Unveiling Frailty: Comprehensive and Sex-Specific Characterization in Prematurely Aging PolgA Mice

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Abstract

Frailty, a geriatric syndrome, is assessed using the frailty phenotype (FP) and frailty index (FI). While these approaches have been applied to aging mice, their effectiveness in prematurely aging mouse models such as PolgA$^{D257A/D257A}$ (PolgA) has not been completely explored. We demonstrated that frailty became evident in PolgA mice around 40 weeks, validated through body weight loss, reduced walking speed, decreased physical activity, and weaker grip strength. Moreover, we also identified sex differences in these mice with females exhibiting higher frailty compared to males. Frailty prevalence in PolgA mice at 40 weeks parallels that observed in naturally aging mice at 27 months and aging humans at 65-70 years. These findings contribute to understanding frailty onset and sex-specific patterns, emphasizing the significance of the PolgA mouse model in investigating aging and related disorders.

Introduction

Frailty is a complex geriatric syndrome that is characterized by a diminished ability to respond to stressors and maintain homeostasis (Milte and Crotty, 2014). Two widely used approaches to evaluate frailty are the frailty phenotype (FP) and the frailty index (FI)(Fried et al., 2001, Rockwood et al., 2005). The FP assesses individuals based on the presence or absence of specific criteria, which are: unintentional weight loss, slow walking speed, self-reported exhaustion, weakness, and low physical activity. Individuals, meeting three or more of these criteria are classified as frail[2]. On the other hand, the FI is based on the accumulation of health deficits, where the total number of deficits is divided by the number of deficits assessed, providing a quantitative measure of frailty (Rockwood et al., 2005). Both the FP and FI are valuable approaches for identifying frailty in individuals, enabling a better understanding of its impact on health outcomes and aging research.

With the intention of investigating frailty, mouse models that accurately replicate the complex nature of frailty in humans have been developed(Yilmaz et al., 2023). Several research groups established frailty assessment tools designated for mice based on human criteria (FP or FI)(Baumann et al., 2016, Baumann et al., 2020, Liu et al., 2014, Parks et al., 2012). Slowness (rotarod), weakness (grip strength), physical activity (rotarod), and endurance (combined

rotarod and grip strength) were assessed to develop frailty in mice by adopting the FP approach for humans(Liu et al., 2014). Similarly, frailty was defined in mice by using the clinical human FI approach, which involved 31 parameters, including basal metabolism, body composition, and activity levels in evaluating signs of discomfort(Whitehead et al., 2014) and the modified versions of these methods were used by other groups to assess frailty(Baumann et al., 2018, Baumann et al., 2019, Baumann et al., 2020, Gomez-Cabrera et al., 2017, Kwak et al., 2020, Sukoff Rizzo et al., 2018). However, the methods employed for assessing frailty in mice have primarily been applied to C57BL/6 (naturally aging) mice aged 17 months and older(von Zglinicki et al., 2016). Therefore, it remains unclear whether these methods are equally effective in identifying frailty in prematurely aging mice.

In this regard, prematurely aging mouse models such as PolgA$^{D257A/D257A}$ (referred to as PolgA) offer numerous advantages over naturally aging mice(Yilmaz et al., 2023). Notably, PolgA mice demonstrate the premature manifestation of age-related phenotypes within a short time frame(Trifunovic et al., 2004). Around just 40 weeks of age, these mice exhibit multiple hallmarks of aging, including kyphosis, hearing impairment, progressive weight loss, osteosarcopenia, and osteoporosis(Kujoth et al., 2006, Trifunovic et al., 2004, Scheuren et al., 2020). In addition, age-related changes in these mice were evaluated in females using the clinical mouse FI method, exhibiting higher FI scores in PolgA mice compared to their age-matched wild-type littermates (WT)(Scheuren et al., 2020). Although the PolgA mouse model holds promise for investigating frailty and age-related musculoskeletal disorders, there are certain limitations in its frailty scoring. Despite being a non-invasive method, FI scoring, in general, lacks assessments such as walking speed and endurance to measure the functional abilities of mice. Moreover, the sex-dependent component of frailty has not been addressed in these mice. Therefore, our first objective is to identify the frailty phenotype in prematurely aging PolgA mice around 40 weeks by evaluating weight loss, grip strength (weakness), walking speed (slowness), and home cage walking distance (physical activity) in comparison to their age-matched WT littermates. Our second objective is to show the sex-dependent differences in frailty in PolgA mice.

