uptake of an effective treatment for osteoporosis due to patient concern. The risk of AFF is increased 6- to 7-fold in patients of Asian ethnicity compared with Europeans. Genetic factors may underlie the AFF phenotype. Identifying genetic associations with AFF is important to facilitate precision medicine in osteoporosis treatment. Given the rarity of AFFs, studying familial AFF cases is valuable in providing insights into any genetic predisposition.

Methods: We present two Singaporean families, one of which comprising a mother (I-1) and a daughter (I-2), and the other comprising two sisters (II-1 and II-2). All 4 cases presented with bisphosphonate-associated AFF. Whole exome sequencing was performed on I-2, II-1 and II-2. DNA for I-1 was not available. Variants were then examined using a candidate gene approach comprising a list of genes previously associated with AFF in the literature, as well as using hypothesis-free models of dominant and recessive inheritance.

Findings: Using a candidate gene approach, rare variants shared between all three cases were not identified. A rare variant in *TMEM25,* shared by the two sisters with AFF (II-1 and II-2), was identified. A rare heterozygous *PLOD2* variant was present in the daughter case with AFF (I-2), but not the sisters. A list of potential genetic variants for AFF was identified with inheritance model-based analyses in the two sisters (II-1 and II-2), including Gly35Arg variant in TRAF4, a gene required for normal skeletal development.

Conclusion: Although the findings from this genetic analysis are inconclusive, the existence of AFFs in families is suggestive of a genetic component in AFF pathogenesis. We provide a comprehensive list of rare variants identified in these AFF familial cases to aid future genetic studies.

P164
Gene burden testing of a large patient cohort identifies potential gene candidates for atypical femur fractures

Wei Zhou[a], Jeroen van Rooij[a], Cat Shore-Lorenti[b], Hanh Nguyen[b], Denise M. van de Laarschot[a], Joost Verlouw[a], Shoshana Sztal-Mazer[c], Vivian Grill[d], Bram C.J. van der Eerden[a], Roderick Clifton-Bligh[e], Annemieke J.M.H. Verkerk[a], Peter R. Ebeling[b], M. Carola Zillikens[a]

[a]*Erasmus Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*
[b]*Monash University, Department of Medicine, Clayton, Australia*
[c]*The Alfred Hospital, Department of Endocrinology and Diabetes, Melbourne, Australia*
[d]*Western Health, Department of Endocrinology and Diabetes, Melbourne, Australia*
[e]*University of Sydney, Faculty of Medicine and Health, Sydney, Australia*

Background: Genetic factors have been suggested to play a pathogenetic role in atypical femur fractures (AFFs) in addition to bisphosphonates. Understanding the role of genetics in AFFs may help elucidate the AFF pathogenesis and allow precision medicine in osteoporosis. Although several studies have investigated the role of genetics in AFFs, results have not been replicated. The current study aims to identify candidate genes involved in AFFs by gene-based burden testing in a relatively large set of AFF cases using whole exome sequencing (WES) data.

Methods: A total of 145 AFF patients were recruited from the Netherlands and Australia. 196 controls without AFF, but with >3 years bisphosphonate use, were recruited from the Rotterdam Study, a well-established, population-based cohort study. Gene-based burden testing was performed with protein-altering variants that have a population frequency <0.01 in gnomAD. Genes with nominal

p-value < 0.01 by Fisher's exact test were considered suggestive. Top genes were compared with previous genetic studies of AFF and their bone-related functions were evaluated through multiple data sources.

Results: We identified 29 suggestive genes. The *SORD* gene's expression is associated with a SNP eQTL in cultured fibroblasts in the GTEx project, which is in LD ($r^2 = 0.29$) with rs62026663, a previously identified AFF GWAS SNP. Four variants were included for burden testing in *SORD*, with 10 carriers in the cases compared to 2 carriers in the controls (OR = 7.15, 95% CI: 1.49~68.22). Other suggestive genes for AFFs based on bone-related functions include *SLC29A3*, *COL18A1*, *PCNT*, *ZHX3* and *SPTA1*.

Conclusions: This study identified multiple genes potentially underlying bone biology in AFF and describes the largest AFF-patient WES dataset to date. In addition, it provides a stepping-stone for future genetic studies of AFF. The current findings merit replication through larger international collaborations.

