GeroScience (2021) 43:1947–1957
https://doi.org/10.1007/s11357-021-00401-6

ORIGINAL ARTICLE

Early manifestation of gait alterations in the Tg2576 mouse model of Alzheimer's disease

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Received: 9 April 2021 / Accepted: 7 June 2021 / Published online: 23 June 2021
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Abstract There is strong clinical evidence that multifaceted gait abnormalities may be manifested at early stages of Alzheimer's disease (AD), are related to cognitive decline, and can be used as an early biomarker to identify patients at risk of progressing to full-blown dementia. Despite their importance, gait abnormalities have not been investigated in mouse models of AD, which replicate important aspects of the human disease. The Tg2576 is frequently used in AD research to test therapeutic interventions targeting cellular mechanisms contributing to the genesis of AD. This transgenic mouse strain overexpresses a mutant form of the 695 amino acid isoform of human amyloid precursor protein with K670N and M671L mutations (APPK670/671L) linked to early-onset familial AD. Tg2576 mice exhibit impaired cognitive functions and increased cortical and hippocampal soluble β-amyloid levels starting from 5 months of age and increased insoluble β-amyloid levels and amyloid plaques that resemble senile plaques associated with human AD by 13 months of age. To demonstrate early manifestations of gait dysfunction in this relevant preclinical model, we characterized gait and motor performance in 10-month-old Tg2576 mice and age-matched littermate controls using the semi-automated, highly sensitive, Catwalk XT system. We found that Tg2576 mice at the pre-plaque stage exhibited significantly altered duty cycle and step patterns and decreased stride length and stride time.

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Base-of-support, stride time variability, stride length variability, cadence, phase dispersions and gait symmetry indices were unaltered. The presence of measurable early gait abnormalities during the pre-plaque stages of AD in this relevant preclinical mouse model has direct translational relevance and supports the view that longitudinal monitoring of gait performance could be used in addition to behavioral testing to evaluate progression of the disease and to assess treatment efficacy.

Keywords Brain aging · Neurodegeneration · Motor performance · Balance · Gait · Neurodegenerative disease

Introduction

Alzheimer's disease (AD) is the most common cause of age-induced dementia, and the fifth leading cause of death among Americans age 65 and older. Currently, almost 6 million elderly Americans are living with AD, and this number is projected to double by 2040. During the past decade, the Alzheimer research field has witnessed a conceptual shift favoring the understanding of the disease as a continuum.

Although the main clinical hallmark of AD is cognitive impairment, a number of studies reported that AD pathogenesis is also associated with motor impairments, including bradykinesia, extrapyramidal rigidity, and gait disorders [1]. More importantly, clinical studies have demonstrated that a range of gait impairments, including slowing gait, may be manifested at early stages of AD and can be used as an early biomarker to identify patients at risk of progressing to full-blown dementia [2–20]. Gait depends on a complex interplay of central nervous system networks including motor, sensory and cognitive functions. Gait alterations can be caused by pathological processes affecting any part of these networks, including both pathologies affecting the grey matter and white matter damage. Gait alterations and cognitive impairments frequently co-occur, as the underlying brain regions overlap [21]. For example, frontal subcortical circuits play a critical role in gait control, which overlap with neuronal circuits controlling executive function and attention [21]. Aging-induced and AD-related pathologies (including amyloid pathologies, neuroinflammation, microvascular pathologies, neurodegeneration) similarly affect neuronal circuits mediating gait control and contributing to cognitive function, which can result in combined cognitive and gait impairments. In most people with AD, cognitive symptoms and sub-clinical gait alterations first manifest much sooner before the diagnosis (the average age of diagnosis is 80 years). The large time gap between AD-related gait dysfunction at the preclinical stage and diagnosis of AD represents a missed opportunity for intervention.

Over the past decades several transgenic mouse models that recapitulate various aspects of AD have been generated to assess the effects of a wide range of genetic, pharmacological, and nutritional interventions to prevent/slow the progression of the disease. Assessment of gait performance in these models has important translational relevance. Preliminary evidence suggests that mouse gait is altered in aging and in pathophysiological conditions associated with accelerated brain aging [22, 23]. Despite these advances and the emerging clinical and diagnostic importance for gait in AD, preclinical studies on the impact of AD on mouse gait are scarce and the available mouse models are not well characterized.

