The Retrotransposon/Inflammaging Axis Governs Common Gait Disturbance in Alzheimer's Disease and Osteoarthritis Mouse Models

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INTRODUCTION: Alzheimer's disease (AD) and Osteoarthritis (OA) are both aging-associated pro-inflammatory, degenerative diseases. Studies have established that the OA onset increases the risk of developing AD and accelerates the onset of AD, indicating potential common mechanisms for pathogenesis of both aging-associated diseases [1-4]. However, such a common molecular mechanism underlying both diseases is unclear. While OA causes pain and mobility difficulties, AD also leads to gait disturbances, which often render late-stage AD patients to bed rest. In this study, we compared the characteristics of gait disturbance in an AD mouse model (APP/PS1) and aging OA transgenic mouse model (Col2a1Cre; MiR-365Flox) with the goal of discovering a potential common mechanism underlying both diseases. We found that OA and AD share common gait disturbance characteristics. The gait disturbance can be corrected in both OA and AD mice by inhibiting retrotransposon LINE1 (L1) with an FDA-approved anti-viral drug, the nucleoside reverse transcriptase inhibitor (NRTI) Lamivudine (3TC). L1 is an abundant transposable element occupying 17% of the human genome [5]. Although repressed in normal somatic cells, it is de-repressed in both brain and joint tissues during aging [6]. We demonstrated here that systemic inhibition of L1 suppressed inflammation, including IL-1β levels in the CNS in AD mice and in joint cartilage in OA mice. Thus, we established an important biological axis as a common mechanism linking two distinct tissues and aging-associated diseases.

METHODS: This study utilized 16 APP/PS1 transgenic mice (10M, 6F) as AD models, Col2a1Cre; miR-365Flox mice as OA models [7], and 28 of their wild-type littermates as controls (17M, 11F), respectively. Beginning at 3 months of age, mice were randomized to receive 2mM of 3TC in their drinking water. This water was replaced twice per week. From 7 to 10 months of age, mice were subjected to DigiGaitTM analysis. Murine gait was investigated at walking speeds of 15, 20, and 25 cm/s. All animal studies were approved by the IACUC at Rhode Island Hospital and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (eighth edition). DigiGaitTM analysis software processed each run, providing data on 42 different gait metrics. Murine gait was further analyzed using the Common OA Gait (COG) methods established previously [7]. At 10 months of age, mice were subject to CO2 euthanization, before the mouse brains were harvested and dissected into four regions: Front, Middle, Back, and Hippocampus. Whole-brain immunostaining was conducted for Amyloid-Beta plaques, L1, and inflammatory cytokine IL-1β. RealTime-qPCR analysis was performed on these samples to further evaluate levels of L1 and IL-1β. Subsequent NanoString nCounter analysis investigated an additional 700+ genes associated with neuroinflammation. NanoString nSolver Advanced Analysis evaluated expression levels of these genes as proxies for relative cell type abundance and pathway activity, producing scores for both of these attributes respectively.

RESULTS: We previously found that murine OA gait is characterized by decreased stride duration and length, and increased stride frequency, which were collectively referred to as Common OA Gait (COG) [7]. Gait analysis of AD mice indicated that the COG comprised the major gait disturbance in AD mice as well. They included significant decreases in stride duration and stride length, and increased stride duration (Fig. 1). The magnitude of this difference corresponded to significant COG scores of 4 for all three metrics. Thus, AD mice shared common COG gait disturbance characteristics with OA mice. 3TC treatment in drinking water not only corrected COG in the OA mice (data not shown), but also the COG gait disturbance in the AD mice (Fig. 1). 3TC treatment significantly inhibited the elevated L1 levels in the hippocampus and the mid-brain, where the hippocampus resided in the AD mice (Fig. 2, LINE-1). It also led to significant inhibition of the elevated IL-1β mRNA levels in the hippocampus and mid-brain in the AD mice (Fig. 2, IL-1β). RNA sequencing analysis indicated that immune-associated genes Csf1, C1qb, Slamf9, Fcgr2b, and Ctss were significantly upregulated in all regions of the AD brain in comparison to the WT brain. 3TC treatment abolished the increase of these inflammation-related genes in the AD mice (Fig. 3).

DISCUSSION: Although AD occurs in the central nervous system and OA occurs in the joint, both are characterized by inflammaging, a persistent low-grade inflammation in aged tissues. Our findings indicate that AD and OA gait disturbances are characterized by the three common major gait changes comprising the COG, providing a behavioral linkage between these two diseases. 3TC treatment successfully inhibited both AD and OA-associated gait disturbances, suggesting the important function of L1 in regulating the pathogenesis of both AD and OA. The L1 upregulation in AD mice was correlated with IL-1β upregulation in the hippocampus of the mid-brain, important tissues involved in AD pathogenesis. L1 was also elevated in the OA joint cartilage with IL-1β as a major inflammatory cytokine involved in OA pathogenesis. Furthermore, we identified inflammatory gene signatures, including C1qb, associated with L1 elevation and gait disturbance during AD pathogenesis. It was shown previously that complement genes were associated with OA pathogenesis [8]. Taken together, our data support a common axis of retrotransposon/inflammaging in regulating the pathogenesis of both AD and OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study establishes L1 inhibition as an effective therapy for inflammaging and gait disturbances in AD and OA mouse models. The re-purpose of the FDA-approved anti-viral drug is a cost-effective and time-efficient approach to developing new therapeutics for the treatment of AD and OA, two major aging-associated diseases in humans.

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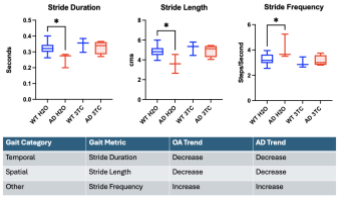
IMAGES AND TABLES:



Gait Category	Gait Metric	OA Trend	AD Trend
Temporal	Stride Duration	Decrease	Decrease
Spatial	Stride Length	Decrease	Decrease
Other	Stride Frequency	Increase	Increase

Figure 1. Upper Panel: Characteristics of gait disturbance in AD mice, which were corrected by 3TC treatment. *p<0.05. Lower Table: Common Gait Disturbances in OA and AD mice.

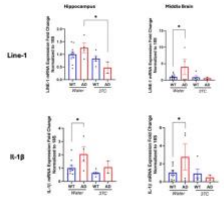


Figure 2. Real-time PCR analysis of LINE1 RNA levels (upper) and IL-1b mRNA (lower) in the hippocampus (left) and mid-brain (right). *p<0.05

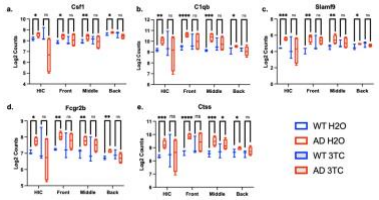


Figure 3. L1 targeted immune and inflammation genes in different parts of the brain. HIC, Hippocampus, Front, Front Brain, Middle, Mid-brain, Back, Back Brain. 3TC, 3TC treatment in drinking water; H2O, control treatment in drinking water only. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns: not significant.