Results

When considering age-related changes in the PolgA mice, there were notable differences compared to WT mice for both sexes (Figure 1). Firstly, PolgA males were observed to be 13% lighter than their WT counterparts, whereas this difference was -17% for females, reflecting the unintentional body weight loss observed in sarcopenia patients(Bales and Ritchie, 2002) (Figure 1A). In terms of slowness, PolgA males exhibited a 23% reduction in walking speed compared to WT males, while PolgA females showed a 14% decrease compared to WT females (Figure 1B). Although there was no significant difference in grip strength among males, PolgA males exhibited an 11% decrease in grip strength, whereas there was a 23% decrease in grip strength among females compared to WT females (Figure 1C). In the case of physical activity, there was an 11% and 18% decrease for males and females respectively between PolgA and WT mice (Figure 1D). Hence, the age-related differences were notably prominent in PolgA mice, with the differences being more pronounced in females around 40 weeks.

To assess the frailty of PolgA mice, cutoff values, and the ranking order of mice for each criterion from best to worst, were identified (Figure 2A-B). Mice were considered frail if their performance fell below the cutoff values for the designated parameters for body weight (31.3 g, 24.4 g), grip strength (101.4 gf, 73.8 gf), average speed (23.3 cm/s; 21.6 cm/s) and average distance (769.1 cm, 1025.9 cm) for male and female mice respectively using the reference values obtained from WT mice around 40 weeks. According to these criteria, 5 PolgA male mice and 4 PolgA female mice were categorized as frail, while 3 PolgA male and female mice were classified as pre-frail. None of the PolgA mice were identified as non-frail. In contrast, among the WT mice, 2 males and females were designated as pre-frail, and 6 males and females were categorized as non-frail based on the same criteria. Regardless of sex, none of the WT mice were identified as frail (Figure 2A-B). In addition, these cut-off values obtained from WT mice for each sex were utilized as a reference to assess the prevalence of frailty in PolgA mice (Figure 2C). 62.5% of PolgA male mice were classified as frail, while 37.5% were categorized as pre-frail. On the other hand, 57.1% of PolgA females were identified as frail,

while 42.9% were designated as pre-frail. In contrast, the majority of the WT males, 85.7%, were designated as non-frail while only 14.3% of them were identified as pre-frail. Similarly,75 % of WT females were categorized as non-frail and 25% as pre-frail (Figure 2C).

To identify the morphological differences between the mice identified as frail, pre-frail, and non-frail, we systematically compared these subgroups by evaluating each criterion to assess frailty. No distinct difference was observed in body weight within the male subgroups of PolgA and WT mice. However, non-frail female WT mice exhibited a higher body weight compared to pre-frail WT mice. Likewise, pre-frail PolgA females had a slightly greater body weight compared to their frail counterparts (Figure 3A). Regarding the slowness, there were no distinctive variations in walking speed among subgroups of PolgA and WT males. In contrast, female PolgA frail mice displayed a slower walking speed in comparison to the pre-frail group while no variance in walking speed among WT females was observed (Figure 3B). For comparison of the weakness, pre-frail PolgA mice had higher grip strength compared to frail PolgA mice, regardless of sex. Whereas non-frail WT mice exhibited higher grip strength compared to pre-frail mice for both sexes (Figure 3C). A similar trend was observed for comparison of physical activity assessed by average walking distance. Pre-frail PolgA mice showed increased walking distances compared to frail PolgA mice, irrespective of sex for both groups while non-frail WT mice covered longer distances compared to their pre-frail counterparts (Figure 3D).

Furthermore, individual frailty scores were calculated as percentages, determined by dividing the total number of failed tests by the total number of tests performed for each group (Martinez de Toda et al., 2018) (Supplementary Table 1). PolgA males had a frailty score of 65.6%, while females had a score of 75%. In contrast, the frailty scores for WT mice were 25% for both males and females, indicating a higher level of frailty in female PolgA mice compared to males and WT females.

The calculation of frailty scores has revealed that the key contributors to frailty in our study include alterations in body weight, walking speed, and grip strength (Supplementary Table 2).

Therefore, we compared these primary contributors used to evaluate frailty across different ages (Figure 4).

