Heike Weidner
Yohan Jouan
Miki Maeda
Maria-Bernardette Madel
Sofie Hertz Rønn

2018 ECTS Academy Clinical ePoster Award

Irma de Bruin

2018 ECTS Academy Basic ePoster Award

Florian Henning

ECTS Travel Awards

Petar Milovanovic
Victoria Leitch
Alaeddine El Jamal
K. Berit Sellars
Kazuhiko Matsuoka
Alexander Rodriguez
Ferran Jardi
Maude Gerbaix
Annegreet Veldhuis-Vlug
Francesca Brito

ECTS East-Meets-West Research Award

Yuuki Imai
Koichi Morimoto
Saori Kunii
Sung-Kil Lim
Yun-Sil Lee
Kwi Young Kang
Janak Lal Pathak
Yue Ding
Yu Jiang
Subhashis Pal
Gatha Thackes
Amit Saraf

The 2018 East-Meets-West Research Award is supported by AgNovos

2018 ECTS onsite-selected Clinical Oral Presentation Award

Katrine Hygum

2018 ECTS onsite-selected Basic Oral Presentation Award

Kazuhiko Matsuoka

Feedback on ECTS 2018

From twitter

@Chuesa Great talks on FGF23 in rare bone diseases by Wolfgang Hogler and Michael Econs during the pre-congress programme #ECTS2018

@AndreaSPollard What can zebrafish tell us about genetic regulation of skeletal growth? Lots, it turns out! Super interesting talk by Matthew Harris #ECTS2018

@Osteoporosis_NLSuperb critical overview about anabolic / sequential therapies Lorenz Hofbauer #ECTS2018 ...how I would treat my mother..

@germdave Thank you, Valencia, #ECTS2018, @ECTS_soc, and all delegates for a wonderful conference! Deiby out!

From congress delegates

This is the second time I came to ECTS, I do enjoy the mix of clinical and research put together....its really good for researchers to see the clinical context more than strongly you would through papers, the really big thing about ECTS is that cross talk (post-doc)

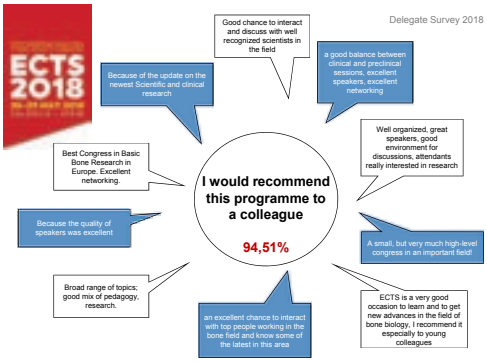
We both had 1 minute SNAP presentations...it's a good opportunity to attract some people to your poster, even if its frustrating as researchers not to be able to talk as much as you would like! (post-doc)

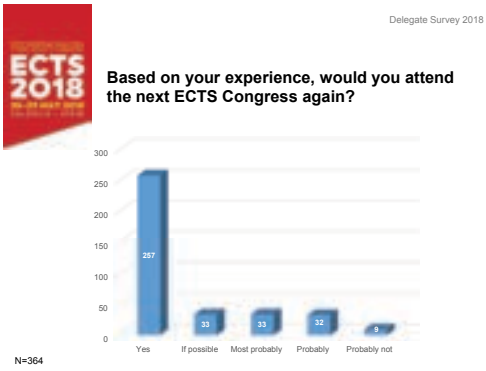
I'm from South Korea, this is my second time at ECTS. I'm totally satisfied with the scientific programme covering from the basic research to the clinics. I get many new ideas from the plenary lectures and I'm hoping to develop some joint projects between East and West (orthopaedic surgeon)

From the delegate survey

Over 350 delegates completed the delegate survey through the ECTS 2018 congress app, with the results now analysed showing high levels of satisfaction with the congress quality and organization (see below).

Overall, 94.5% of responding delegates would recommend the programme to a colleague, and 97.5% would be definitely or probably be interested to attend ECTS congress again.





ECTS through the year

ECTS is the main organisation in Europe for researchers and clinicians working in the musculoskeletal field, and in addition to the annual congress there is a busy programme of activities through the year.

ECTS Educational activities include the ECTS PhD training course (next one is September 1st-4th 2018 in the Netherlands), webinar series' "Bone, Muscle and Beyond" and "ERC grant winners" and online resources including webcasts from our past congresses, presentations, webinar recordings, abstracts and many other e-learning tools.

The Society also provides a range of travel awards, grants and fellowships for young investigators.

ECTS is also active in promoting the field of bone research in cooperation with other national societies worldwide and there is an ongoing publication of position and policy papers on relevant topics.

Members of the ECTS receive benefits including reduced registration fees for ECTS events and enhanced access to online resources. For more details on how to join, please see our website.

ECTS Academy

The daughter society of ECTS started in 2015 and supports new investigators in developing their careers in the musculoskeletal field, including both basic and clinical researchers.

As well as organising its own programme during the annual congress, the ECTS Academy organises networking and mentoring, and benefits include free registration at the ECTS annual congress and a personal research grant.

Membership lasts 5 years and is awarded through an annual application process. Profiles of the current members can be viewed here. Congratulations to the latest members of ECTS Academy members were announced in Valencia!

Natalie Butterfield
Melanie Haffner-Luntzer
Marietta Herman
Ciro Menale
Petar Milovanovic
Andrea Palermo
Ling Oei

Applications will next be open in autumn 2018, see here for more details.

Twitter @AcademyEcts