

Review

Sexual Dimorphism in the 3xTg-AD Mouse Model and Its Impact on Pre-Clinical Research

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Abstract. Female sex is a leading risk factor for developing Alzheimer's disease (AD). Sexual dimorphism in AD is gaining attention as clinical data show that women are not only more likely to develop AD but also to experience worse pathology and faster cognitive decline. Pre-clinical AD research in animal models often neglects to address sexual dimorphism in evaluation of behavioral or molecular characteristics and outcomes. This can compromise its translation to a clinical setting. The triple-transgenic AD mouse model (3xTg-AD) is a commonly used but unique AD model because it exhibits both amyloid and tau pathology, essential features of the human AD phenotype. Mounting evidence has revealed important sexually dimorphic characteristics of this animal model that have yet to be reviewed and thus, are often overlooked in studies using the 3xTg-AD model. In this review we conduct a thorough analysis of reports of sexual dimorphism in the 3xTg-AD model including findings of molecular, behavioral, and longevity-related sex differences in original research articles through August 2020. Importantly, we find results to be inconsistent, and that strain source and differing methodologies are major contributors to lack of consensus regarding traits of each sex. We first touch on the nature of sexual dimorphism in clinical AD, followed by a brief summary of sexual dimorphism in other major AD murine models before discussing the 3xTg-AD model in depth. We conclude by offering four suggestions to help unify pre-clinical mouse model AD research inspired by the NIH expectations for considering sex as a biological variable.

Keywords: Alzheimer's disease, mouse models, sex as a biological variable (SABV), sexual dimorphism, triple transgenic, 3xTg-AD

INTRODUCTION

Sexual dimorphism has recently come into focus in the Alzheimer's disease (AD) community as clinical data show that women are not only more likely to develop AD but also to experience more rapid

cognitive decline [1–3]. This finding is even more pronounced in those who are carriers of the $\epsilon 4$ allele of apolipoprotein E (*APOE4*), the strongest and most prevalent known genetic risk factor for AD [4, 5]. Reasoning behind sexual dimorphism in AD suggests that it is due to intrinsic risk factors like genetics, sex hormones, and differing inflammatory responses, in addition to the longer life expectancy of females [1]. Moreover, females are documented to respond better than males to two acetylcholinesterase inhibitors (donepezil and rivastigmine), 2 out of 4 of the only currently FDA-approved drugs for treatment

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of AD symptoms [6, 7]. Despite this evidence of sexual dimorphism in AD, there is a lack of sex-stratified data from clinical trials of AD as well as in pre-clinical and basic research using animal models [7–9].

Scrutiny over the utility of animal models for therapeutic discovery in AD has intensified over the last decade [10, 11]. One animal model, the triple transgenic AD (3xTg-AD) mouse, has been widely used for almost 20 years because it is one of the only two mouse models in which interventions against both human amyloid and tau pathology can be studied simultaneously [12]. The 3xTg-AD mouse overexpresses three human transgenes throughout the central nervous system that contribute to the AD-like phenotype of this model. Two of these transgenes cause early-onset AD in patients, namely the amyloid precursor protein with the Swedish familial double mutation ($APP_{KM670/671NL}$) and the presenilin 1 with a substitution mutation ($PS1_{M146V}$). Together, these mutations accelerate production of pathological A β and deposition into amyloid plaques [12, 13]. The third transgene is microtubule associated protein tau (MAPT) with a P301L mutation that causes increased aggregation of hyperphosphorylated tau into neurofibrillary tau tangles [14]. While this is the major AD mouse model in which both human amyloid and tau pathology can be studied simultaneously, it is important to note that the MAPT mutation is a familial determinant for early-onset frontotemporal dementia [15]. We have summarized the evidence confirming that male and female 3xTg-AD mice are sexually dimorphic in both the presentation of AD molecular pathology and in behavioral studies examining short-term recognition and spatial memory. Surprisingly, we could not identify any well-powered studies that compared total transgene expression levels between sexes.