Body weight gradually increases with aging in PolgA and WT mice from 12 to 40 weeks, with a more pronounced increase in WT mice. However, PolgA mice begin to experience weight loss after 40 weeks for both sexes. The differences in body weight between PolgA and WT mice become statistically significant at 34 and 40 weeks for males, while for females this significance is observed only at 40 weeks (Figure 4A). Considering the slowness, there was no distinct difference between PolgA and WT mice males until 40 weeks. However, at 40 weeks, a significant decrease in walking speed is observed between WT and PolgA mice. In females, although there is a trend of decreasing speed with age in PolgA females, there is no notable difference between WT mice at any age (Figure 4A). Regarding the grip strength, there were no notable differences in males. However, in females, PolgA mice showed a gradual decrease over time, and at 40 weeks, this decrease was significant compared to WT females (Figure 4A).

Finally, we assessed FI in PolgA mice at these various ages. Our results showed that the frailty of PolgA male and female mice increased with age which confirms our observations. PolgA male mice in particular demonstrated slightly higher FI compared to their WT and female counterparts at 40 weeks (Figure 4B). This particular outcome might be attributed to the fact that females exhibited greater impairments in physical functions at 40 weeks while males accumulated a higher cumulative burden of health deficiencies although they performed better in these physical function measures.

Discussion

In this study, we applied the human frailty phenotype approach(Fried et al., 2001) to evaluate frailty characteristics of prematurely aging PolgA mice by assessing body weight loss, impaired strength, reduced walking speed, and limited physical activity. By applying this approach, we were able to effectively identify the onset and prevalence of frailty in PolgA mice and further elucidate the sex differences. Previous studies had evaluated frailty in WT mice

as early as 17 months old, indicating the onset of frailty at approximately 27 months(Baumann et al., 2020). In contrast, PolgA mice developed frailty as early as 40 weeks, roughly equivalent to 20-23 months in C57BL/6 mice, and corresponds to frailty onset at 65–70 years in humans(Fried et al., 2001, Dutta and Sengupta, 2016, Justice et al., 2016).

At 29 months of age, 52.4% of WT mice were shown to be frail and another study reported frailty rates of 44.4%, for males and 73.7% for females at 26 months(Baumann et al., 2019, Baumann et al., 2020). Using a similar assessment method, PolgA mice exhibited a prevalence of frailty approximately at 40 weeks with rates of 63% for males and 57% for females. In addition, the calculated frailty scores for PolgA mice were 65.6 % for males and 75% for females while the frailty score for the prematurely aging mice was previously reported as 40%(Martinez de Toda et al., 2018).

Collectively, our data show that PolgA mice have higher frailty scores compared to their age-matched WT counterparts irrespective of sex. Furthermore, the prevalence of frailty among PolgA mice at 40 weeks is comparable to that observed in WT mice at around 27 months of age. Considering the sex differences, female PolgA is frailer compared to male PolgA. This observation parallels with what was similarly observed in humans, where women were known to exhibit higher frailty compared to men(Gordon et al., 2017). While there has been some controversy regarding increased frailty in female mice, some studies have indicated no significant sex differences while some demonstrated female C57BL/6 mice were frailer compared to males(Baumann et al., 2019, Whitehead et al., 2014, Parks et al., 2012). Moreover, it was argued that the prevalence of frailty increases with age, ultimately resulting in early mortality among frail mice, regardless of sex(Baumann et al., 2019, Baumann et al., 2018, Kwak et al., 2020, Kane et al., 2018). Nevertheless, our findings underscore the existence of sex differences in aging PolgA mice, aligning with observations related to the frailer female naturally aging mice and humans.

One limitation of our study is the small sample size contributing to non-significant results and altering the outcomes for the prevalence of frailty. In addition, the average walking distance of males might be impacted by their single housing conditions, potentially leading to no notable

differences between the groups. Moreover, our frailty phenotype assessment is based on the 4 criteria adopted from the mouse frailty phenotype approach, which was derived from the human approach(Fried et al., 2001, Liu et al., 2014). However, the mouse FP approach lacked a weight factor and did not include an established endurance test. On that note, we could have incorporated an endurance test into our studies to provide a more comprehensive overview of the frailty phenotype status of PolgA mice. However, the traditional treadmill running test used to evaluate exhaustion in animals has faced significant scrutiny from an animal welfare point-of-view due to the stress it imposes on the animals.