P166
β2-adrenoceptor-deficient mice exhibit exacerbated subchondral bone remodeling in experimental osteoarthritis

Gundula Rösch[a], Dominique Muschter[b], Shahed Taheri[c], Karima El Bagdadi[a], Christoph Dorn[d], Andrea Meurer[a], Frank Zaucke[a], Arndt F. Schilling[c], Susanne Grässel[b], Rainer H. Straub[e], Zsuzsa Jenei-Lanzl[a]

[a]*Dr. Rolf M. Schwiete Research Unit for Osteoarthritis, Department of Orthopedics Friedrichsheim- University Hospital Frankfurt- Goethe University, Frankfurt, Germany*
[b]*Department of Orthopedic Surgery- Experimental Orthopedics- Centre for Medical Biotechnology, University of Regensburg, Regensburg, Germany*
[c]*Department of Trauma Surgery- Orthopedic Surgery and Plastic Surgery, Universitätsmedizin Göttingen, Göttingen, Germany*
[d]*Institute of Pharmacy, University of Regensburg, Regensburg, Germany*
[e]*Laboratory of Experimental Rheumatology and Neuroendocrine Immunology, Department of Internal Medicine- University Hospital Regensburg, Regensburg, Germany*

Introduction: Previous studies demonstrated, that adrenoceptors (ARs) are expressed in all joint tissues indicating a contribution of the ARs to osteoarthritis (OA) progression. Based on *in vitro* experiments, the β2-AR subtype appears to have a major role.

Purpose: To unravel the role of the β2-AR during OA pathogenesis *in vivo*, we examined the progression of surgically-induced OA in β2-AR-deficient (Adrb2$^{-/-}$) mice.

Methods: OA was induced by destabilization of the medial meniscus (DMM) in wildtype (WT) and Adrb2$^{-/-}$ mice. 8 weeks after DMM or sham surgery, subchondral bone remodeling (µCT), osteoblast and osteoclast activity, cartilage degradation, as well as synovial inflammation (histological scorings), body weight and serum leptin levels were analyzed.

Results: Bone volume fraction (WT: 0.563±0.029, Adrb2$^{-/-}$: 0.713±0.024; p=0.006) and subchondral bone plate thickness (WT: 100.7±3.102 µm, Adrb2$^{-/-}$: 170.5±11.84 µm; p<0.001) as well as calcified cartilage thickness (WT: 64.54±2.072 µm, Adrb2$^{-/-}$: 74.68±2.789 µm; p=0.01) significantly increased, while osteoclast activity decreased (WT: 2.316±0.572 %, Adrb2$^{-/-}$: 0.526±0.068 %; p=0.002) in Adrb2$^{-/-}$ compared to WT mice after DMM. There were no significant differences between WT and Adrb2$^{-/-}$ animals after sham surgery. The progression in cartilage degeneration and synovial inflammation was comparable in WT and Adrb2$^{-/-}$ DMM mice without significant differences. Adrb2$^{-/-}$ mice had significantly

higher body weight and fat mass compared to WT mice (WT: 28.56 $\pm$0.275 g, Adrb2$^{-/-}$: 33.54$\pm$1.181 g; p<0.001). DMM in Adrb2$^{-/-}$ mice lead to elevated serum leptin levels (WT: 8.483$\pm$2.449 ng/ml, Adrb2$^{-/-}$: 85.396$\pm$40.07 ng/ml; p<0.001).

Conclusion: We assume, that the changes in the subchondral bone remodeling in Adrb2$^{-/-}$ DMM were not only β2-AR deficiency-dependent, but also due to a synergistic effect of OA and elevated leptin concentration Taken together, the β2-AR plays a major role in OA-related subchondral bone changes and is therefore an attractive target for novel therapeutic avenues.

P167

A predictive human 3D cartilage-on-chip model for screening anti-osteoarthritis drugs and medical devices

Paola Occhetta[a], Stefano Piazza[a], Tiina Kähkönen[b], Jussi Halleen[c], Roberta Visone[a], Marco Rasponi[a]

[a]BiomimX, Na, Milan, Italy
[b]OncoBone Ltd, Na, Oulu, Finland
[c]OncoBone, Na, Oulu, Finland

Osteoarthritis (OA) is a prevalent musculoskeletal disease with no treatments available to restore degraded cartilage or decelerate OA progression. Predictive in vitro screening models are needed to advance drug development of disease-modifying OA drugs (DMOADs).