The present study was designed to characterize gait function in the Tg2576 mouse model of AD and to provide a comprehensive analysis of potential gait disturbances associated with amyloid pathology. The Tg2576 mouse model is frequently used in AD research to test therapeutic interventions targeting cellular mechanisms contributing to the genesis of AD. This transgenic mouse strain overexpresses a mutant form of the 695 amino acid isoform of human amyloid precursor protein with K670N and M671L mutations (APPK670/671L) linked to early-onset familial AD. Tg2576 mice exhibit impaired cognitive functions and increased cortical and hippocampal soluble β-amyloid levels starting from 5 months of age and increased insoluble β-amyloid levels and amyloid plaques that resemble senile plaques associated with human AD by 13 months of age. To determine whether AD pathologies are associated with gait alterations in this relevant preclinical model, gait function in 10-month-old Tg2576 mice and age-matched littermate controls was characterized using the semi-automated, highly sensitive, Catwalk XT system. We analyzed gait parameters that have direct translational relevance (e.g., gait speed, swing speed, cadence, stride length, stride time, base of support) and adopted and developed methods to analyze stride time and stride length variability, which are considered sensitive indices of AD-related human gait abnormalities with predictive potential [20].

Methods

Experimental animals

Age matched (10-month-old) Tg2576 and WT controls mice (n=10 in each group) were used. Mice were kept under

specific pathogen-free barrier conditions in the Rodent Barrier Facility at University of Oklahoma Health Sciences Center under a controlled photoperiod (12 h light; 12 h dark). All the performed procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma Health Sciences Center.

Analysis of gait function

To determine the impact of amyloid pathologies on gait coordination, we tested both groups of mice using a highly sensitive, automated computer assisted method (CatWalk; Noldus Information Technology Inc.) [24]. The Catwalk system is a sophisticated apparatus that allows for quantitative assessment of footfall and motor performance. Mouse paw location and placement patterns were recorded by a high-speed high-resolution camera while the animals were allowed to freely walk on an illuminated glass platform, providing accurate and repeatable measurements of gait function and spatial and temporal aspects of interlimb coordination [22, 25]. Briefly, animals from both groups were acclimatized and trained to voluntarily walk across the illuminated walkway in a dark and quiet room dedicated for behavioral experimentation. Mice gait function was acquired for over 20 consecutive runs, producing over 200 steps for each animal. The resulting data was averaged across the ~ 20 runs in which mice maintained a relatively constant speed across the walkway. Subsequently, computer-aided analysis of the gait data and manual paw identification and labeling of each footprint was carried out blindly and spatial and temporal gait parameters were calculated. The variability of the data has been assessed using quartile dispersion. We adopted a common outlier definition, labeling points more than 1.5 interquartile ranges away from the sample median as extreme values. Mean gait characteristics calculated included speed, swing speed, cadence, stride length, stride time, duty cycle, and base of support. Cadence is the measurement of stride frequency and is expressed in steps/second. Stride length is the distance (in cm) between successive placements of the same paw. Stride time is the interval lapsed (s) between each successive paw contact. Duty cycle (%) expresses the interval of time during stand as a percentage of the whole step cycle:

$$Duty\ cycle = \frac{Stand\ (s)}{Stand\ (s) + Swing(s)} \times (100\%)$$

$$Regularity\ Index = \frac{\#\ of\ normal\ step\ sequence\ patterns \times 4}{total\ \#\ of\ paw\ placements} \times (100\%)$$

Base of support is the average width between either the front paws or the hind paws. Variability characteristics and measures of gait coordination were also acquired using the CatWalk system. The regularity index (%) is a fractional measure of inter-paw coordination, which expresses the number of normal step sequence patterns relative to the total number of paw placements.

In young healthy, fully coordinated mice, the regularity index value is closer to 100%. Investigating gait variability [26, 27], the stride-to-stride fluctuations in gait parameters, offers a sensitive, novel method of quantifying subtle changes in locomotion in mice. Step time and step length variability were analyzed by computing the median absolute deviation (MAD) for datasets that contained > 200 steps for each animal, obtained in consecutive runs at similar speeds where each step value is X (X_1, X_2, …, X_{200}). MAD is a robust measure of statistical dispersion, which is more resilient to outliers in a data set than the standard deviation [28].