In conclusion, our study comprehensively characterized frailty in prematurely aging PolgA mice. The onset of frailty in PolgA mice was evident around 40 weeks, thereby confirming the validity of the observed frailty phenotypes including body weight loss, reduced walking speed, grip strength, and physical activity. FI scoring corroborated these findings and highlighted an age-associated increase in frailty with the onset observed at 40 weeks in PolgA mice.

Furthermore, our study revealed sex differences, with females displaying a slightly higher susceptibility to frailty compared to males. We contextualized these results by comparing them with their WT littermates and referencing established literature on natural aging in mice and humans, emphasizing PolgA mice as a valuable model for investigating aging and age-related disorders.

Methods

Animal models

All animal procedures were carried out by the regulations set by the local authorities (license: ZH35/2019) and approved by the Verterinäramt des Kantons Zürich, Zurich, Switzerland. A colony of mice expressing an exonuclease-deficient version of the mitochondrial DNA polymerase γ (PolgAD257A, B6.129S7(Cg)-Polgtm1Prol/J, JAX stock 017341, The Jackson Laboratory) was bred and maintained at the ETH Phenomics Center. The mice were housed under standard conditions, including a 12:12 h light-dark cycle, with access to maintenance feed and water ad libitum, and three to five animals per cage. The mice were bred as previously described(Scheuren et al., 2020) and genotyped before the experiments

(Transnetyx, Cordova, USA). Homozygous PolgA mice and age- and sex-matched WT littermates (controls) were used for all experiments.

Grip strength

Grip strength was assessed using a force tension apparatus (Grip Strength Meter, model 47200, Ugo Basile) approximately at 40 weeks in female and male PolgA and WT mice as previously described(Scheuren et al., 2020). The mice held the stationary bar with their forepaws and were then gently pulled horizontally by their tail until they released their grip. This procedure was repeated five times for each mouse, and the maximum force (gram-force) value was used for the analysis. All measurements were conducted by the same trained operator.

Gait analysis

Gait data was collected from female and male PolgA and WT mice around 40 weeks of age using the Noldus CatWalk XT system. Run speed, stand, duty cycle, swing speed, maximum variation, and stride length were analyzed to assess the gait characteristics of the mice.

Physical activity

The average distance was measured from the Phenomaster experiment and used to assess physical activity level. The PhenoMaster metabolic cage system was utilized to record various physiological and behavioral measurements including the measurement of drinking and feeding amounts, CO_2 and O_2 consumption, walking speed, and walking distance.

Quantification of frailty index

The Frailty index (FI) in the study was quantified by implementing the Mouse Clinical Frailty Index (FI) consisting of a comprehensive evaluation of 31 non-invasive clinical parameters(Whitehead et al., 2014). Each parameter except body weight and body surface temperature received a score of 0 for absence, 0.5 for a mild deficit, and 1 for a severe deficit. Body weight and body surface temperature were scored relative to the standard deviations from a reference mean in young adult mice (12 weeks old). However, body surface temperature was excluded in the calculation of the FI due to a lack of appropriate tools for measurement.

Frailty phenotype criteria

To establish a frailty phenotype, we identified 4 criteria that are indicative of frailty: body weight loss, grip strength, walking speed, and physical activity. These criteria were deliberately chosen due to their similarity to the clinical criteria for assessing frailty in humans(Fried et al., 2001). By incorporating these key indicators, we aimed to create a comprehensive and reliable frailty phenotype that aligns closely with established clinical mouse and human frailty standards (Table 1). For each parameter, a 20% cut-off value was chosen, except for body weight. In the case of body weight, which was measured prior to the experiments, a 10% cut-off value was applied. This choice was made to maintain alignment with established human criteria and to ensure consistency in assessments between mice and humans, as indicated in the previous publication(Fried et al., 2001, Hill, 2009, Hill et al., 2009, Koch et al., 2016, Leibel, 2008).

Table 1: The criteria for defining mouse frailty for PolgA mice based on the human frailty criteria

Human frailty criteria	Mouse frailty criteria	Cut off values
Unintentional weight loss	Weight loss: Body weight (g) - Males: 31.3 g - - Females: 24.4 g	Lowest 10%
Weakness - Low grip strength	Weakness: Grip strength (gf) - Males: 101.4 gf - - Females: 73.8 gf	Lowest 20%
Slow walking speed	Slowness: Average walking speed (cm/s) - Males: 23.3 cm/s - Females: 21.6 cm/s	Lowest 20%
Low physical activity level	Low physical activity: Average walking distance (Liang et al.) - Males: 769.1 cm - Females: 1025.9 cm	Lowest 20%

Statistical analysis

Data analysis was performed with GraphPad Prism software. The normality of the samples was confirmed by the Shapiro-Wilk test. Mann-Whitney test or one-way ANOVA with mixed

effect analysis was performed as indicated in the figure legends, and $p<0.05$ was considered significant.