Both the amyloid and tau pathologies are required for a confirmed diagnosis of AD; however, they are not the only factors that contribute to neurodegeneration and memory loss; neuroinflammation, cell death, and widespread metabolic changes are concomitant [16, 17]. Concerns regarding the consistency of the data obtained from this animal model have recently arisen and have yet to be reviewed, including colony drift and considerable sexually dimorphic presentation of AD pathology and cognitive deficits [18, 19]. Understanding sexually dimorphic responses to preclinical therapeutic interventions will elucidate mechanisms of both the disease and the interventions. Despite the noted sexual dimorphism of this mouse model, there are a limited number of studies that use both sexes (Fig. 1), and even fewer that report results separately by sex or directly compared results between males and females. Preclinical animal models should be considered on the basis of their ability to recapitulate both the universal hallmarks of AD as well as the sexually dimorphic nature of AD [20]. In this review, we evaluate the literature regarding sexual dimorphism in molecular and behavioral presentations of disease progression in the 3xTg-AD mouse model along with sexually dimorphic responses to preclinical therapeutic interventions.

SEXUAL DIMORPHISM IN THE CLINICAL PRESENTATION OF ALZHEIMER'S DISEASE PATIENTS

Although the mechanisms behind sexual dimorphism in AD are the subject of ongoing investigations, it has been well-documented that AD disproportionately affects women, at a ratio of approximately two to one [21]. One explanation for the higher prevalence in females may be the link to *APOE4*, an allele

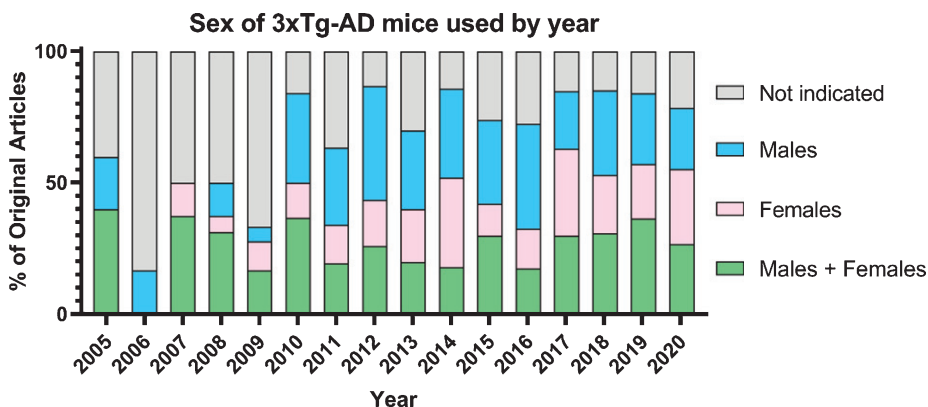


Fig. 1. Graph of 613 original publications using 3xTg-AD mice by sex over time.

variant believed to affect mechanisms associated with clearance of amyloid- β (A β), hyperphosphorylation of tau, microglial activation, and synaptic plasticity. *APOE4* is strongly correlated to risk for sporadic and late-onset familial AD, and this correlation is more prominent in women than men [4]. Studies in human subjects have shown that female *APOE4* carriers have worse performance in memory tasks and more rapid cognitive decline than men, even in non-AD cohorts [22]. It has also been suggested that *APOE4* allele carriers respond differently with respect to safety and efficacy in clinical trials [23]. Clinically, sex differences in A β plaque burden are still being studied, with some trials reporting an increase in women [24, 25], while others have found no sex differences in A β deposition [26]. Studies comparing tau between males and females are also finding a greater burden in women. Oveisgharan et al. analyzed postmortem brains from AD patients and report significantly higher densities of tau tangles in women [27]. Another study, in a cohort of individuals at risk of developing AD based on A β load, tau deposits (as measured by positron emission tomography; PET) in the entorhinal cortex appeared earlier in women compared to men, further supporting the notion that sex influences AD risk [28]. Taken together, this recent literature points towards a trend of worse pathology in women.

Behind these discrepancies between sexes in AD pathology are several inherently sex-based differences including sex hormones, epigenetics, immune response, and lifespan. One hypothesis for the higher prevalence of AD in women lies in the drastic changes in sex hormones women experience as they traverse menopause, resulting in a depletion of estrogen post-menopause [29]. In addition to the reported neuroprotective aspects of estrogen [30], female estrogen and male androgen act upon gene expression through epigenetic modifications such as methylation and acetylation [31]. These sex hormone-based epigenetic effects may influence susceptibility to certain disease or possibly response to drug therapies. However, their impact in AD pathology remains largely elusive.

