Human Apolipoprotein E4 Drives Disrupted Osteocyte Function and Bone Fragility

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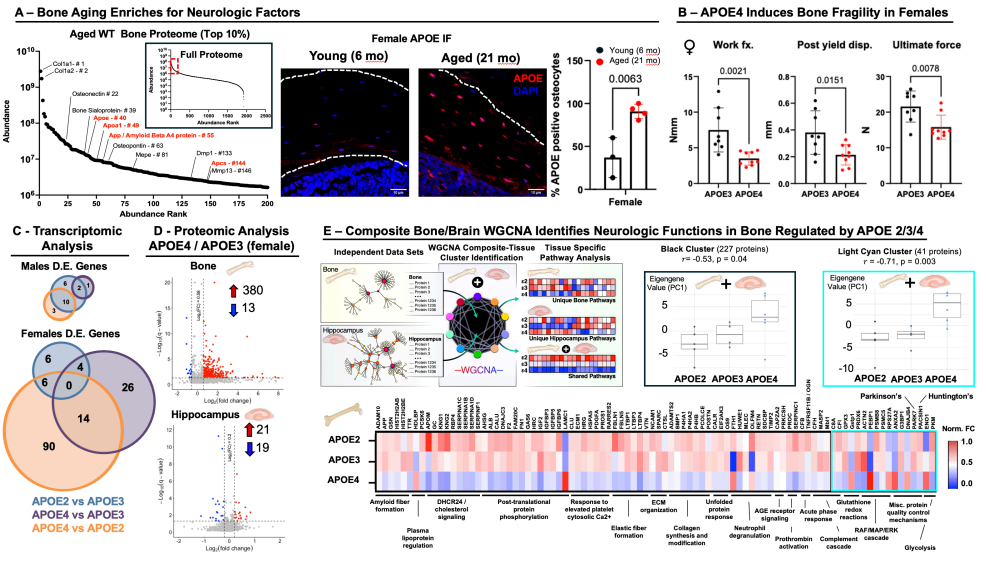
INTRODUCTION: Individuals diagnosed with Alzheimer's disease (AD) are at 2-2.5 times increased risk of bone fracture than healthy individuals[1]. Conversely, a diagnosis of osteoporosis in females is the earliest predictor for AD (odds ratio 1.8)[2]. While mechanisms connecting these conditions remain unclear, the AD risk factor Apolipoprotein E (APOE) can exert influence on bone density and fracture healing[3,4]. In humans, allele variants of *APOE* carry differing risk for the development of AD with age (*APOE2* protective, *APOE3* neutral, and *APOE4* increased AD risk factor). This study aims to determine the effects of human *APOE* allele variants on bone fragility using a humanized *APOE* knock-in mouse model.

METHODS: Male and female APOE-TR mice (N=5-8 per group) were purchased from Taconics Biosciences (B6.129P2-Apoe$^{tm1(APOE*2)Mac}$N9 (*APOE2*), B6.129P2-Apoe$^{tm1(APOE*3)Mac}$N9 (*APOE3*), B6.129P2-Apoe$^{tm1(APOE*4)Mac}$N9) (*APOE4*). These animals carry homozygous copies of the human APOE ε2, ε3, and ε4 alleles. Mice were maintained in accordance with the Institutional Animal Care and Use Committee (IACUC) at the Buck Institute for Research on Aging using approved procedures and collected at 16 months of age prior to the full manifestation of cognitive alterations. Young (6 mo.) and aged (21 mo.) "Wild Type" (WT) C57BL/6 mice (Male and Female, N=6 per group) were bred and maintained at the University of California, San Francisco (UCSF) in accordance with the UCSF IACUC. Humeri were collected for RNA sequencing, tibia were collected for histological analysis and immunofluorescence, and femurs were collected for mechanical testing, μCT structural analysis, and unbiased Data Independent Acquisition - Mass Spectrometry (DIA-MS) proteomics. Epiphyses, periosteum, and marrow were removed from humeri and femora prior to analysis. Hippocampi of *APOE* mice were also collected for DIA-MS.

RESULTS: In aged male and female WT mice, proteomic analysis of cortical bone unexpectedly revealed an over-representation of neurological disease-associated factors, including APOE, Apolipoprotein A1 (Apoa1), Amyloid precursor protein (App), and serum amyloid P component (Apcs), in the top 10% most abundant proteins. Immunofluorescent analysis of APOE in cortical tibia sections showed a dramatic increase of APOE protein expression in osteocytes of old, relative to young, bone, but only in female mice. Even with no change in cortical bone structural parameters by μCT, bone from female *APOE4* mice displayed reduced work to fracture, post-yield displacement, and ultimate force compared to *APOE3* (healthy) controls, indicating bone fragility due to poor bone quality. *APOE4* males did not display this bone quality deficit. Fragility in *APOE4* females was accompanied by truncated osteocyte lacunocanalicular networks and reduced Cathepsin K, Sclerostin, and Mmp13 staining, demonstrating suppressed osteocytic bone remodeling. In *APOE2/3/4* mice, *APOE4* drove the largest difference in differentially expressed genes (RNAseq: Females:140 DE genes, Males:16 DE genes, FDR-0.1). Proteomic analysis of female bone demonstrated a similar but more profound relationship, with 472 significantly altered proteins of 2002 uniquely detected proteins. Males displayed 463 significantly altered proteins amongst the three APOE genotypes ($Q<0.05$, $Log2FC>0.58$). However, the direction of regulation was opposite for each sex, with strong downregulation in females and upregulation in males. In contrast, proteomics of the female hippocampus showed minimal protein changes across the APOE alleles (40 proteins significantly regulated out of 3138 proteins with $Log2FC>0.2$). Composite-tissue Weighted Gene Co-expression Network Analysis (WGCNA) utilizing both bone and hippocampal proteomic data sets from female *APOE2/3/4* animals found two composite clusters of proteins with similar regulation by the APOE ε4 allele (Black, 227 proteins & Light Cyan, 41 proteins). Bone proteins from these clusters were involved in bone remodeling and ECM regulation and functions related to neurodegeneration (i.e. amyloid fiber formation, cholesterol signaling, Parkinson's disease, Huntington's disease).

DISCUSSION: We found that aging is associated with a concentration of neurodegeneration-associated proteins in bone, including the accumulation of APOE in osteocytes, specifically in female bone. In addition, we demonstrate that AD risk factor *APOE4* severely disrupts the bone transcriptome and proteome in females, leading to impaired bone quality independent of changes in bone mass. These changes are associated with disrupted osteocytic bone remodeling, pointing to a direct effect of the neurodegenerative risk factor *APOE4* on the skeleton. These skeletal changes also occurred with minimal alterations to the hippocampal proteome at 16 months of age. WGCNA analysis identified female-specific modules in bone that enriched to neurodegenerative risk factors and bone remodeling factors that shared similar regulation by APOE4 – further linking neurodegenerative risk factors to bone fragility in female aging.

SIGNIFICANCE/CLINICAL RELEVANCE: Given the increased susceptibility of women to both AD and osteoporosis, our findings highlight a sex-specific vulnerability that adds to the growing evidence that this neurodegenerative disease extends beyond the brain and suggests that addressing osteocyte-control of bone quality may be an important, and actionable, part of managing AD in women. Moreover, this work, and clinical and preclinical evidence, suggests that bone deficits may precede overt cognitive decline and points to the potential of bone as an early biomarker of AD. These findings open new opportunities for earlier detection, risk assessment, or diagnosis of age-related neurodegeneration or dementias through skeletal assessment and highlight the skeleton as a novel therapeutic target in Alzheimer's disease.



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