The Phase Dispersions parameter describes the temporal relationship between placement of two paws (anchor and target) within a step cycle and is a parameter used to study inter-paw coordination across six pairs of paws (diagonal pairs: RF-LH, LF-RH, ipsilateral pairs: RF-RH, LF-LH, and girdle pairs: LF-RF, LH-RH). In healthy young animals with normal gait, the diagonal paw pairs typically move synchronously with the anchor, so the initial contacts (IC) occur simultaneously resulting in a phase dispersions value of 0%. Ipsilateral and girdle pairs typically alternate resulting in an expected phase dispersion of 50%. Phase Dispersions is calculated by measuring the timing between Initial Contacts of a paw pair and expressing it as a percentage of the step cycle time of the anchor paw. As an index for asynchronous stepping, the absolute deviation from the expected coupling

value can be calculated. For instance, if diagonal limbs move synchronously, representing high gait coordination, the expected value of inter-limb coupling is 0. In contrast to diagonal limb pairs, lateral pairs are known to alternate during walking. When the two limbs alternate perfectly, the inter-limb coupling value is 50%. Deviation from the "expected value" (representative of high level of gait coordination) is shown as Δ Phase dispersion.

$$Median\ Absolute\ Deviation\ (MAD) = median\left(\left|X_i - median(X)\right|\right)$$

$$Phase\ Dispersion = \frac{IC_{Target_m} - IC_{Anchor_n}}{Step\ cycle_{anchor}}$$

Rodents can potentially alternate between multiple step sequence patterns during spontaneous walk. The Catwalk system records the actual order of footfalls as they occur and categorizes them based on common and uncommon sequence patterns. The 4 most commonly used gait patterns in rodents, known as the "normal step patterns", are the alternate patterns AA (RF-RH-LF-LH) and AB (LF-RH-RF-LH), and the cruciate patterns CA (RF-LF-RH-LH) and CB (LF-RF-LH-RH). The rotate patterns are seldomly used: [Ra paw sequence: RF-LF-LH-RH and Rb paw sequence: LF-RF-RH-LH]. To define the pattern utilized by each mouse the right front paw was arbitrarily chosen as the initial step.

Statistical analysis

Statistical analysis was carried out by unpaired or paired t test, as appropriate, using Prism 5.0 for Windows (Graphpad Software, La Jolla, CA). A p value less than 0.05 was considered statistically significant. Data are expressed as mean ± S.E.M.

Results

Gait alterations in Tg2576 mice

In the present study, the regularity index, a comprehensive measure of inter-paw coordination, did not differ between freely moving Tg2576 and WT mice (Fig. 1A). No significant difference in cadence (Fig. 1B) was detected between freely moving Tg2576 and WT mice. We also did not observe differences between Tg2576 and WT mice in base of support (hind paws; Fig. 1D), and average speed (Fig. 1E).

An important consideration in gait studies is speed, as the velocity with which the mouse walks affects gait patterns. In the CatWalk, the mice are allowed to freely walk at their preferential speed. From the data it is evident that body speeds were maintained uniform without significant differences between groups (WT: 27 ± 2 cm/s Tg2576: 28 ± 2 cm/s. Similarly, no differences were observed in body weights between the two groups (data not shown). These results indicate that all differences in gait are due to gait mechanics and not speed or weight/size of the animals.

Tg2576 mice showed significantly larger duty cycle (Fig. 1C), decreased stride time (Fig. 1F), and stride length (Fig. 1G), consistently with previous finding from other laboratories [29]. The study of gait variability, the stride-to-stride fluctuations in walking, offers a sensitive method of quantifying and characterizing locomotion. Previous studies in patients with AD suggest that measures of gait variability [30] are more closely related to cognitive decline or falls than other measures of gait characteristics [3, 12, 19, 31–37]. However, in the present study, we did not find statistically significant changes in stride length or stride time variability in Tg2576 mice (Fig. 2A and 2B). Phase dispersion measures inter-paw coordination across girdle, ipsilateral and diagonal paw pairs. Increased deviation from expected phase dispersion values is representative of worsening inter-paw coordination. In general, Tg2576 mice displayed increased deviation in phase dispersion across all paw pairs when compared to WT littermate controls (Fig. 3A–E). The regularity index was similar in Tg2576 and WT animals (Fig. 1A), suggesting that both groups used predominantly the four natural gait patterns (see Methods). Yet, we found differences in the gait-pattern frequency distribution between WT and Tg2576 mice (Fig. 3G). Tg2576 mice exhibited a 20% increase in frequency of the radial pattern AA, known as