Studies have shown significant differences in immune system activation between the sexes, specifically in innate and adaptive immunity [32]. Importantly, immune response has been implicated as a critical factor in AD development, with inflammatory cytokines, chemokines, and gliosis increased in AD patients [33]. Autoimmunity differs between sexes in clinical presentation and prevalence, with

women representing almost 80% of all known cases of autoimmunity in the US [34]. Furthermore, men and women show different responses to vaccines, as measured by antibody response [35]. For example, females, compared to males, had a better humoral response to influenza vaccines and hepatitis vaccines, but a worse response to vaccines for measles and yellow fever. The mechanisms behind these sex differences are still under investigation but are thought to be a combination of differences in genetics and gonadal hormones. This is of interest to AD research as vaccines against A β are under investigation in clinical trials [36, 37].

Lifespan is also a confounding variable. Women, on average, live longer than men, increasing the probability of developing AD. However, longitudinal and epidemiological studies that have controlled for this have concluded a higher lifetime risk for women compared to men [38]. These factors are, of course, not mutually exclusive and influence each other significantly, which makes distinguishing a precise mechanism behind sexual dimorphism in AD unrealistic but makes sex-based stratifications all the more important.

Sex differences in clinical trial outcomes are often overlooked and underreported, making it difficult to determine how sex differences influence AD and possibly other neurodegenerative diseases. In fact, there was a strong male bias in clinical trials until it was required by law in 1993, that female enrollment must be included in phase III clinical trials [39]. There are widespread reports of differences in therapeutic response between sexes, yet stratification of results remains limited [40]. Among 54 clinical trials for the AD therapeutics cholinesterase inhibitors (donepezil, rivastigmine, and galantamine) and the N-methyl-D-aspartate receptor agonist memantine, 48 did not report data separately by sex [41]. This lack of stratification in clinical data may be problematic since sex differences have been reported in the Mini-Mental State Examination [42], verbal memory tests [43], cholinergic systems [44], and drug metabolism [45]. A similar lack of stratification is found in preclinical studies, as described below.

SEXUAL DIMORPHISM IN THE 3xTg-AD MOUSE MODEL

Overall, sex differences are underreported in preclinical AD research as most studies do not equally recruit animals from each sex, especially not in numbers

large enough to reach statistical power [7]. Sexual dimorphism has been reported in many of the most commonly used AD mouse models, which employ various familial mutations in human amyloid-beta protein precursor (A β PP or APP) and presenilin 1 (PS1). Reports include increased A β plaque burden in females of the APP/PS1 [46–49], TASTPM [50], and Tg2576 [1, 51, 52] mouse models, and sex differences in tau phosphorylation in the P301L mouse [53, 54].

Sex differences in behavior have also been noted in various AD models including stress response in 5xFAD [55, 56], impaired reference memory in female Tg2576 [52], and impaired spatial learning in female TgCRND8 mice [57]. However, the 3xTg-AD mouse has been particularly noted for the model's variable sex differences, which have yet to be reviewed [19]. These sex differences in AD mouse models often do not correlate, and sometimes even contradict differences seen clinically, which could

hinder the development of AD treatments if they are not accounted for.

Sexual dimorphism in AD pathology

Accumulation of A β in serum and brain tissue is reported to occur earlier and progress at an accelerated rate in female 3xTg-AD mice in the majority of studies that compare sex (Table 1) [58–70]. A significant difference in A β pathology in this mouse model proves problematic for the pre-clinical screening of A β -targeted therapies, since the age of onset and subsequent A β plaque accumulation both differ between males and females. The Mufson laboratory reported A β immunoreactive-plaque deposition in the subiculum of females at an age of 8-9 months and males at 11-12 months [59, 62]. Hebda-Bauer et al. saw A β PP/A β immunoreactivity in the hippocampus of both males and females at 3 months, yet also reported greater immunoreactivity in females [70].

