

associated with generalized muscle weakness. Cluster B patients had a significantly lower arginine concentration ( $21.39 \pm 17.83 \mu\text{M}$ ) than clusters A and C ( $41.76 \pm 19.48 \mu\text{M}$ ;  $p = 3.44 \times 10^{-16}$ ) and controls ( $115.08 \pm 50.77 \mu\text{M}$ ;  $p = 7.98 \times 10^{-11}$ ). The cluster C was distinguished from other two clusters by the lower concentration of lyso-phosphatidylcholine (lysoPC a C16:0) ( $79.02 \pm 25.46 \mu\text{M}$  vs.  $130.47 \pm 65.10 \mu\text{M}$ ;  $p = 1.42 \times 10^{-12}$ ) and controls ( $82.61 \pm 37.02 \mu\text{M}$ ;  $p = 3.79 \times 10^{-6}$ ), but higher phosphatidylcholine acyl-alkyl (PC ae C38:2) concentrations than other patients ( $1.94 \pm 1.44 \mu\text{M}$  vs.  $1.74 \pm 1.20 \mu\text{M}$ ;  $p = 5.8 \times 10^{-3}$ ), but lower than controls ( $4.54 \pm 2.30 \mu\text{M}$ ;  $p = 1.33 \times 10^{-7}$ ). These phospholipids are thought to have pro-inflammatory effects. Further, we found that 55% of cluster A patients were diabetic in comparing to other clusters (13%;  $p = 5.05 \times 10^{-13}$ ), about 8% of cluster B patients had coronary heart disease compared to other OA patients (3.4%;  $p = 0.003$ ), and 41% of cluster C patients had higher cholesterol concentration than other study participants (38%;  $p = 0.02$ ).

**Conclusions:** Our data demonstrated that at least three distinct endotypes existed in primary OA, suggesting muscle weakness OA, arginine deficient OA, and inflammatory OA that can be distinguished by specific blood metabolic markers. While confirmation is needed, these findings provide us better understanding of OA pathogenesis and hold promising in developing personalized tools for OA management.

## 7

### THE ASSOCIATION OF DECLINING KLOTHO EXPRESSION WITH AN ONSET OF KNEE OSTEOARTHRITIS IN PRE-CLINICAL MODEL AND HUMAN SAMPLES

H. Iijima<sup>1,2</sup>, Y. He<sup>3</sup>, N. Wang<sup>3</sup>, H. Lin<sup>3,4</sup>, F. Ambrosio<sup>1,4</sup>. <sup>1</sup>Dept. of Physical Med. and Rehabilitation, Univ. of Pittsburgh, Pittsburgh, PA, USA; <sup>2</sup>Japan Society for the Promotion of Sci., Tokyo, Japan; <sup>3</sup>Dept. of Orthopaedic Surgery, Univ. of Pittsburgh, Pittsburgh, PA, USA; <sup>4</sup>McGowan Inst. for Regenerative Med., Univ. of Pittsburgh, Pittsburgh, PA, USA

**Purpose:** Aging represents a major risk factor for knee osteoarthritis (OA). However, systematic review of the literature reveals that the underlying mechanisms of the age-related knee OA are poorly understood. While the  $\alpha$ -Klotho has been shown to regulate senescence and autophagy in a variety of cell types in an age-dependent manner, to our best of knowledge, there have been no studies addressing the role of  $\alpha$ -Klotho in the pathogenesis of age-related knee OA. This study aims to elucidate the role of  $\alpha$ -Klotho in the pathogenesis of age-related knee OA. We hypothesize that  $\alpha$ -Klotho plays a protective role for knee joint, and the reduction of  $\alpha$ -Klotho level, caused by aging or gene knock-down, will result in the increased development of knee OA.

**Methods:** The knee joints of young (3–6 months old), aged (21–24 months old), and young  $\alpha$ -Klotho<sup>+/−</sup> mice (3–6 months, which displayed a reduced expression level of  $\alpha$ -Klotho) were harvested ( $n = 5$  in each group). Normal human articular cartilage tissue from young (15 years old;  $n = 1$ ) and older (69 years old;  $n = 1$ ) donors were collected with the approval from the Committee for Oversight of Research and Clinical Training Involving Decedents (CORID). Knee joints were decalcified and embedded in paraffin. Safranin O/Fast green staining and immunofluorescence were performed to evaluate: (i) the cartilage degeneration, (ii)  $\alpha$ -Klotho expression level in chondrocytes. ImageJ and Matlab were used to perform quantification of irregularity of the knee cartilage surface, and NIS Elements software was used to quantify  $\alpha$ -Klotho expression per cell.

**Results:** Histology results indicated that aged mice displayed more severe cartilage degeneration with increased cartilage surface irregularity compared to young mice. Similar results were found in the cartilage from humans. Klotho-signal intensity per cell in aged mice and older donor was significantly lower than that in young mice and young donor, respectively. In addition, the aged-relevant OA phenotype was recapitulated in Klotho<sup>+/−</sup> mice, as evidenced by an accelerated articular cartilage degeneration, increased cartilage surface irregularity and reduced cellularity when compared to age-matched wild type mice.

