Fracture Healing in a Late-Onset Alzheimer's Disease (LOAD2) Mouse Model

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Abstract

Osteoporotic fractures are a major cause of morbidity in the aging population. Alzheimer's Disease (AD) has been shown to increase fracture risk by ~2-fold (1). Mechanistic studies show that AD-associated neuroinflammation and the late-onset AD (LOAD2) risk alleles *APOE4* and *TREM2 R34H* promote RANKL-mediated osteoclastogenesis, which, along with reduced activity, may lower bone mineral density (2, 3). We also remain uncertain whether fracture itself or the anesthesia required for surgery might modify the trajectory of AD pathology in mice. We hypothesized that APOE4/TREM2-driven neuroinflammation in LOAD2 mice impairs fracture healing.

To test this, we used a LOAD2 mouse model carrying humanized APOE4 and TREM2 R34H, which recapitulates AD-like neuroinflammation without extensive amyloid or tau deposition (4). Mice were assigned to four groups: C57BL/6J unfractured, C57BL/6J fractured, LOAD2 unfractured, and LOAD2 fractured. C57BL/6J is henceforth referred to as B6. Femoral fractures were created and stabilized using a surgical osteotomy technique at 1 year of age. Animals that were not in the fracture group were shaved, given Ethica**,** and received equal isoflurane exposure. Grip strength, Y-maze (distance and % spontaneous alternation), and light/dark testing were performed at baseline, 10 weeks, and 20 weeks post-fracture. Healing was monitored by serial X-rays (biweekly from day 7 to week 5, then weekly from weeks 6-12, and every other week thereafter until sacrifice) and quantified at 40 weeks post-fracture using micro-computed tomography (μCT).

Male LOAD2 control femurs showed a smaller cross-sectional area (B.Ar) and a lower bone-volume-fraction (BV/TV) relative to B6. At 40 weeks post-fracture, female LOAD2 fractured femurs displayed a higher BV/TV with unchanged B.Ar. Female LOAD2 control femurs showed greater cortical thickness. Behaviorally, 20-wk post-fracture testing demonstrated no genotype-specific deficits; the only significant difference was a modest reduction in Y-maze distance in fractured B6 males compared to B6 control males. Spontaneous alternation, dark-phase locomotor time, and grip strength were unaffected across groups. These findings suggest

that LOAD2 produces subtle sex-specific increases in cortical bone density without compromising functional recovery after femoral fracture in mice.