Table 1
Summary of sex differences in molecular and behavioral studies using the 3xTg-AD model

Age (mo)	Molecular summary	Behavioral summary	Author	Year
4, 12, 18	F ↓ NLGN1	F ↔ M performance in Y-maze, SOR, and MWM	Dufort-Gervais et al. [115]	2020
12	F ↑ A β ₄₂	M ↑ impairment in OR; F ↔ M impairment in OLM	Creighton et al. [66]	2019
6	F ↑ A β	F ↑ impairment in spatial reorientation	Stimmell et al. [58]	2019
12	F ↑ A β and p-tau	F ↑ impairment in MWM	Yang et al. [71]	2018
9	F ↑ A β ₄₀ and A β ₄₂		Omori et al. [69]	2017
12 and 18	F ↑ A β ₄₀ and A β ₄₂		Vandal et al. [68]	2015
2, 6, 12, 15		M ↑ impairment in RAM	Stevens et al. [92]	2015
6		M ↑ impairment in CFC; F ↔ M performance in NOR and Y-maze	Stover et al. [88]	2015
4		F ↑ impairment in PM, HB, DLB, TM, and ACT	Cañete et al. [91]	2015
12 and 15		F ↑ impairment in MWM, M ↑ inhibition in CT and OF	Blazquez et al. [89]	2014
3-4	F ↑ A β		Hebda-Bauer et al. [70]	2013
12 and 18	F ↑ A β ₄₀ and A β ₄₂		Bories et al. [67]	2012
2-3, 13–15, 18–20	F ↑ A β		Perez et al. [59]	2011
6	F ↑ A β ₄₀ and A β ₄₂		Gimenez-Llort et al. [61]	2010
12 to 14	F ↑ A β	F ↑ impairment in Y-maze	Carroll et al. [60]	2010
8-9 and 18–20	F ↑ A β and p-tau		Oh et al. [62]	2010
2, 3, 4, 6, 9, 12	F ↑ A β		Rodriguez et al. [63]	2008
9, 16, 23	F ↑ A β ₄₀ and A β ₄₂ ; F ↔ M tau		Hirata-Fukae et al. [64]	2008
2, 4, 6, 9, 12, 15	F ↔ M A β and p-tau	F ↔ M performance in MWM, IA, NOR at 2, 4, 12 months; F ↑ impairment in MWM and IA at 6 and 9 months	Clinton et al. [72]	2007
10	F ↑ A β ; M ↑ tau	F ↔ M performance in MWM and OF	Nelson et al. [65]	2007

↑ = increase, ↓ = decrease, ↔ = no change or difference. F, female; M, male; A β , amyloid- β ; p-tau, phosphorylated tau; NLGN1, Neuroigin-1; ACT, spontaneous circadian motor activity test; CFC, cued fear conditioning; CT, corner test; DLB, dark-light box; HB, Boissier's hole-board test; IA, inhibitory avoidance; MWM, Morris water maze; NOR, novel object recognition; OF, open field; OLM, object location memory; PM, elevated plus maze; RAM, radial arm maze; SOR, spatial object recognition; TM, tunnel-maze.

Stimmell et al. and Yang et al. also found significantly greater A β (6E10) immunopositive-plaque deposition in the hippocampus of female mice at 6 months and 12 months, respectively [58, 71]. Creighton et al. report nearly 4-fold greater A β_{42} deposition in the hippocampus, as measured by ELISA, in female compared to male 3xTg-AD [66]. Omori et al. report A β_{42} levels approximately 20-fold higher in females than males using ELISA [69]. The LaFerla laboratory, which described the generation of the 3xTg-AD model, found no sex differences in whole brain A β_{40} and A β_{42} at 2, 6, 12, and 15 months of age [72]. We identified only one other study that measured cortical A β_{40} and A β_{42} at 6 months and observed no difference between sexes [73]. Notably, no study has reported a significantly greater amount in A β levels in male 3xTg-AD mice compared to females. Recently, Russo-Savage et al. measured greater levels of human APP in the somatosensory cortex of females at 3 months old using western blot [74]. However, this was only evaluated between a single mouse from each sex and no analysis was performed on A β PP processing enzymes or A β production.

