Methods FAPα expression in vivo was determined by immunohistochemistry in human synovial tissues of patients with RA and osteoarthritis (OA) as well as in hind paws of tumour necrosis factor-alpha transgenic (hTNFtg) mice, which develop a RA-like destructive arthritis. In vitro expression and regulation of FAPα was analysed in differentiated osteoclasts cocultured with osteoblasts, RA or OA synovial fibroblasts (SF) by PCR. To scrutinise the role of FAPα in osteoclastogenesis, we analysed the in vitro differentiation of osteoclasts from wildtype (WT) and $FAP^{-/-}$ mice (Oncology Research, Boehringer Ingelheim RCV, Vienna) by TRAP staining. In order to assess the role of FAP in arthritis severity, we crossed $FAP^{-/-}$ and hTNFtg mice and performed TRAP staining.
Results RA synovial tissues demonstrated a high expression of FAPα throughout the tissue whereas in OA samples FAPα was expressed only in the lining layer. In vitro, no expression of FAPα was found in differentiated preosteoclasts and osteoclasts, but coculture experiments showed that RASF, but not OASF or osteoblasts, induce the expression of FAPα in preosteoclasts and osteoclasts. Consistent with the selective induction of FAPα in osteoclasts by RASF, FAPα expression was detected in osteoclasts at the invasion front of the hyperplastic synovial tissues in joints of hTNFtg mice. $FAP^{-/-}$ mice show a severely diminished osteoclast formation compared to WT. We also found a lesser amount of osteoclasts in the hind paws of $FAP^{-/-}$hTNFtg compared to hTNFtg.
Conclusions The disease-dependent expression of FAPα by osteoclasts in human RA and hTNFtg mice suggests an important role of FAPα in joint destruction in RA. The selective induction of FAPα in preosteoclasts and osteoclasts by RASF indicate that FAPα may be regulated through the interaction with the pannus tissue. The fact, that under inflammatory conditions the loss of FAP led to a reduced number of osteoclasts in the hind paws and FAP deficient bone marrow derived macrophages showed a reduced osteoclast formation, suggest a role of this serine protease in macrophage/osteoclast precursor migration and differentiation.

A8.6 FRZB IS A CRITICAL MODULATOR OF CANONICAL WNT SIGNALLING IN CARTILAGE BIOLOGY

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Background and Objectives Polymorphisms in Frizzled-related protein (*FRZB*), a WNT antagonist, have been associated with osteoarthritis (OA). However, a recent meta-analysis failed to find a consistent effect of FRZB genetic variants on OA susceptibility. Our transcriptomics analysis in $FRZB^{-/-}$ mice provided evidence for a tight regulation of WNT signalling and highlighted the complex role for *FRZB* in joint homeostasis. We previously demonstrated that $FRZB^{-/-}$ mice have increased damage when dramatically challenged by papain, collagenase or severe inflammation. As these models are acute and short-term, we aimed to further investigate the effect of *FRZB* loss in a true translational model of OA and to study molecular interactions in the ATDC5 micromass in vitro model using RT-PCR and Western blot analysis.
Materials and Methods Surgical destabilisation of the medial meniscus (DMM) was performed on the right knee of eight-week-old male $FRZB^{-/-}$ and wild-type mice. Eight weeks after surgery, mice were sacrificed and histological scores were determined for the femoral and tibial articular surfaces following the OARSI histopathology initiative guidelines.
Results Overexpression of *FRZB* in ATDC5 micro-masses boosted chondrogenesis with up-regulation of *Col2a1* and *Aggrecan* transcription, whereas downregulation of *FRZB* lead to a decreased expression of *Col2a1* and *Aggrecan*. These results corresponded with a reduction or increase in the activation of canonical WNT signalling pathway, respectively. Fluctuating levels of *FRZB* did not influence the Wnt/CamKII signalling pathway. The semi-quantitative *OARSI* score showed a significant increase in cartilage erosion in DMM-operated $FRZB^{-/-}$ mice compared to wild-type.
Conclusions Our data show that, in addition to the higher susceptibility to OA in acute induced models, $FRZB^{-/-}$ mice are more prone to OA in a full translational model of the disease characterised by slowly progressive joint damage. Overexpression of *FRZB* stimulates chondrogenesis by its inhibitory role on Wnt/beta-catenin signalling.

A8.7 FUNCTIONAL IMPAIRMENT IN AN ANIMAL MODEL FOR RHEUMATOID ARTHRITIS ASSESSED AS CHANGES IN GAIT IS DUE TO JOINT DESTRUCTION BUT NOT SYNOVIAL INFLAMMATION *PER SE*

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Objective To investigate the individual impact of synovial inflammation, subchondral bone erosion or cartilage damage on functional impairment in an animal model of Rheumatoid Arthritis (RA).
Methods We analysed gait profiles in human tumour necrosis factor transgene (hTNFtg) animals, using the video-based Catwalk gait analysis system (from Noldus, Netherlands). In this system, mice run along an illuminated glass plate. A digital camera measures light emissions resulting from the contact of paws on the glass plate. We evaluated gait profiles at different time points of disease (6, 10, 15 week of age) in hTNFtg animals. Wildtype littermates served as controls. Bodyweight and clinical signs of arthritis including paw swelling and grip strength were also evaluated. To investigate whether gait changes are pain related, we treated hTNFtg animals with diclofenac (50 mg/kg, i.p.) at week 10 and week 15 after birth and analysed gait profiles before, as well as 1 h and 3 h after treatment. To investigate reversibility in impaired gait profils, we treated hTNFtg mice with anti-TNF (Infliximab 10 mg/kg body weight, 2× per week) for 5 weeks starting 6 and 10 weeks after birth. To analyse inflammatory joint destruction, we quantitatively assessed the extend of synovial inflammation, subchondral bone erosion and cartilage damage on hematoxylin and eosine (H&E), tartrate-resistant acid phosphatase (TRAP) and toluidine-blue stained paw sections. We performed correlation studies between gait parameters and the histopathological components as well as clinical signs.
Results We identified several gait parameters among them weight bearing, stride length and contact area of the paw to be significantly decreased in hTNFtg animals compared to sex- and age-matched wildtype animals. Moreover, we found a marked reduction in maximum intensity, an indicator for weight bearing, in week 10 and 15 compared to week 6 old hTNFtg mice. Similar effects were seen in print width, print area, print length, max contact max intensity and max contact area at different stages of disease. Interestingly, analgesic treatment with diclofenac (50 mg/kg, i.p.), resulted in a better improvement of weight-bearing parameters in 10 week old hTNFtg mice than in 15 week old hTNFtg animals indicating an pain independent, irreversible functional impairment in progressed disease. To further investigate to which extend synovial inflammation, subchondral bone erosion or cartilage damage are responsible for the functional impairment of joints, we correlated these components with changes in different gait parameters. We observed strong correlations of various gait parameters with the amount of cartilage damage, whereas subchondral bone erosions correlated to a lesser extend and synovial inflammation did not correlate at all.