Data availability

The authors declare that all data supporting the findings of the study are available within the paper and could be shared upon reasonable request from the corresponding author.

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Author Contributions

DY prepared figures, performed the data analysis, and drafted the manuscript. AS contributed to obtaining physical activity data while NM oversaw the analysis of the gait data. GAK designed and performed the phenotyping experiments used in the study for this manuscript. DY, NM, AS, EW, GAK, and RM, edited and revised the manuscript; DY, NM, AS, EW, GAK, and RM approved the final version.

Declaration of Interest

The authors have no conflict of interest to declare.

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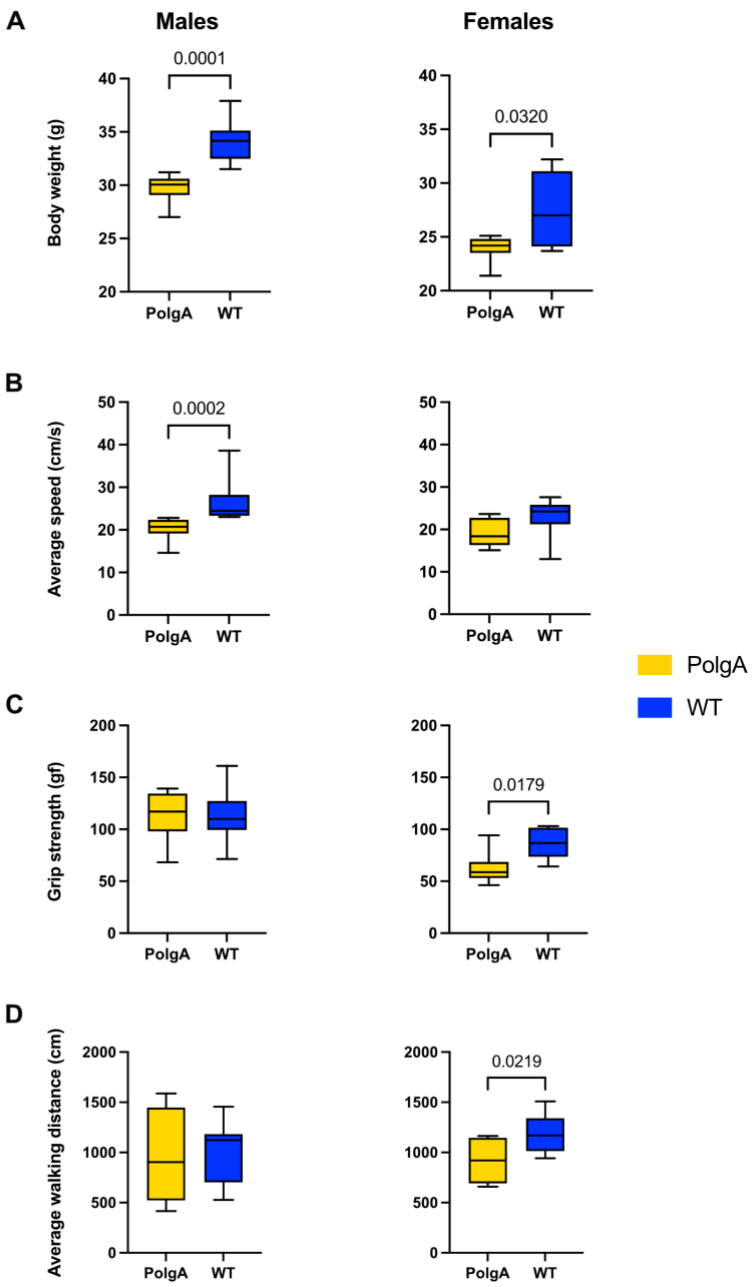

Figure 1: Age and sex-related changes in the PolgA mice. Comparison of each criteria used to assess frailty phenotype between PolgA mice and WT littermates at 40 weeks A) body weight B) average walking speed C), max grip strength D), and average walking distance (n=7-8 mice/group). Mann-Whitney test. Data are presented as mean ± s.d. Exact P value is indicated in the figures.