Sexually dimorphic tau burden has received less attention and studies that have addressed it produced variable and inconclusive results [61, 62, 64, 65, 71]. Gimenez-Llort et al. did not report sex-based differences in PHF-tau immunostaining in hippocampus and amygdala [61]. Oh et al. on the other hand, used four different immunohistochemical stains for different tau residues (MC1, AT8, AT180, and PHF-1) and found greater tau staining in 18- to 20-month old females compared to males [62]. Additionally, Yang et al. report increased phospho-tau (T231) positive cells in the hippocampus of 12-month-old females [71]. In two of the studies that reported greater A β burden in females, the authors did not observe a correlation between sex and tau burden [61, 64]. Nelson et al. reported less hippocampal and amygdala tau in 5-month-old females relative to males using HT7 immunostaining, although A β ELISA uncovered A β_{40} to be three-fold greater in females than males [65].

Sexual dimorphism in physiological responses

Kapadia et al. report worse autoimmunity in male 3xTg-AD mouse model, including reduced CD+T-splenocytes, higher serum autoantibody levels, and greater splenomegaly in 6-month-old males compared to females [73]. They hypothesize that early autoimmunity in the males may protect them from

plaque deposition and go on to suggest that the observed sex-related immune differences might be linked to differences in behavior. Of note, no sex differences were observed in cortical tau, A β , or BDNF levels in these mice. An investigation of immunoenocrine aging in 3xTg-AD mice reported a worse neuroimmunoendocrine network in males, involving lower splenic lymphocyte chemotaxis and proliferation and lower thymic natural killer cell activity [75, 76].

Adding further complexity to the observed pathological sex differences in 3xTg-AD mice are the effects of sex and stress hormones. Studies using ovariectomized (OVX) 3xTg-AD mice report earlier and accelerated AD pathology and impaired learning compared to sham-operated mice, while 17 β -estradiol treatment was enough to prevent the accelerated decline [77–79]. Gonadectomized (GDX) male 3xTg-AD mice also show increased A β and increased memory-related behavioral deficits, which are attenuated with testosterone treatment [80–82]. Together, these studies suggest that androgen and estrogen pathways may influence regulation of A β and tau pathology in 3xTg-AD mice. Furthermore, studies have found sex-specific corticosteroid responses in 3xTg-AD mice. Clinton et al. report that 9-month-old 3xTg-AD females, but not males, have an elevated corticosterone response following stressful tasks, which consequently affects their performance in memory-related tasks [83]. A recent study corroborates this finding, reporting increased corticosterone response to restraint stress in 4-month-old females compared to males and wild type females, proposing that this increased stress response may precede and contribute to AD pathology in these mice [84]. Given that chronic stress is a risk factor for AD [85], these studies suggest that sex-specific stress responses may help explain sex differences in AD pathology. Influences of the neuroendocrine system on AD pathology are of great importance in the context of AD sexual dimorphism and should be considered in pre-clinical research.

Despite worse pathology described by many of the studies discussed here, female 3xTg-AD mice typically outlive their male counterparts. Kane et al. measured survival of 3xTg-AD mice and found females to live an average of 130 days longer than males [86]. This study also described males to have higher frailty index scores than females and concluded frailty index to be an adequate predictor of health span in this model. Rae and Brown tested lifespans of a number of AD mouse models and found

female 3xTg-AD mice had an average lifespan of 744 days while males lived an average of 469 days [87], suggesting that lifespan should be taken into account when predicting health span of a particular mouse model. According to their findings, age-related disorders in mice should consider sex differences in life expectancy, so that the relative age of female mice in the 3xTg-AD model is “younger” than males given the females longer life expectancy. This, however, contradicts studies that note worse molecular and cognitive responses in females compared to males.

Sexual dimorphism in behavior

Non-memory-related behavioral discrepancies between 3xTg-AD males and females are not robust and are often influenced by the background strain. At 6.5 months of age, one study reported that 3xTg-AD females displayed higher average velocity than males in the Barnes maze test [88]. These authors also performed fear conditioning and demonstrated that 6.5-month-old 3xTg-AD females spend more time freezing than males. However, this was true for the wild type strain as well. Additionally, Blázquez et al. reported that 3xTg-AD males and their wild type strain exhibit higher behavioral inhibition/fearfulness than females as measured in both the Corner and in the open field (OF) tests at 12 and 15 months of age [89]. In contrast, Fertan et al. showed that female 3xTg-AD mice, regardless of treatment, had longer latency to fall times in the rotarod task compared to males and this was independent of the background strain [90].

