Fatty Acid Binding Protein 4 (FABP4) As A Biomarker for Knee Osteoarthritis

Chaofan Zhang, MD, MS[1,2], Kwong Yuen Chiu, MBBS[1], Aimin Xu, MD, PhD[3], Chun Hoi Yan, MBBS[1,4] 1. Department of Orthopaedics & Traumatology, The University of Hong Kong, Hong Kong SAR, China; 2. Department of Orthopedics, The First Affiliated Hospital of Fujian Medical University, Fuzhou, China; 3. Department of Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; 4. Shenzhen Key Laboratory for Innovative Technology in Orthopaedic Trauma, Department of Orthopaedics and Traumatology, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China.

Objectives:
Osteoarthritis (OA) is a common joint disorder that affects millions of people all around the world. The etiology of OA, however, is still poorly understood. Recent studies have suggested that adipokines play important roles in the pathogenesis of OA. Fatty acid binding protein 4 (FABP4) is a novel adipokine that is closely associated with obesity and metabolic diseases. Yet no previous studies have examined FABP4 in OA. The aim of this study is to explore the potential role of FABP4 in the pathogenesis of OA.

Methods:
Both clinical study and animal study were performed. For the clinical study, we included patients with radiologically-confirmed knee OA and non-OA controls. Plasma level of FABP4 was determined by ELISA method. Regression analysis of FABP4 and knee OA severity, which was presented as Kellgren-Lawrence (K-L) grade was performed. For the animal study, we included 36 FABP4 knockout mice (KO) and 36 wild-type littermates (WT) (all male, 6-week-old), and fed the mice with a very high-fat diet (HFD, fat 60% calorie) or standard diet (STD, fat 11.6% calorie), for 3 months, 6 months and 9 months. At each time point, we measured animals' body weight and body fat. We then evaluated knee OA via examination of serum Cartilage Oligomeric Matric Protein (COMP) level, knee histologic assessment, and subchondral bone analyses. In the parallel study, we included 48 WT mice and fed the mice with HFD or STD, and simultaneously treated them with daily oral gavage of FABP4 selective inhibitor BMS309403 (15mg/kg/d) or vehicle (PBS solution), for 4 months and 6 months. At each time point, OA evaluation was performed same as above.

Results:
A total of 226 patients were included. 58 were males and 168 were females. The mean plasma level of non-OA patients, KL-2 patients, KL-3 patients, and KL-4 patients were 12.3±3.0 ng/ml (n=6), 14.6±6.9 ng/ml (n=72), 18.2±11.3 ng/ml (n=65), and 19.3±12.3 ng/ml (n=83), respectively (p=0.023, one-way ANOVA). After stratifying FABP4 into 4 level groups (<10 ng/ml, 10-15 ng/ml, 15-20 ng/ml, >20 ng/ml), FABP4 was positively associated with the severity of knee OA (p=0.031, ordinal regression analysis, age and sex adjusted). (Figure 1) For the animal study, HFD induced significant obesity in mice. However, KO mice were much fatter than WT mice with significantly higher body weight and higher percent body fat. (Figure 2) At 3 months of HFD, KO mice showed less cartilage degradation than WT mice with significantly lower serum COMP ((1.6±0.2)U/L vs (2.3±0.3) U/L, p=0.01) and OARSI score ((0.8±0.4) vs (2.9±0.4), p=0.00). Daily oral gavage of BMS309403 in mice for 4 months protected cartilage from degradation as well, in which the mice with BMS309403 for 4 months showed significantly lower COMP ((1.7±0.2)U/L vs (2.1±0.3) U/L, p=0.04) and OARSI score (5.4±6.0 vs 6.0±0.4, p=0.04) than mice with PBS. (Figure 3) At 9 months of HFD, WT mice underwent serious OA changes with significant osteophyte formation and subchondral bone sclerosis. While in KO mice, the changes were much alleviated. The subchondral bone BMD (p=0.00), bone volume percentage (p=0.00), trabecular thickness (p=0.00), and trabecular number (p=0.01) was significantly lower in KO mice. However, chronic treatment with BMS309403 did not seem to have significant effects on the subchondral bone strcuture. (Figure 4) For lean mice (fed with STD), either genetically knocking out or pharmaceutical inhibition of FABP4 had no significant effects on knee OA.

Conclusions:
Plasma FABP4 level was positively associated with the severity of knee OA. Knocking out or pharmaceutical inhibition of FABP4 alleviated OA induced by a very high-fat diet in mice. FABP4 may be a potential biomarker for knee OA.