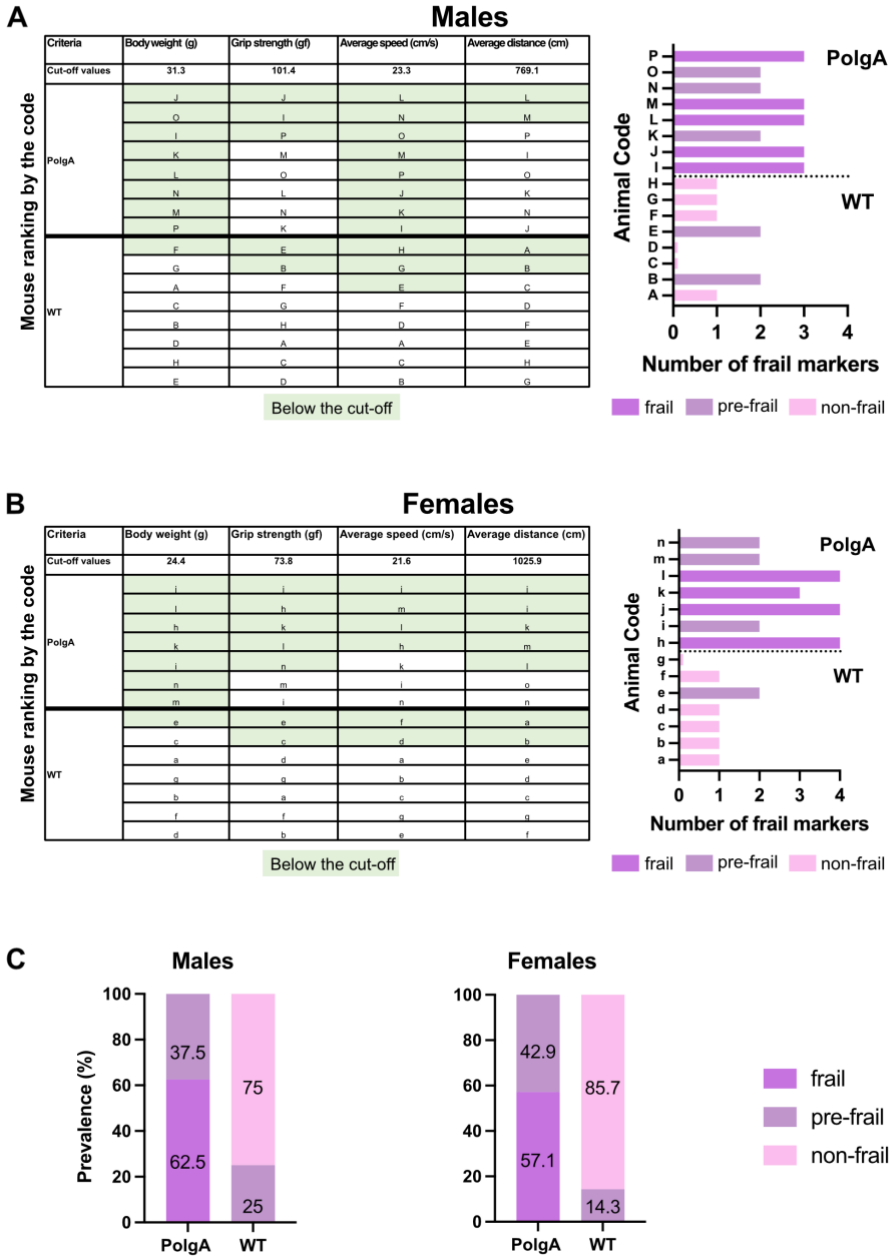

Figure 2: Frailty status of PolgA mice at 40 weeks. A) The mice were coded A-P for males and a-o for females and ranked by their performance. Frailty for PolgA mice of both sexes was calculated using reference values obtained from WT mice for each criterion. The green highlight indicates the mice in the below 20% of the performance for each criterion except body weight (below 10%). B) The number of frailty markers for each mouse at 40 weeks was presented. Frailty was defined if a mouse exhibited three or more of the criterion markers below the cut-off values. Pre-frailty was assigned if a mouse displayed two frailty markers, while non-frailty was indicated if a mouse had one or no markers. C) Prevalence for frailty was determined using the cutoff values obtained from 40-week-old WT mice for each sex and criterion categorized as frail, pre-frail, and non-frail

