molecular chaperone activity (clustering). Interestingly, other proteomic studies have identified clustering and CILP-1 as being upregulated in early human OA. These biomarkers may be involved in early repair responses in cartilage and thus may be useful for the detection of early changes in OA.

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**A41** EPHB4 RECEPTOR ACTIVATION BY ITS SPECIFIC LIGAND EPHRIN B2 INDUCED A REDUCED REMODELING PROCESS IN HUMAN SUBCHONDRAL BONE OSTEОСLASTS

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**Purpose:** Ephrin is well documented in the literature for its functions in the nervous and cardiovascular systems. However, it has recently been shown that the interaction of the EphB4 receptor with its specific ligand ephrin B2 could be implicated in the physiological regulation of bone homeostasis. Osteoarthritis (OA), although characterized by cartilage breakdown and inflammation of the synovial membrane, also demonstrates important changes in the subchondral bone. Recent studies have shown that an abnormal remodeling process of this tissue is intimately involved in the genesis of OA. We recently classified human OA sub-chondral bone osteoclasts into two subgroups according to their low (L) or high (H) PGE2 levels. Further data demonstrated that the L-OA showed bone pro-resorption activities and the H-OA pro-formation properties. In this study we determined the presence, level and modulation of EphB4 receptors on each of these subgroups. Further, we investigated the modulation of EphB4 receptors and the effect of their activation by ephrin B2 on OA osteoclasts.

**Methods:** The in situ presence of EphB4 receptors in the subchondral bone was determined by immunohistochemistry. The EphB4 receptor expression level, modulation upon treatment with osteotropic factors, and effect of activation by ephrin B2 on bone catabolic mediators were determined using real-time PCR. The ephrin B2-activated EphB4 receptor effect on the bone resorption activity was also determined using a co-culture system of differentiated human PBMC and human subchondral bone osteoclasts. The intra-cellular signaling pathways employed by the EphB4 receptor activation were investigated by specific ELISA.

**Results:** Data showed that EphB4 receptors were present in the human subchondral bone osteoclasts and osteocytes. A significant increase in EphB4 receptor expression level was found in the L-OA osteoclasts compared to the normal (p < 0.0002) and the H-OA (p < 0.0007). However, there was no difference between the normal and the H-OA. EphB4 receptor levels in the L-OA osteoclasts were significantly up-regulated by PGE2 and IL-17. Interestingly, ephrin B2, PGE2 and IL-17 significantly inhibited the bone resorption activity in L-OA osteoclasts. EphB4 activation by ephrin B2 significantly inhibited the expression level of the pro-inflammatory cytokines II-1β and II-6, the metalloproteases MMP-1, MMP-9 and MMP-13, as well as RANKL. The factors MMP-2 and OPG were not modulated. The EphB4 receptor activation significantly reduced the PI3K/Akt pathway, but had no significant effect on the MAP kinases.

**Conclusions:** This study, for the first time, provides evidence that EphB4 receptor activation by ephrin B2 in human OA subchondral bone could impact abnormal metabolism in this tissue by inhibiting resorption factors and their activities. The differential level of EphB4 receptors in the L-OA and H-OA subchondral bone osteoclasts also indicates that these cells harbor the potential to start different pathological pathways. Data from this study will help to light that ephrin B2 could be targeted as a specific therapeutic approach in the development of a disease modifying OA drug, as this factor could exert a protective effect on OA articular tissue structural changes.

**A42** A GENOME-WIDE ASSOCIATION STUDY REVEALS A NOVEL LOCUS FOR HAND OSTEOARTHRITIS

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**Purpose:** Although a significant genetic effect on hand osteoarthritis (OA) has been reported, confirmed replicated genetic factors have not yet been identified. The aim of this study, therefore, was to identify specific genetic polymorphisms for hand OA by means of the genome-wide association study (GWAS) in Caucasian population.

**Methods:** A three stage approach was utilized. First, we conducted a GWAS implementing 317,818 SNPs across the entire genome in 651 women of the North European origin from the TwinsUK registry. Next, we tested the top 100 SNPs identified in our GWAS in the Rotterdam cohort, consisting of 1438 men and women aged over 50. In the final stage, we searched for confirmation of the association between the OA and SNPs that showed the most significant association results in the second stage. To this aim we used additional independent population-based sample – the Chingford Study, including 671 British women. All participants in the study had their both hands X-rayed, and 15 joints on each hand (DIP, PIP, MCP, and first CMC) were scored for OA, using Kellgren-Lawrence (KL) system. The total KL score adjusted for age (and sex) was used in the analysis.

**Results:** We identified replicated evidence of an association between the SNP which is located in chromosome 16p13 and hand OA. The minor allele with a frequency of 32% significantly decreased (OR=0.86; p=4.86×10−11) in all three cohorts combined. When we categorized the participants of the TwinsUK and the Chingford study into two groups: people who had at least two joints of hands affected, defined by KL ≥ 2, vs individuals who didn’t have any hand’s joints affected, the minor allele was associated with a 33% reduction of risk in the development of hand OA (p=0.00002). The risk was further reduced to 41% if the cases are defined as at least three joints affected (p=0.00001).

**Conclusions:** This genome-wide scan identified a significant association between OA and a novel locus which has a potential role in transcriptional regulation of calciitonin. This finding provides insight into previously unknown genetic mechanisms in the development of hand OA.

**A43** THE RELATIONSHIP OF DENUDED SUBCHONDRAL BONE AREA TO KNEE PAIN SEVERITY AND INCIDENT FREQUENT KNEE PAIN

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**Purpose:** Subchondral bone has attracted attention as a potential source of pain in knee OA in part because of its intimate relationship to overlying cartilage and its load bearing function. With full thickness cartilage defects, exposed bone may be more likely to generate joint pain during contact and weightbearing activity. We hypothesized that (1) increased %denuded bone in OA knees is associated with increased pain and (2) greater %denuded bone predicts incident frequent knee pain.

**Methods:** All participants had knee OA by definite osteophytes. Axial and radiolucent oblique c-spine SPGR sequences were acquired on a 1.5T or a 3T scanner. Images were processed with proprietary software (Chondrometrics). Manual segmentation by trained readers was used to compute total area of subchondral bone, cartilage surface area, and cartilage-covered and denuded areas of bone for the medial tibia, lateral tibia, weightbearing medial and lateral femur, and patella. Pain severity was assessed with a knee-specific 100 mm VAS for pain in the past week. Frequent knee pain was defined as pain in or around the knee for most days in the past month. To test hypothesis 1, we used median quartile regression to determine the increase in median pain score per 10% increase in denuded bone, analyzing the more painful knee. To test hypothesis 2, we used logistic regression with GEE to estimate the odds ratio (OR) per 10% increase in denuded bone at baseline for baseline-to-2 year incident frequent knee pain. Analyses were adjusted for age, gender, BMI, K/L grade, and bone edema score.

**Results:** 184 participants (age 66, BMI 30, 78% women) made up the sample. As in Table 1, greater %denuded bone in the noted medial surfaces was associated with increased median pain score. 176 knees in 119 persons (age 68, BMI 29, 74% women) did not have frequent knee pain at baseline and made up the sample for hypothesis 2. Of the 176 knees, 53 developed frequent knee pain over the next 2 years. As in Table 2, greater %denuded bone at baseline was associated with an increased odds for incident frequent knee pain.

**Conclusions:** Greater %denuded bone was associated with greater knee pain severity. In knees without frequent pain at baseline, %denuded bone predicted incident frequent knee pain. These results support that the exposure of subchondral bone may be a source of pain in knee OA.