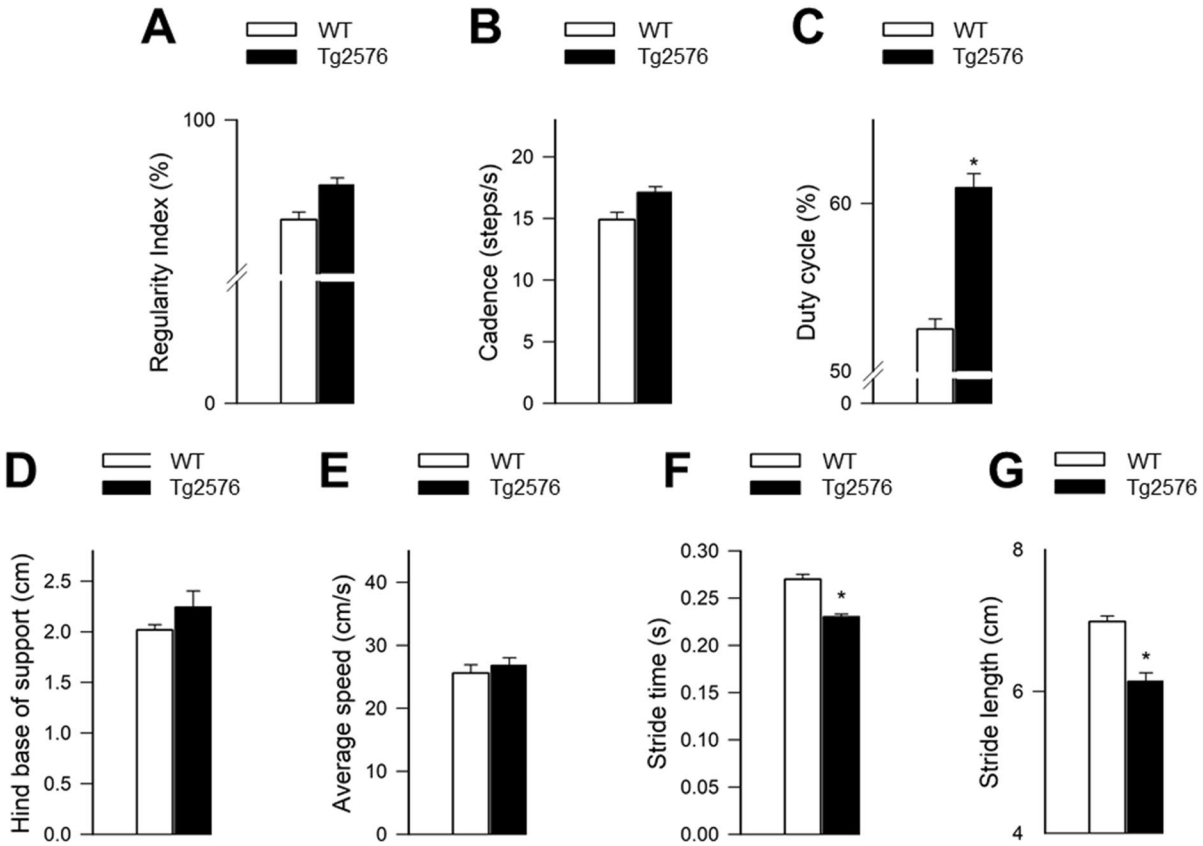


Fig. 1 Changes in gait parameters in the Tg2576 mouse model of AD. (**A**) Regulatory index, (**B**) cadence, (**C**) duty cycle, (**D**) hind base of support, (**E**) average speed, (**F**) stride time, and (**G**) stride length in freely moving wild type control and Tg2576 mice. Data are mean ± SEM. ($n = 10$ in each group). *$P < 0.05$ vs. control

