INTRODUCTION: Osteoarthritis (OA) is an aging-associated degenerative disease that involves cartilage degeneration and osteophyte formation at the joint surfaces. The condition affects more than 300 million people worldwide and has a higher prevalence in women than men. The condition is frequently more severe in women leaving them at higher risk for more pain from knee OA than men. A wide variety of factors are thought to contribute to the development of OA including inflammation and injuries or mechanical stress. Thus, sensitive detection methods are important for identifying early OA phenotypes. Previously, we have shown that a transgenic mouse model with overexpression of mechanos- and inflammatory stress sensitive miR-365 in Col2 lineage specific cells develop early onset of OA. In late stages of OA, there is significant damage to the joint that can be visualized by histology and quantified by OARSI score. Joint structural damage leads to changes in gait as the joint degenerates. Gait analysis has been successfully used to detect differences in a wide variety of conditions including spinal cord injuries, joint injuries, Parkinson’s, and acute limb ischemia by comparing a variety of parameters such as stride duration, paw drag, stride length, stride frequency, or midline distance. We believe similar detections could be applied to detect early onset of OA phenotype. In this study, we quantified gait differences in key parameters (stride length, stride duration, and midline distance) in the 3-month midline distance 365 transgenic mice prior to any visible cartilage structural damage by histology. Female mice showed more significantly different gait parameters compared to male mice in response to miR-365 overexpression. Since OARSI scores showed no difference at 3 months, the gait analysis may provide a more sensitive method for OA phenotype detection.

METHODS: The Institutional Animal Care and Use Committee at Lifespan approved all handling and treatment of mice for this study. In order to overexpress miR-365 in cartilage tissues, miR-365Flox mice were crossed with Col2-Cre (Cre-only) mice to produce Col2-Cre/+miR-365Flox+ transgenic mice (miR-365TG). Col2-Cre mice are the control group (Cre-only) for this study. For gait analysis, mice were run on a flat treadmill at 25 cm/s, and each run (3-5s) was recorded using the DigiGait Imaging software. Data was processed and analyzed to generate gait parameters. Knee joints were also collected from Cre-only and miR-365TG mice, processed, and stained with Safranin-O. Slides were then imaged and scored using the OARSI system. Statistical analysis was calculated using student’s t-tests.

RESULTS: We previously found that female but not male miR-365TG mice developed early onset of OA at 7-month-old, as quantified by the joint histology based OARSI score (Data not shown). However, at 3-month-old, there is no significant joint histology changes (Fig 1A) or difference in OARSI scores (Fig 1B) between miR-365TG or control (Cre-only) mice in either female or male. Female showed no significant difference in weight between miR-365TG and control, while male miR-365TG mice were lighter than the control male (Fig 1C). To determine whether we can detect early stage of OA development by gait analysis, we measured the gait of female miR-365TG mice, which develop OA later, and also male mice, which do not develop OA. At 3 months, there is a statistically significant difference in the stride length, stride duration, and midline distance in female miR-365TG mice in comparison to control mice (Fig. 2). Female miR-365TG mice take shorter strides (Fig 2A) and have a shorter stride duration (Fig 2B) than control mice. Female miR-365TG mice also have a significantly shorter midline distance than control mice while running (Fig 2C). In contrast, there is no significant difference in the stride length, duration, or midline distance in male miR-365TG mice in comparison to control mice (Fig. 2).

DISCUSSION: Here we have shown that the effects of OA on gait alterations may be detected prior to the joint histology changes in an aging-associated early onset OA mouse model. The gait changes were observed in female stress-microRNA transgenic mice at 3 months old. These mice develop OA at 7 months old as measured by OARSI scores. The early OA characteristic gait changes include decreases of stride length and duration, and an increase of midline distance. Stride length is a spatial parameter that measures the distance between consecutive paw contacts of the same paw, while stride duration is a temporal parameter that measures the amount of time between consecutive paw contacts from the same paw. Stride duration and length are closely related that in shorter strides are typically going to be seen in strides of a shorter duration. Our data reveal that OA mice, even at early stages when joint morphological changes are undetectable by histology, have smaller and shorter steps. Such walking patterns mimic those in human OA patients. Midline distance measures the distance between the center of the paw and the transverse midline of the animal during peak stance. It is closely related to balance and often decreases with injuries or neurological disorders. Thus, the early OA mice not only take strides of shorter length and duration but also have a smaller midline distance suggesting poorer coordination. These gait differences were not seen in male 3-month-old mice which do not develop OA at 7-month-old. Our data suggests that the impact of OA can be detected by gait changes prior to detectable visual changes at the joint surface. Subtle changes of the joint and surrounding nerve and muscle tissues, potentially as a result of chronic inflammation, may contribute to the gait changes. Persistent stress accumulated during aging may exacerbate the gait changes and eventually cause cartilage surface degradation associated with OA morphological phenotype.

SIGNIFICANCE: Our study shows that gait analysis might be more sensitive to OA changes than OARSI scores and therefore allow for earlier detection of phenotype OA changes.

REFERENCES:

Figure 1. A. Safranin-O histology of the knee joints of 3-month-old miR-365 and control mice (Cre-only). B. OARSI Scores, n ≥ 4. C. Weight of mice (grams), n ≥ 16, * p < 0.05.

Figure 2. Gait analysis of 3-month-old mice. A. Stride length (cm), n ≥ 22, * p < 0.05. B. Stride Duration (sec), n ≥ 22, *** p < 0.05. C. Midline Distance (cm), n ≥ 22, * p < 0.05.