analysis for creatinine and the TIINE peptide and stored at −70°C until analysis.

Results: Method performance and validation characteristics were established for the immunoaffinity LC-MS/MS assay aimed at measuring the 45-mer with 5HyPin in human urine. Validation experiments were designed to address specific challenges related to quantification of endogenous analytes. The validated method has been shown to be sensitive (lower limit of quantification (LLOQ) 0.156 ng/mL), selective, accurate (<15% RE), and precise (<15% CV) over a linear range of 0.156–7.5 ng/mL. The recovery of the 45-mer peptide from the urine samples of 6 subjects was ranging from 94.2%–108%, which indicated that the recovery was urine matrix independent. Accuracy and precision of spiked dilution linearity samples at 2, 4, 10, and 20-fold dilutions were <15% RE and <15% CV, respectively. Sample stability and inter- and intra-subject variability have been evaluated in the urine of normal and OA populations. The method has been applied to analyze human urine samples from clinical studies. Urinary TIINE 45-mer was consistently found to be elevated in symptomatic patients with X-ray OA in the knee or hip (135 studies. Urinary TIINE 45-mer was consistently found to be elevated in CV, respectively. Sample stability and inter- and intra-subject variability has been successfully employed in clinical studies aimed at validating the 45-mer TIINE peptide as a biomarker for MMP activity and its role in osteoarthritis.

Microarray Gene Expression Profiling of Human Osteoarthritic Bone Suggests Altered Bone Remodelling, WNT and TGF Beta/BMP Signalling

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Purpose: In addition to degenerative changes in the articular cartilage, osteoarthritis (OA) is characterised by alterations to subchondral bone. Changes to bone in OA, which include increased bone volume fraction and reduced bone mineralisation, have also been identified at sites distal to the affected joint. Altered bone remodelling has been proposed to underlie these bone changes in OA. To investigate the molecular basis for these changes, we performed gene expression analysis in bone from the proximal femur of OA and control individuals.

Methods: Messenger RNA prepared from cancellous bone samples, obtained from the proximal femur, were used for both targeted gene analysis, investigating genes thought to be centrally involved in bone turnover, and gene microarray, using Compgen human 19K-oligo microarray slides. In the latter experiments, we compared the gene expression profiles of four sets of OA, CTL and OP bone samples (40 comparisons in total), comprising 10 OA – CTL female, 10 OA – CTL male, 10 OA – OP female and 10 OP – CTL female sample pairs.

Results: We identified clear expression differences between genes such as osteocalcin, osteopontin and type I collagen, between control and OA bone. In the microarray experiments, a large number of differentially expressed genes were identified, and twenty-five of these genes were confirmed to be differentially expressed (p<0.01) by real time PCR analysis. A substantial number of the top-ranking differentially expressed genes identified in OA bone are known to have roles in osteoblasts, osteocytes and osteoclasts. Many of these genes are targets of either the WNT or TGFβ/BMP signalling pathways. Other differentially expressed genes included WNT or TGFβ/BMP identified as differentially expressed in OA bone between females and males, consistent with our other data showing biochemical differences between males and females with OA.

Conclusions: The limitation of this work is that samples were taken at end stage disease. However, altered expression of sets of genes involved in bone turnover suggests a molecular basis for the altered bone remodelling observed throughout OA progression. These data may also in part explain the gender disparity observed in OA.

Patterns of Joint Distribution in Hand Osteoarthritis: Genetic Considerations

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Purpose: 1. To examine the pattern of joint distribution in radiographic hand osteoarthritis (HOA) development in apparently healthy human population and to assess contribution of sex and age on it. 2. To test the effect of the various joint degeneration features to Kellgren-Lawrence (K-L) score of the different joints and in total. 3. To evaluate contribution of the putative genetic factors to inter-individual variation of HOA. 4. To test the association of the extracellular pyrophosphate channel genes (specifically ENPP1) with the variation of the radiographic HOA in the above population.

Methods: The study sample was comprised of Chuvashains (Europeans living along Volga river in Russia): 1,200 individuals of both sexes, with age range between 19 and 90 years. HOA development was assessed radiographically for 15 joints of each hand according to K-L grading system, with modifications. First, using extensive statistical analysis we examined the rate and pattern of age related changes of each type of joints (DIP,PIP etc) and each finger separately in both sexes. We also assessed quantitatively the relative contribution of the specific joint degeneration characteristics (such as osteophytes, OS; joint space nar-

Phenotyping Erosive Osteoarthritis of the Hand

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Purpose: Erosive osteoarthritides of the hand (EOA) is characterised by an aggressive clinical course and the presence of erosive radiographic changes. Over time, EOA has been considered as a variant of OA, a subset of OA, a severe form of OA, an inflammatory phase of OA, and an entity distinct from OA. Our aim is to phenotype EOA in order to demonstrate that it is a distinctive subset of hand OA.

Methods: We examined clinical and radiographic features of EOA, including clinical, radiographic and laboratory aspects. The diagnosis of EOA was usually based on ACR clinical criteria for hand OA and radiographic aspects of articular erosions, at least two in two different IPs and with the exclusion of MCPs. The definition of erosion is still a matter for debate. Most frequently, lesions begin at the central portion of the joint as a sharply marginated defect, usually preceded by subchondral bone lysis. Progression commonly leads to the so-called ‘gull-wing’ deformity due to marginal sclerosis and osteophytes of the distal side of the joints, while the proximal side is centrally eroded or collapsed and thinned. Subchondral pseudocysts may be observed on the proximal side. Another