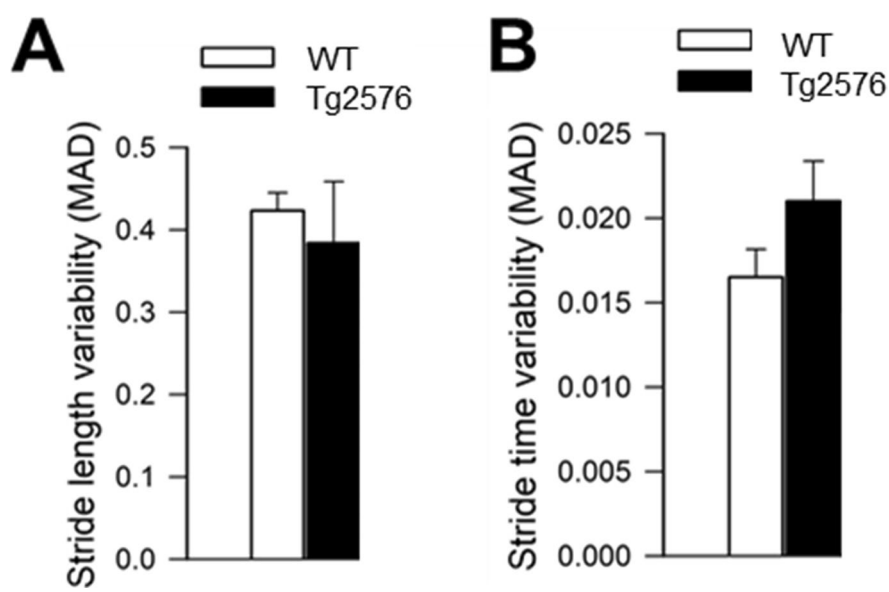
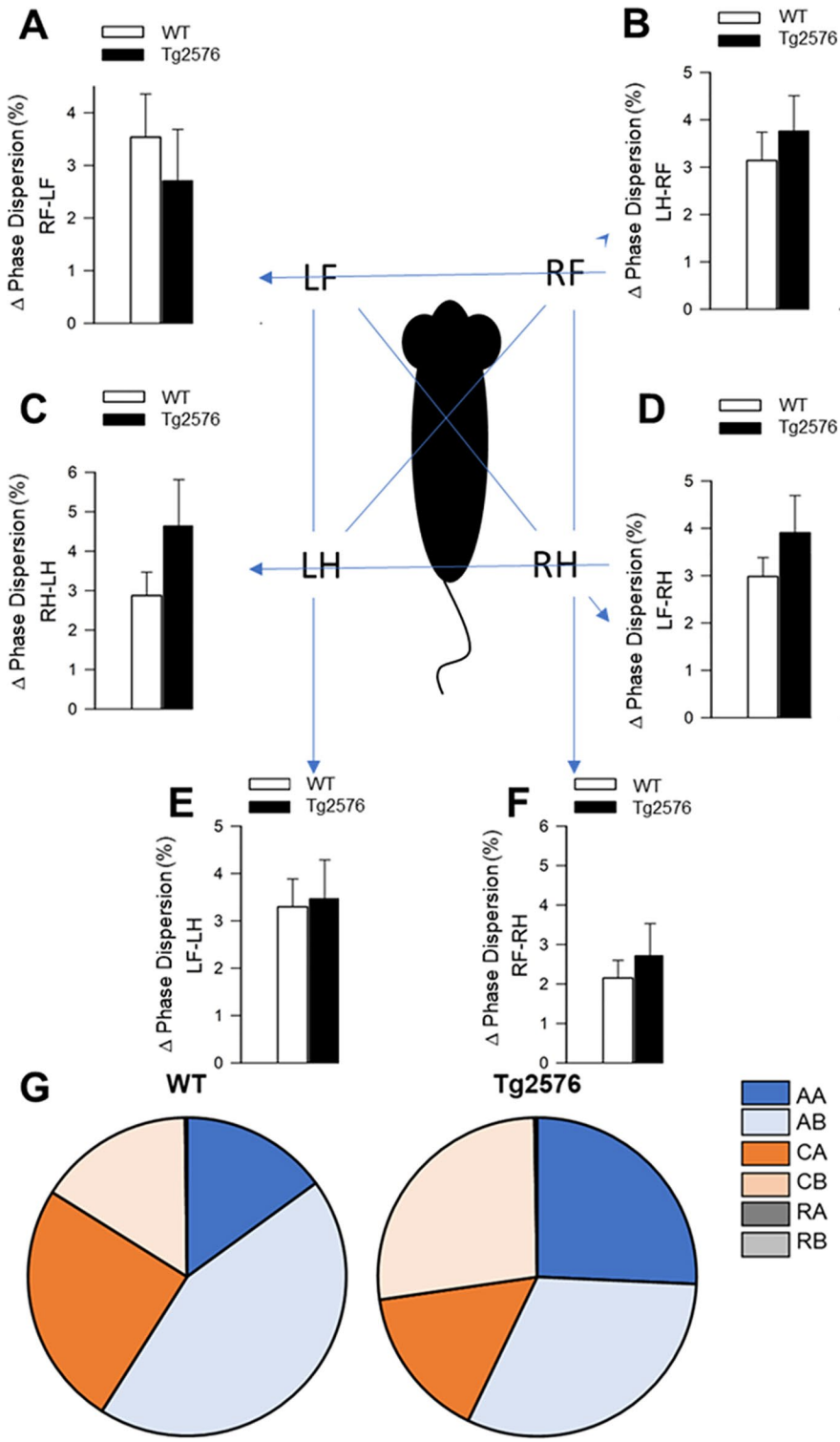