Novel object recognition (NOR) tasks assessing working memory appear to be weak discriminators of possible sexual dimorphism for recognition memory as Clinton et al. did not detect any evidence of sexual dimorphism at 2, 6, 9, and 12 months of age in either short-term (3 h) or long term (24 h) intertrial interval (ITI) NOR paradigms [72]. Creighton et al. detected slightly greater impairments in males than females with an ITI of 5 min which disappeared when groups were tested using a 90 min ITI [66]. Importantly, the NOR apparatus used in this study had clear walls, allowing the mice to use spatial cues which may have affected these results.

Multiple studies assessing spatial memory observed that females perform overall worse in the Morris water maze (MWM) task assessing spatial memory. This is supposedly linked to the often-advanced amyloid pathology of female 3xTg-AD mice. Young (4–6 mo) and old (12–15 mo) females show poorer

place learning acquisition than males in the MWM and the older female mice also displayed impaired reference memory during platform removal [71, 72, 89, 91]. 12–14 month-old females are shown to perform worse than males in the Y-maze task assessing working spatial memory [60]. In a novel test of spatial memory, the spatial reorientation task, only at 6 months of age were deficits noticeable between female 3xTg-AD mice and non-Tg controls. This deficit was not observed in male mice at 3, 6, nor 12 months of age and also not observed in female mice at 3 months of age. This study also demonstrated lesser staining for A β in the dorsal CA1 region of the hippocampus in males compared to females [58].

In contrast, Stover et al. found that 6.5-month-old females performed better during both the acquisition phase and the reversal phase of the Barnes maze task, making fewer errors and exhibiting a lesser latency to goal time [88]. Additionally, in the radial arm maze (testing working and reference spatial memory), 3xTg-AD male mice commit more errors than female mice beginning at 2 months of age. Importantly, in this task, separate cohorts of increasingly older mice did not perform worse than younger mice and there were no noticeable sex differences in the background strain, B6129SF2/J [92].

Sexual dimorphism in therapeutic response

Lastly, we identified only two studies that were sufficiently powered ($n = 10–19$ /treatment/sex) to detect sex differences in response to a preclinical therapeutic intervention. In 2016, Sawmiller et al. reported that the naturally occurring flavonoid, diosmin, reduced cerebral A β oligomers only in female 3xTg-AD mice while it improved markers of phospho-tau and neuroinflammation in both sexes [93]. Additionally, diosmin was shown to improve memory in both sexes of the 3xTg-AD model in the fear conditioning task with no reports of sexual dimorphism in the B6129SF2/J background strain. In 2019, Fertan et al. showed that a novel Indolamine 2,3-dioxygenase (IDO) inhibitor, DWG-1036, exerted sex-dependent side effects and behavioral changes [90]. They reported that DWG-1036 caused excessive weight loss in females of both WT strain (B6129SF2/J) and the 3xTg-AD strain but not in males. In the trace fear conditioning task of working memory, Fertan et al. observed improved memory (measured by increased duration of freezing) only in DWG-1036-treated 3xTg-AD males. DWG-1036

inhibits IDO, an essential enzyme in kynurenine synthesis pathway which is modulated by sex hormone levels [94]. Interestingly, publicly available studies of anti-amyloid antibody therapies, which are the most recent clinical therapeutic candidates for AD, have mostly been conducted in only female 3xTg-AD mice [95–104]. The only exceptions to this do not present sex-stratified data or comment on sexual dimorphism in AD or the 3xTg-AD model [105–108].

SUMMARY

Sexual dimorphisms are commonly observed in patients and pre-clinical models yet are often overlooked when critically interpreting results. In humans, the incidence, pathology, and presentation of AD differs strikingly between the sexes, yet sex-stratification of data is often performed as an afterthought. In smaller Phase I and II trials, this may potentially veil both sexually dimorphic improvement and exacerbation of disease condition. In animal models of AD, sex differences in A β plaque load, immune response, lifespan, memory, and non-memory-related behavior are observed. However, few studies report, or have the power to report, these differences.