**Conclusions:** Age-related cartilage degeneration in knee joint was associated with decreased  $\alpha$ -Klotho expression in both murine and human models. Genetically engineered mice that express decreased  $\alpha$ -

Klotho displayed an aged phenotype with the accelerated development of OA, suggesting that age-related decline in  $\alpha$ -Klotho may contribute to the onset of OA. These studies also suggest that the development of  $\alpha$ -Klotho therapeutics as a means to counteract the effect of age on cartilage degeneration may be an interesting direction for future research.

## 8

### POLYGENIC RISK SCORE AND ITS POTENTIAL TO IMPROVE DIAGNOSTIC ABILITY IN KNEE AND HIP OSTEOARTHRITIS

B. Sedaghati-Khayat<sup>1</sup>, C.G. Boer<sup>1</sup>, L. Broer<sup>1</sup>, A. Verkerk<sup>1</sup>, E. Zeggini<sup>2</sup>, GO consortium<sup>1</sup>, J.G. van Rooij<sup>1</sup>, A.G. Uitterlinden<sup>1</sup>, J.B. van Meurs<sup>1</sup>. <sup>1</sup>Dept. of Internal medicine, Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Inst. of Translational Genomics, Helmholtz Zentrum München, German Res. Ctr. for Environmental Hlth., Neuherberg, Germany

**Purpose:** Osteoarthritis (OA) is the most common joint disorder worldwide, and due to pain and disability it markedly decreases the quality of life of affected patients. Estimates of heritability of hip osteoarthritis (HipOA) and knee osteoarthritis (KneeOA) have fluctuated around 40 to 60%. A polygenic risk score (PRS) is a practical tool used by precision medicine that stratifies an individual's genetic risk of disease using common single nucleotide polymorphisms (SNPs). Applications toward the clinic are currently explored in a diverse number of diseases and are reaching clinical application fast (ref). The value and applicability of a PRS is dependent on the genetic knowledge of the disease under study. The largest genome-wide association study (GWAS) meta-analysis in OA has recently been performed by the Genetics of Osteoarthritis (GO)-consortium, and discovered >100 loci associated with OA. The purpose of this study was to evaluate the ability of the 63 SNPs as a PRS in predicting hip and knee OA prevalence and incidence. This project is part of the ErasmusMC project "Genotyping On All Patients (GOALL)", in which the aim is to genotype all patients coming into the clinic and return valuable information back to the patient and/or clinician.

**Methods:** Data from a total of 7,983 individuals from the Rotterdam Study-I was used. Knee and hip OA was defined as a KL-score of 2 or higher. We studied prevalent knee ( $n = 1177$  cases) as well as hip ( $n = 561$  cases). In addition, incident knee and hip OA was examined during a mean follow-up time of mean 10 years ( $n = 463$  for incident knee,  $n = 404$  for incident hip). We constructed a 43-SNPs PRS for HipOA and a 23-SNPs PRS for KneeOA. Eleven SNPs are shared in both scores. Standardized PRSs (zPRS) were constructed for each subject, weighted by the effect sizes reported by the GO Consortium. Age and sex adjusted logistic regression models were applied to estimate the odds ratios per standard deviation (SD) of zPRS. We also assessed the PRS performance in the highest ten percentiles. All primary analyses were done in the Rotterdam Study-I (RS-I) cohort. Subsequently, external validation of the PRS is being performed in the Cohort Hip and Cohort Knee (CHECK) study and RS cohorts II and III.

**Results:** The mean zPRS was  $-0.004$  ( $SD = 1$ ) in controls and  $0.033$  ( $SD = 0.99$ ) in prevalent HipOA patients and was  $-0.03$  ( $SD = 1$ ) in unaffected participants and  $0.13$  ( $SD = 1$ ) prevalent KneeOA cases. The zPRS were averaged to 0 with a SD of 1. The zPRS for HipOA significantly predicted prevalent HipOA ( $p = 6.7 \times 10^{-4}$ ;  $OR = 1.1$ ) and incident HipOA ( $6.2 \times 10^{-4}$ ;  $OR = 1.4$ ). The zPRS for KneeOA significantly predicted prevalent KneeOA ( $p = 1.5 \times 10^{-6}$ ;  $OR = 1.19$ ) but failed to reach significance for incident KneeOA ( $p = 0.08$ ;  $OR = 1.11$ ). Subsequently, we examined individuals in the top 10 percentile of the polygenic risk score and compared them with the rest of the population for incident OA risk. We observed an almost 2 times higher risk for HipOA incidence ( $p = 7.7 \times 10^{-4}$ ;  $OR = 1.95$ ) and 50% higher risk for KneeOA incidence ( $p = 3.7 \times 10^{-2}$ ;  $OR = 1.55$ ).

**Conclusions:** Our results show that the OA polygenic risk score is associated with prevalent and incident Hip and Knee OA. Importantly, individuals with a PRS in the highest 10% of the population, had an almost 2 times higher risk for incident hip OA, and 50% increased risk for knee OA. To evaluate the power of PRS fully, future studies should focus on age of onset of disease. In addition, the predictive power of genetics can be different across (patient) populations and ongoing efforts will address this by validating the predictive power of the genetic risk scores in diverse populations.