Gait Disturbance of Alzheimer's Disease Mimics Osteoarthritis: Characterization, Treatment and Correction by Suppression of Retrotransposon LINE1

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INTRODUCTION: Osteoarthritis (OA) and Alzheimer's disease (AD) are inflammatory, degenerative diseases with high prevalence in aging populations. OA is a musculoskeletal condition that remodels articular cartilage and subchondral bone, while AD is a neurological condition characterized by deposits of β-amyloid and tau-tangles in the brain. Although these conditions affect different tissues and have distinct pathophysiology, research evidence suggests that there is an association between OA and AD at both biological and clinical levels in humans [1, 2]. At the biological level, LINE1 (L1) is a retrotransposon that makes up 17% of the human genome. It is heavily upregulated in the brain as well as in joint tissues during aging [3]. Inhibition of LINE1 with nucleoside reverse transcriptase inhibitor (NRTI) has been shown to be effective for suppressing tissue inflammaging [3]. At the clinical level, while OA is characterized by joint pain and difficulties in mobility, many AD patients experience gait disturbance in addition to memory loss. Induction of OA is known to accelerate AD pathogenesis in the APP/PS1 transgenic mouse model of AD [4]. However, gait disturbance has not been characterized in an AD mouse model. Understanding of the biological mechanism of AD-associated gait disturbance will lead to treatment and correction of gait, thereby increasing the life quality of AD patients. In this study, we characterized gait disturbance patterns in the APP/PS1 AD mouse model. We determined whether LINE1 levels increase in AD mice, and if so, in which tissue and regions L1 is over-expressed. We further determined whether oral NRTI treatment can lower LINE1 levels in AD mice, and if so, whether it corrects gait disturbance.

METHODS: The use of animals was approved by the Lifespan IACUC animal studies committee and all procedures were performed in accordance with institutional guidelines. APP/PS1 transgenic mice lines (AD) and their wildtype littermates (WT) were divided into four groups. The first and second group consisted of AD and WT mice respectively, which were given 2 mM of 3TC (FDA-approved NRTI for HIV treatment) in drinking water replaced twice per week starting at 2 months. The third and fourth group consisted of AD and WT mice respectively, which were given drinking water without any 3TC. At 10 months, the mice were euthanized using CO_2, and the brains and knee joint cartilage were then collected for qPCR analysis. RNA extraction was performed using the Qiagen miRNeasy Mini Kit. Real-time RT-PCR reactions with LINE1 primers were performed after RNA reverse transcription. Gait analysis was performed using Digigait (Mouse Specifics, Inc.). Three different running speeds were used: 15, 20, and 25 cm/second.

RESULTS: Since onset of the neurological phenotypes of APP/PS1 transgenic mice occur at 6 months old, we tested gait characteristics of AD mice between 8.2 to 9.75 months old. Mice were divided into four groups for testing: WT (H_2O), AD (H_2O), WT treated (3TC), AD treated (3TC) (**Fig. 1**). Analysis of thirty-eight gait features at three running speeds indicated that at least four gait characteristics were significantly altered in AD mice. Compared to WT mice, AD mice presented a significant decrease of stride length, stride duration, and swing duration, and a significant increase of stride frequency (**Fig. 2**). This indicates that AD mice have shorter and more frequent strides than WT mice. The gait characteristics of AD mice not only mimic those of AD patients, but also those of OA mouse models and patients. Strikingly, systemic oral treatment of AD mice with 3TC, an NRTI anti-retroviral drug approved by FDA, corrected all four abnormal gait metrics in AD mice (**Fig. 2**). This suggests that systemic inhibition of L1 levels may contribute to correction of gait in AD mice. To identify the tissues in which L1 levels were inhibited by 3TC, we isolated different tissues including cartilage joint and three regions of the brain (front, middle, and back) from AD mice (**Fig. 3A**). We found that the L1 RNA level is greatly increased in the middle region of the brain of AD untreated mice (**Fig. 3B**). In contrast, we did not observe significant increase of L1 in the joint cartilage of AD mice (data not shown). 3TC treatment of the AD mice, which corrected the abnormal gait, inhibited L1 levels, which were comparable to the basal levels of L1 in WT mice (**Fig. 3B**).

DISCUSSION: This study demonstrated for the first time that gait differences were not only present in AD mice, but also was a consequence of increased L1 levels in the mid-brain. Gait changes in AD mice include shorter and more frequent steps, which could be a compensatory mechanism for musculoskeletal and neuronal weakness. A decreased step length and speed is a gait characteristic change not only in degenerative neurological diseases including AD, but also aging-associated joint diseases such as OA. Revealed by this study, the similar gait changes between AD and OA mice prompted us to determine whether there are common biological mechanisms responsible for gait changes in these two aging associated diseases. L1 retrotransposon has been proposed to play a critical role in inflammaging [3]. Indeed, we found that, in AD mice, L1 levels were increased in the middle region of the brain containing the motor cortex and limbic system. 3TC treatment inhibited L1 increases and corrected gait disturbances in AD mice.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study suggested that NRTI drug 3TC may be used to correct gait disturbance of AD patients by inhibiting L1 levels in the mid-brain that induces inflammaging of the motor cortex and limbic system. It supports a clinical trial to test the efficacy of repurposing the FDA-approved anti-viral drug 3TC to improve mobility and quality of life for AD patients.

REFERENCES: [1] Weber et al. *Medicine* (2019) 98:10 [2] Ikram et al. *Osteoarthritis and Cartilage* (2019) 27:10 [3] Gorbunova et al. *Nature* (2021) 596:7870 [4] Kyrkanides et al. *Journal of Neuroinflammation* (2011) 8:112

IMAGES AND TABLES:

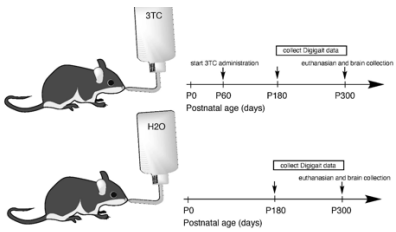

Fig. 1. Experimental design of treatment of AD and WT mice with NRTI 3TC and the timetable of 3TC treatment, gait analysis, and tissue collection for L1 quantification in the mice.

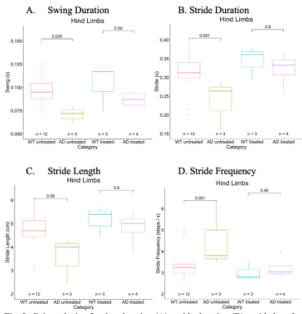

Fig. 2. Gait analysis of swing duration (A), stride duration (B), stride length (C), and stride frequency (D) in the hind limbs of WT and AD mice treated or untreated with 3TC. The number of tested mice and the p value of testing groups are indicated.

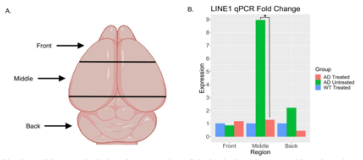

Fig. 3. A. Diagram depicting the separation of the brain into three equal length regions. B. Bar graph showing the normalized expression of LINE1 across the WT 3TC, AD 3TC, and AD H2O. The groups are separated into the front, middle, and back of the brain with an average of all three groups included. N=3, * p<0.02.