Fig. 2 Gait variability in the Tg2576 mouse model of AD. Stride length variability (**A**) and stride time variability (**B**) do not significantly change in 10-month-old Tg2576 mice compared to control wild type mice. MAD: median absolute deviation. Data are mean ± SEM. ($n = 10$ in each group)


A
WT
Tg2576
Δ Phase Dispersion (%)
RF-LF
B
LH-RF
C
RH-LH
D
LF-RH
E
LF-LH
F
RF-RH
LF
RF
LH
RH
G
WT
Tg2576
AA
AB
CA
CB
RA
RB

◂**Fig. 3** Inter-limb coupling in the Tg2576 mouse model of AD. (**A**–**F**) Inter-limb coupling during spontaneous walk in 10-month-old Tg2576 mice and control wild type mice. To assess genotype-related impairment of gait coordination, we compared the degree of synchronization between diagonal, horizontal and lateral limb couplets in each group. Inter-limb coupling was assessed by computing the interval between onset times of each paw in a couplet contacting the floor. As an index for asynchronous stepping, the absolute deviation from the expected coupling value was calculated. For example, if diagonal limbs move synchronously, the expected value of inter-limb coupling is 0. In contrast to diagonal limb pairs, lateral pairs are known to alternate during walking. When the two limbs alternate perfectly, the inter-limb coupling value is 50%. Bar graphs are summary data, indicating that WT mice showed a narrow distribution around the expected value, Tg2576 tended to exhibit a more dispersed distribution. (**G**) Mice utilize six different gait footfall patterns (Cruciate [Ca paw sequence: RF—LF—RH – LH and Cb paw sequence: LF—RF—LH – RH]; Alternate: [Aa paw sequence: RF—RH—LF – LH and Ab paw sequence: LF—RH—RF – LH]; Rotate: [Ra paw sequence: RF—LF—LH – RH and Rb paw sequence: LF—RF—RH – LH]. The relative frequency of footfall patterns utilized by mice in each group is shown, Tg2576 mice the frequency of preferred step sequences was altered, as they used more frequently the radial AA and the alternate CB pattern than wild type control mice and compensated with a decreased use of the AB and CA pattern

the "giraffe walk". This gait pattern was found to be elevated in aged mice [24] and in mouse models with increased gait instability [38]. The alternating contralateral footfall pattern CB was used with increased frequency in Tg2576 mice compared to WT controls. Thus, the compensatory adjustments in gait mechanics in Tg2576 mice, observed via gait-pattern frequency distribution, revealed that Tg2576 mice preferentially relied on the AA radial pattern and alternating CB pattern over the AB and CA gait patterns preferentially used by WT mice.

Discussion

Our study provides evidence that 10-month-old Tg2576 mice partially recapitulate important aspects of gait alterations observed in AD patients in a translationally relevant mouse model of AD. The Tg2576 model is one of the most well characterized, and widely used, mouse models of AD. It overexpresses the APP isoform 695 mutant with the K670N, M671L Swedish mutation, resulting in elevated levels of Aβ and ultimately amyloid plaques in the brain. By 11–13 months of age, hemizygous mice develop numerous parenchymal Aβ plaques along with some vascular amyloid. They also show oxidative lipid damage but no evidence of neurofibrillary tangles or neuronal loss [39]. At 10 months of age, Tg2576 mice do not manifest parenchymal Aβ plaques and are devoid of neurofibrillary tangles. Changes in gait characteristics were studied in 10-month-old Tg2576 mice, which is ideal to investigate the presence of measurable early gait abnormalities during the pre-plaque stages of the disease. This stage of the AD pathogenesis in this model corresponds to the preclinical phase of AD in humans, in which gait alterations are already manifested [2–5].