In this study we demonstrate validation of a novel human mechanically active 3D osteoarthritic cartilage-on-chip model, uKnee. In this unique model, 3D cartilage-like constructs are subjected to a hyper-physiological mechanical microenvironment provided by a proprietary uBeat® technology that triggers OA-induced changes in primary human articular chondrocytes. The cartilage-on-chip model was used for studying effects of standard-of-care (SOC) therapies.

The development of a stable cartilage phenotype was evident after a 2-week culture of human primary chondrocytes as analysed by increased expression of specific genes (e.g. *ACAN* and *PRG4* for articular cartilage; *GDF5* and *ATX* for joint interzone; *FRZB and GREM1* as hypertrophy brakes) and by the deposition of a cartilage-like matrix assessed with immunofluorescence staining (e.g. Aggrecan, Collagen II). When these OA microtissues were subjected to a hyper-physiological confined compression for 7 days (uBeat, 30% at 1 Hz), induction of OA-like gene expressions was observed, including enhancement of catabolic and inflammatory responses (e.g. increased *IL-8* gene expression, MMP13 gene and protein upregulation), and switching towards hypertrophic cartilage phenotype (e.g. *COL10A1* upregulation, *FRZB*, *GREM1* downregulation). When the OA microtissues were treated with SOCs (i.e., Rapamycin, Celecoxib, IL-1Ra and dexamethasone) or two hyaluronic acid -based medical devices for 3 days, a reduction in the expression of *MMP13* and *IL8* was observed.

As a conclusion, hyper-physiological mechanical stimulation is fundamental for eliciting OA pathogenesis in the validated uKnee cartilage-on-chip model of OA, and uKnee is a validated model for predictive screening of DMOADs and medical devices.

P168

Mechanical loading prevents arthritis-induced bone loss in male mice

Suchita Desai[a], Jianyao Wu[b], Karin Gustafsson[b], Maria Norgard[a], Claes Ohlsson[b], Sara Windahl[a], Cecilia Engdahl[c]

[a]Karolinska institute, Laboratory Medicine- division of pathology, Stockholm, Sweden
[b]Gothenberg, Department of Internal Medicine and Clinical Nutrition, Gothenberg, Sweden
[c]Gothenberg, Rheumatology and inflammation research, Gothenberg, Sweden

Arthritis induces inflammation-mediated bone loss. This bone loss can be prevented by loading in female mice, but loading may also cause damage to the joint. It is unknown whether non-steroidal anti-inflammatory drugs (NSAIDs) can reduce inflammation without affecting the loading-associated bone formation in male mice. The aim of this study was to investigate whether loading and NSAIDs can prevent arthritis-induced bone loss and inflammation in male mice. Four-months-old male C57BL/6J mice were loaded 3 times/week for 2 weeks. Local mono-arthritis was induced with a systemic injection of mBSA at the first loading session, followed by a local injection one week later in one knee. Arthritis was evaluated by assessment of the swelling over the knee and histological scoring. Cortical and trabecular bone parameters were assessed by DEXA and µCT. Markers of bone remodelling (CTX and PINP) were analysed in serum. Loading of the arthritic knee enhanced swelling of the knee (+226% and +84% respectively, p<0.001), synovitis (+60% and +93% respectively, p<0.01), and articular cartilage damage (+75% and +43% respectively, p<0.01) compared to the arthritis or loading alone. Addition of NSAIDs reduced swelling of the loaded arthritic knee (-13%, p<0.001). Loading prevented the arthritis-induced decrease in metaphyseal trabecular thickness (+196%, p<=0.001) and epiphyseal BMD (+108%, p<0.05). Cortical area was increased by loading (16%, p<0.01) but unaffected by arthritis. NSAIDs did not alter the bone protective effects of loading. Serum CTX and PINP were not altered by any treatment. In conclusion, loading prevented arthritis-induced trabecular bone loss, and NSAIDs reduced inflammation in the knee without affecting the bone protective effects of loading. If our results can be extrapolated to the human situation, NSAIDs could be used in combination with loading exercise to prevent pain, inflammation, and arthritis-induced bone loss.

P169

Association between long-term exposure to air pollution and immune-mediated diseases: a population-based cohort study

Giovanni Adami[a], Angelo Fassio[a], Marco Pontalti[a], Camilla Benini[a], Davide Gatti[a], Maurizio Rossini[a]

[a]University of Verona, Rheumatology Unit, Verona, Italy

Background: Environmental air pollution has been associated with disruption of the immune system at a molecular level.

Purpose: The primary aim of the present study is to describe the association between long-term exposure to air pollution and risk of developing immune-mediated conditions.