INTRODUCTION: Biomechanical studies of cartilage have often focused on the contributions of the primary extracellular matrix (ECM) constituents, collagen and aggrecan [1], while the roles of other ECM constituents have received less attention. With the advent of new high resolution nanotechnological tools, the direct quantification of cartilage biomechanical properties using murine models can provide important insights into how secondary ECM molecules affect the function and pathology of cartilage. Previous nanoindentation studies of murine cartilage have assessed the effects of maturation and osteoarthritis-like degeneration of cartilage on its biomechanical properties [2, 3]. Recently, murine models have received increased attention because of the availability of specific gene-knockout and gene alteration technologies [4]. For example, chondroadherin (CHAD) is a non-collagenous small leucine-rich proteoglycan (SLRP) with α-helix and β-sheet secondary structure, spatially localized in the territorial matrix (MW = 38 kDa) [5]. CHAD binds to type II collagen and the αβ integrin and is hypothesized to function in the communication between chondrocytes and their surrounding matrix, as well as in the regulation of collagen fibril assembly [6, 7] (Fig. 1). In this study, the effect of CHAD on the cartilage biomechanical properties was assessed via atomic force microscopy (AFM)-based nanoindentation of murine knee cartilage from wild type control and CHAD-knockout specimens.

METHODS: CHAD wild type and knockout mice were obtained in selected age groups (1 year, 4 months, and 11 weeks). Femoral condyles from knee joints were dissected from both the right and left hind legs of female mice in the presence of phosphate buffered saline solution (PBS, 0.15 M, pH = 7.4) and mounted on a magnetic disc for AFM-based nanoindentation. AFM-based nanoindentation (Veeco Multimode AFM) was performed according to established procedures on the femoral condylar cartilage of each specimen using a gold-coated SiO2 colloidal probe tip (k ~ 4.5 N/m, R ~ 2.5 μm), functionalized with a neutral, hydroxyl-functionalized self-assembled monolayer (11-mercaptopoundecanol, HS(CH2)11OH), at 0.1-10 μm/s AFM z-piezo displacement rates in PBS [3]. Each medial condyle was indented 3 times at each rate at 5 locations at maximum indentation depths below surface roughness. After indentation, specimens were preserved in formalin solution at 4°C. The effective indentation modulus, Eind, was calculated from the loading portion of force-indentation depth curves using the Hertzian model to account for indentation geometry [8]. Within each age and treatment group, a relatively large variation in Eind was observed (Fig. 2b). Therefore, at least four different joints for each treatment group were tested.

RESULTS: Both the wild type control and CHAD-knockout cartilage exhibited significant rate-dependent mechanical properties (Friedman’s test, p < 0.05, Fig. 2b) with the wild type being more rate-dependent in all the tested age groups. The mean value of Eind, for each joint, was utilized to quantify the effect of CHAD-knockout at each of the tested rates. For all the tested age groups, Eind of CHAD-knockouts was significantly lower by ~67.9-80.3% (p < 0.05, 2-way ANOVA) (Fig. 3). DISCUSSION: A significant reduction in penetration resistance and stiffness of the cartilage tissue was observed for the CHAD knockout mice relative to the wild type controls (Figs. 2, 3). This weakening of cartilage appears to be consistent with the hypothesis that despite its low abundance (compared to collagen and aggrecan) and spatial localization within the territorial matrix, CHAD does have a biomechanically important function as a connecting linkage in the formation of an appropriately assembled fibrillar collagen network [7]. Lack of CHAD appears to slow down development of the load bearing extracellular matrix, possibly due to the assembly and linkages of the fibrillar collagen network. This could affect the effective cross-link density of the network and, hence, the local osmotic swelling and hydraulic permeability of the resident aggrecan. In addition, the significant indentation rate dependence of Eind suggested the presence of poroviscoelastic energy dissipation in both control and CHAD-knockout cartilage [9]. The variation in Eind within the same age and treatment groups, and with different locations along the same joint are likely due to the variations in proteoglycan content, surface geometry and local collagen cross-link density within and between different joints. Bearing in mind that the protocol used primarily tests a narrow superficial zone, where knowledge on detailed composition is limited the current data provide new information of importance to understanding biology in this region. The observed alterations may be directly related to altered content of CHAD, but may also relate to the altered cell signaling when CHAD is not binding to cell surface receptors. The altered mechanical properties are most pronounced at young age and that apparently compensatory mechanisms become activated with age. Ongoing studies are investigating the biochemical properties and nanostructure of CHAD-knockout murine joints, to provide further evidence on the important role of CHAD in cartilage tissue formation and function.