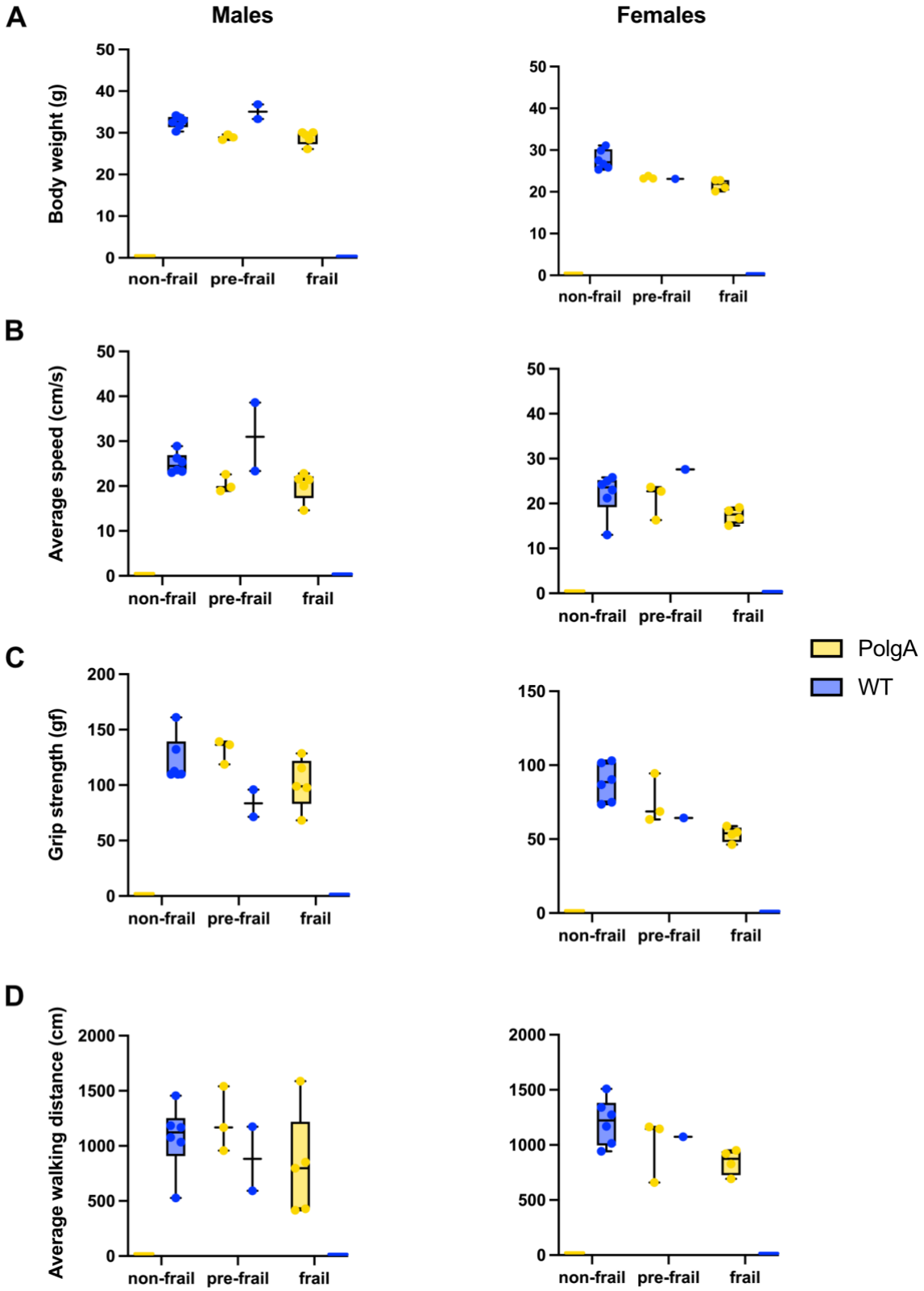

Figure 3: Age-related differences between frail, pre-frail, and non-frail mice. Mice that were identified as frail, pre-frail, and non-frail at 40 weeks were compared for each criterion used to evaluate the frailty phenotype in PolgA and WT mice A) body weight, B) average speed, C) grip strength, D) average walking distance