Key words:
Osteoarthritis, fatty acid binding protein 4, BMS309403, obesity, subchondral bone sclerosis

Figure 1 Serum FABP4 level in 6 non-OA patients and 220 radiologically-confirmed OA patients. The level was 12.3±3.0 ng/ml, 14.6±6.9 ng/ml, 18.2±11.3 ng/ml, and 19.3±12.3 ng/ml in KL-0, KL-2, KL-3, and KL-4, respectively (p=0.023, one-way ANOVA). Post hoc analyses showed FABP4 of KL-2 patients was significantly lower than KL-4 patients (p=0.028, Tukey HSD). After stratifying FABP4 into 4 level groups (<10 ng/ml, 10-15 ng/ml, 15-20 ng/ml, >20 ng/ml), the ordinal regression analysis showed that FABP-4 was positively associated with the severity of knee OA (p=0.031, age and sex adjusted).

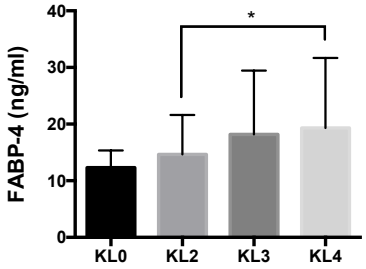

Figure 2 Knocking out or pharmaceutical inhibition of FABP4 significantly increased animals' body weight and body fat percentage. (A) Photos of KO and WT mice after 6-month of HFD and STD showed KO mice were fatter than WT mice, especially under HFD. (B, C) Statistical analyses showed KO mice had significantly higher body weight and body fat. (C) WT mice treated with daily oral gavage of BMS309403 (15mg/kg/d) for 4 months and 6 months had significantly higher body weight and body fat.

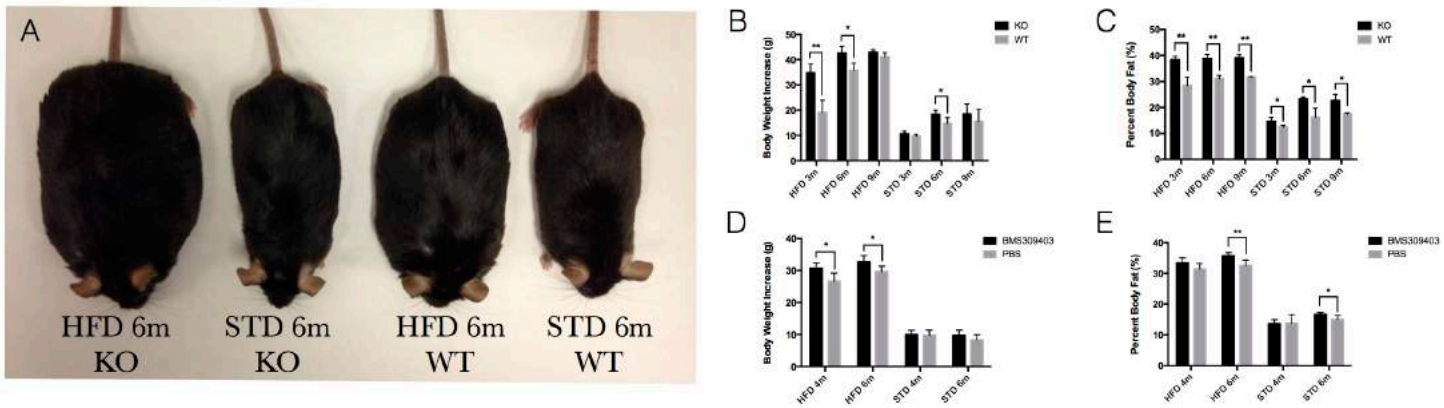

Figure 3 Knocking out or pharmaceutical inhibition of FABP4 significantly alleviated cartilage degradation in mice fed with HFD. The coronal paraffin sections of knees were stained with Safranin O and Fast Green. Loss of Safranin O staining in cartilage indicated a loss of glycosaminoglycan (GAG) content. (A, D, A1, D1) KO and WT mice after 3-month of HFD. Staining loss of cartilage was observed in WT mice but not obviously seen in KO mice. (B, E, B1, E1) KO and WT mice after 6-month of HFD. KO mice started to undergo cartilage degradation, while in WT mice the cartilage were seriously degenerated. In addition, osteophyte formation was seen in WT mice, especially in the medial compartment (arrow). (C, F, C1, F1) KO and WT mice after 9-month of HFD. Both KO and WT mice experienced severe cartilage degradation. In WT mice, serious subchondral bone sclerosis was observed (arrow). (G, I, G1, I1) WT mice treated with BMS309403 or PBS for 4 months. Mice treated with BMS309403 showed less cartilage staining loss than mice treated with PBS. (H, J, H1, J1) WT mice treated with BMS309403 or PBS for 6 months. Mice with PBS showed significant cartilage staining loss. (K) KO mice showed significantly lower level of COMP than WT mice after 3-month of HFD and STD. COMP level at 6-month of HFD in KO mice was significantly higher than WT, which suggested severe cartilage degradation in WT mice. (L) The OARSI score was significantly lower in KO mice than WT mice. (M) Mice treated with BMS309403 for 4 months showed significantly lower serum COMP than mice with PBS. (N) The OARSI score was significantly lower in mice treated with BMS309403 for 4 months. (Data presented as mean±std. Student t-test was performed to compare the means. *: p<0.05.)