The literature reviewed here evaluates the heterogeneity in neuropathology, lifespan, and memory- and non-memory-related behavioral performance reported between sexes of the 3xTg-AD mouse model. In some cases, this sexual dimorphism mimics clinical observations; however in others, the AD-like phenotypes are opposite. There are multiple potential reasons for this large variability, one of which includes the source and maintenance of the mouse colony. Our review of the literature indicated that there are two major sources where researchers procured their 3xTg-AD models: purchased from Jackson Laboratory (JAX) and the Mutant Mouse Resource and Research Center (MMRRC) or donated from the originating colony at the laboratory of Dr. LaFerla. We did not observe a correlation between severity of pathology and source of the 3xTg-AD mice used in these studies. In 2014, the developing laboratory communicated to JAX that male 3xTg-AD mice “may not exhibit the phenotypic traits initially described” [109]. This finding, along with the dimorphisms highlighted above, warrants copy number, transgene expression validation, and baseline behavioral analysis in future applications of this model. These measures are rudimentary yet integral

to yielding informative and reproducible outcomes. Additionally, male mice from both the 3xTg-AD and background strain exhibit increased potential for aggressiveness that may warrant housing in isolation which has known effects on immune and endocrine signaling as well as non-memory and memory-related behavior [110]. This should be considered when deciding how to house the animals depending on what types of data are being collected.

Another possible cause of the heterogeneity is methodological variations in the biochemical analyses. For example, there are at least four common methods to measure amyloid load in the brain including western blotting, ELISA, Luminex, and immunofluorescence. Adding a further layer of complexity is the wide array of antibodies available for A β detection. Many studies analyzed A β deposition by immunohistochemistry using antibodies that react to varying regions of A β or A β PP including abnormal isoforms and precursor forms of A β [59, 62, 70, 71]. Even among antibodies specific for the A β ₄₀ or A β ₄₂ end residues, there are multiple products available. Behavior protocols vary widely between groups as well.

Some studies justify their use of one sex over the other, citing reasons including that females are reported to develop amyloid plaques earlier than males, or that females have a more homogenous pathology than male 3xTg-AD mice. The main reason cited for using only males in many of studies is to avoid the metabolic, hormonal, and behavioral changes that accompany the female estrous cycle. Studies that do utilize both sexes do not always compare sexes separately due to low power. Of the 613 original research articles published since the creation of the 3xTg-AD mouse model in 2003, 23% of the articles do not indicate the sex of the animal used in the study. This causes problems when comparing or recreating these studies, especially given the large differences between sexes seen in some of the studies discussed above [66, 69].

In order to rectify and unify our research efforts moving forward, preclinical and clinical studies need to be well powered so that independent statistical analyses on males and females can be performed within each treatment group. Although this may increase the duration and cost (monetary and in animal lives) of a study, it will help account for the variability observed when analyzing results and improve translatability of the findings. The NIA has acknowledged the need for more unified and transparent communication of AD research by creating

an easily searchable, peer-reviewed database to submit published and unpublished data from preclinical studies, the Alzheimer's Disease Preclinical Efficacy Database (AlzPED) [111]. Moving forward, it is highly encouraged to utilize this database both when designing a study and when sharing results. It will help lay the groundwork to synthesize emerging reports of sexual dimorphism, including less studied areas of the AD landscape like synaptic transmission [112, 113], ultrastructural analysis of plaque and tangle morphology [62], and disruptions in circadian rhythm [114].

When choosing and applying mouse models to preclinical research, we make four recommendations that aid consideration of sex as a biological variable (SABV) in accordance with NIH expectations: 1) a thorough understanding of the published characterizations of the model, 2) highly powered studies where males and females can be analyzed independently within each treatment group, 3) independent validation of transgene expression, and 4) at least partial validation of previously published molecular and behavioral hallmarks to be assessed, including baseline behavior and biometrics. Incorporating these four recommendations into future animal model research will aid the continuity between animal model studies and improve the quality of their findings, leading to a stronger understanding of how and which therapeutic approaches hold translational potential.

DISCLOSURE STATEMENT

Authors' disclosures available online (<https://www.j-alz.com/manuscript-disclosures/20-1014r2>).

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