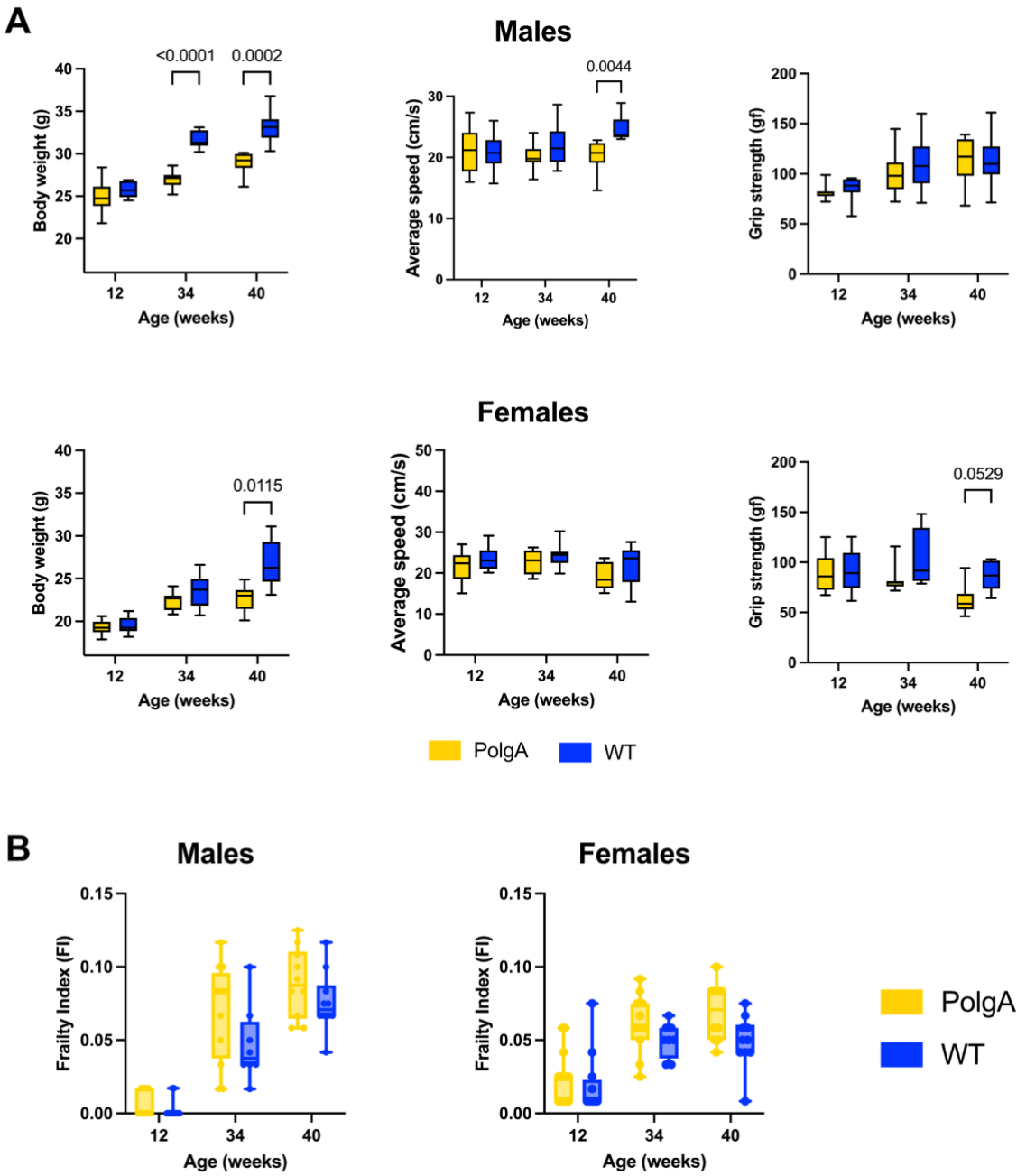

Figure 4: Comparison of key frailty phenotype parameters and frailty index across various age groups in PolgA mice A) Assessment of the primary contributors used to evaluate frailty: body weight, slowness (average speed) and weakness (grip strength) B) Frailty index (FI) in PolgA and wild type (WT) at 12, 34, and 40 weeks (*n* = 7-10/group). One-way ANOVA followed by mixed effect analysis test. Data are presented as mean ± s.d. Exact P value is indicated in the figures.