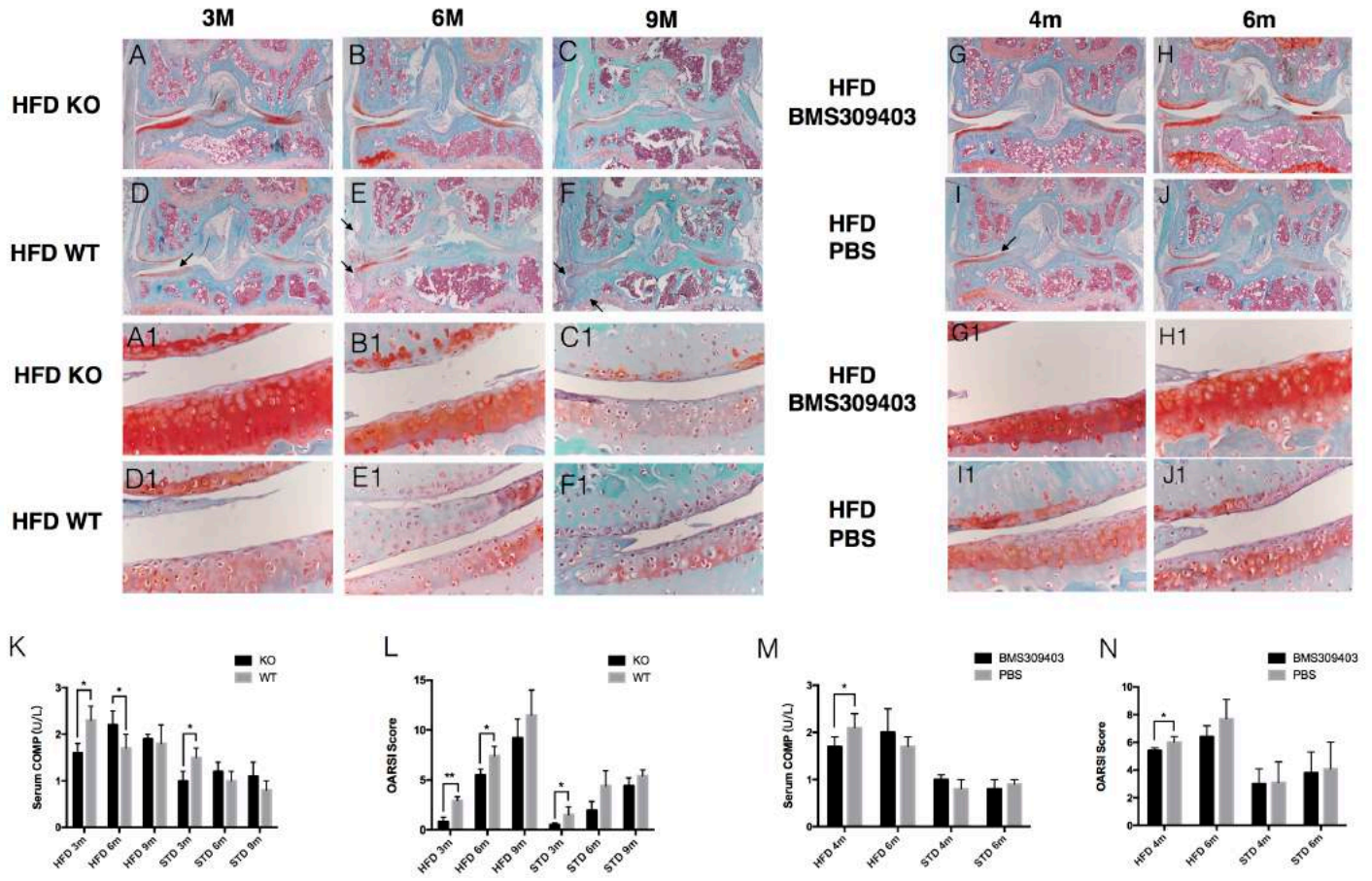

Figure 4. Micro-CT analysis of subchondral bone. Knocking out FABP4 significantly alleviated subchondral bone sclerosis in mice fed with HFD for 6 months and 9 months (A, arrow indicated). The subchondral bone BMD (p=0.00), bone volume percentage (p=0.00), trabecular thickness (p=0.00), and trabecular number (p=0.01) was significantly lower in KO mice after 9-month of HFD. (C, D, E, F) However, chronic treatment of BMS309403 in WT mice for 4 months and 6 months did not seem to have significant effects on the subchondral bone. (B, G, H, I, J) (**:p<0.01)