Using automated, computer-aided gait analysis we demonstrated that freely moving Tg2576 mice exhibited significantly altered gait signatures compared to wild type controls, including changes in stride time, stride length, duty cycle, and footfall pattern distribution. These findings provide important reference values for the design of future studies attempting to characterize the effects of dietary, pharmacological or genetic interventions on AD pathogenesis. Our study extends previous findings which identified early gait alterations in Tg2576 mice. Seo et al. showed that Tg2576 mice had shorter and narrower strides than the non-transgenic control at 10 months of age, whereas Schroer et al. found significantly reduced stride times and increased stride frequency in the Tg2576 mouse model prior to expected plaque accumulation, independently from body size and mass [29, 40]. Gait abnormalities present in Tg2576 mice at the pre-plaque stage recapitulate important aspects of early gait alterations in human AD patients [2–5]. Clinical studies have found that the severity of cognitive decline in human patients closely relates to the severity of gait changes: observable changes mainly include decrease in walking speed, a decrease in stride length, and an increase in support phase [41]. High gait variability is a newly identified marker of cognitive-cortical dysfunction in AD patients [20]. In contrast to our expectation, we found that gait variability was not affected in Tg2576 mice at the age studied. Further studies are warranted to determine how gait variability changes in this model with progression of the plaque development. Additionally, future studies should also determine the impact of amyloid pathologies on gait function, including gait variability, in other mouse models of AD and document the time course of these gait alterations. Follow-up

longitudinal studies are also needed to determine gait performance changes in mouse models of AD combined with assessment of cognitive function. It is significant that mice also exhibit age-related gait alterations in the absence of amyloid pathologies [24], which mimic aspects of age-related gait disturbance in older adults [34, 37, 42–65]. As AD is a disease of aging, the interaction of aging and amyloid pathologies to alter gait should be determined.

The mechanisms underlying gait abnormalities in Tg2576 mice likely include cortical dysfunction induced by β-amyloid, similar to the genesis of AD-related gait dysfunction in human patients. Increasing evidence suggests that in humans dementia-related gait abnormalities are not only attributable to motor disorders, but also associated to problems with the cortical processing of information what may be present at the early stage of AD [66, 67]. Recent mechanistic pre-clinical studies in mice and clinical investigations provide proof-of-concept that impaired microvascular function, including attenuated neurovascular coupling responses and development of microhemorrhages, are causally linked to gait abnormalities [22, 23, 50, 55, 68, 69]. Many of these microvascular aberrations have been observed in Tg2576 mice as well as other models of AD [70–80].

In conclusion, Tg2576 mice exhibit quantifiable, clinically relevant alterations in gait function at 10 months of age which recapitulate findings in human patients at early stages of AD. We recommend that longitudinal gait assessment protocols could be incorporated in the experimental designs of preclinical studies aimed at characterization of the effects of dietary, pharmacological or genetic interventions on AD pathogenesis in mouse models.

Acknowledgements This work was supported by grants from the American Heart Association, the Oklahoma Center for the Advancement of Science and Technology, the National Institute on Aging ((R01-AG055395, R01-AG047879; R01-AG038747; R01-AG072295), the National Institute of Neurological Disorders and Stroke (NINDS; R01-NS100782), the National Cancer Institute (NCI; 1R01CA255840), the Oklahoma Shared Clinical and Translational Resources (OSCTR) program funded by the National Institute of General Medical Sciences (U54GM104938, to AY), the Presbyterian Health Foundation and the NKFIH (Nemzeti Szivlabor). The authors acknowledge the support from the NIA-funded Geroscience Training Program in Oklahoma (T32AG052363), the Oklahoma Nathan Shock Center (P30AG050911), the Cellular and Molecular GeroScience CoBRE (1P20GM125528, sub#5337). The funding sources had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declarations

Competing interests Dr. Anna Csiszar serves as Associate Editor for The Journal of Gerontology, Series A: Biological Sciences and Medical Sciences and GeroScience. Dr. Andriy Yabluchanskiy serves as Guest Editor for The American Journal of Physiology-Heart and Circulatory Physiology. Dr. Zoltan Ungvari serves as Editor-in-Chief for GeroScience and as Consulting Editor for The American Journal of Physiology-Heart and Circulatory Physiology. The authors declare no competing financial interests.

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