

PERSPECTIVE

Biological sex differences in Alzheimer's preclinical research: A call to action

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Abstract

Introduction: For decades, researchers have largely ignored sex as a biological variable (SABV) within preclinical studies. Recent literature indicates scientists are increasingly including male and female subjects in studies, but fewer studies assess for sex differences in study outcome. This is particularly concerning within the field of Alzheimer's disease (AD), as disease burden is higher among women and evidence suggests sex differences exist in etiology and disease course.

Methods: We conducted an informal review of preclinical AD research studies.

Results: Results confirmed that only about one-third of ≈ 150 recent studies included both male and female mice, and <15 of nearly 150 studies examined SABV as an outcome of interest.

Discussion: Previous research supports the idea that better integration of SABV could open new doors in treatment research. We provide examples of best practices and discuss the need for Alzheimer's researchers to account for SABV within preclinical studies.

KEYWORDS

Alzheimer's disease, basic research, preclinical science, sex as a biological variable

1 | SEX DIFFERENCES IN ALZHEIMER'S PRECLINICAL RESEARCH

During her 24 years in the United States House of Representatives, Pat Schroeder was a staunch advocate for women's health. With that in mind, it comes as no surprise she had this to say on the topic of biomedical research: "If anyone thinks women are going to keep paying half the cost of health care and have researchers so elitist they won't even use female rats in the research, they're nuts." This statement, made in 1990,¹ still resonates. Although women are more likely to be included in clinical trials today than in the 1990s, a majority of

preclinical neuroscience research continues to use exclusively male animals or fails to acknowledge sex as a biological variable (SABV) entirely.

With Rep. Schroeder's words on our minds, the Society for Women's Health Research (SWHR) Alzheimer's Disease Network conducted a retrospective and informal review of preclinical Alzheimer's research in the spring of 2019. We examined studies conducted from 2013 to 2018 that used common mouse models (3xTg, 5xFAD, APP-PS1, CRND8, and Tg2576) and sought to determine the efficacy of a potential Alzheimer's disease (AD) therapy. Of the nearly 150 studies reviewed, only about one-third included both male and female mice as

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subjects. A vast majority of the reviewed studies experienced a successful or partially successful result (97%). This preclinical success rate clearly is not translating into successful clinical trials, which begs the question, why not? One potential explanation is the persistent failure to examine SABV: Of those studies that included both sexes, <15 articles examined SABV as an outcome of interest. The exclusion of SABV in Alzheimer's research is concerning, precisely because disease burden is higher among women, who account for almost two-thirds of diagnosed cases in the United States. They also experience different neuropsychiatric symptoms and a markedly steeper decline in cognitive functioning after diagnosis with AD compared to men.² A growing body of research suggests that studies conducted using mouse models of Alzheimer's reveal sex differences in amyloid beta (A β) plaque formation and burden, levels of neuroinflammation, number of pathological tau tangles, and timing and severity of cognitive decline.²

2 | A HISTORICAL TREND

Lack of consideration of SABV in Alzheimer's research is not surprising when understood in historical context. Thirty years ago, a Government Accountability Office report revealed that the National Institutes of Health (NIH) was doing little to ensure women were represented—or even acknowledged—in research funded by the agency.

Congress passed a law in 1993 requiring the NIH to ensure inclusion of women and minorities in agency-funded research. However, it wasn't until 2016 that the NIH enacted a policy requiring grant applicants to consider SABV in basic science research. Based on the 2016 policy, if applicants seek to study only one sex, they must offer strong justification. The policy is limited in impact, however, given not all preclinical research is funded through NIH. Within federally funded research studies, little oversight exists to ensure this policy is followed as intended.

Current research shows that the dominance of male models and ignorance of SABV persists. Overrepresentation of males in preclinical work occurs across fields, from chronic pain to mental health, from autoimmune disorders to stroke research, and within trials for therapeutics and medication. As compared to a 2009 study,³ recent research indicates that significantly more preclinical articles published in 2019 included both sexes in the sample population. However, little progress has been made in analyzing study results by sex. Among studies that included both sexes as subjects, only 42% included sex-disaggregated analyses, down from 50% of articles in 2009.⁴

Traditionally, scientists have pointed to increased hormonal variability of female animals as a reason for exclusion. Yet contemporary literature contradicts this longtime myth. Female animals show no greater hormonal variability than male animals.⁵ Biological sex differences, present even at the cellular level, mean the tendency to use male mice exclusively based on these outdated assumptions may render preclinical findings less applicable at best, and at worst, largely irrelevant to biologically female patients.

HIGHLIGHTS

- Many preclinical Alzheimer's studies do not incorporate both sexes in research.
- Very few preclinical Alzheimer's studies examine sex as a biological variable (SABV) as an outcome of interest.
- The field must develop best practices on SABV within Alzheimer's disease research.
- Institutions must improve SABV training for students and early-career researchers.
- Journals are an important arbiter and should require researchers to include SABV.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional (eg, PubMed) sources. Articles included used common mouse models of Alzheimer's disease (AD) within preclinical research studies.
2. Interpretation: Review of common studies suggested that the majority of preclinical Alzheimer's studies did not include both sexes within their study populations; a very small minority of studies examined sex as a biological variable as an outcome of interest.
3. Future directions: The authors discuss the need for a "best practices" approach across AD preclinical research, as well as improved training for scientists and improved oversight by journals during the article submission and peer-review process. Examples of articles describing best practices are provided.

3 | THE NEED TO ADOPT A BEST PRACTICES APPROACH

While research and policy slowly continue to evolve, the Alzheimer's research community should strive to standardize consideration of SABV in preclinical research. We believe it is past time for scientists to coalesce around standard methodologies for (1) how to incorporate female mice within preclinical research, and (2) how to account insightfully for SABV. A "best practices" approach for stakeholders may include guidance on minimum inclusion standards for both male and female study subjects, evaluation of SABV during preclinical research, and consideration of study outcomes through the lens of SABV.

Biomedical research journals are critical in this endeavor and have an important role as gatekeeper—previous research indicates that the prevalence of sex omission across articles varies by journal. Thus, journals must adopt the consideration of SABV as a criterion for article submission and publication in order to improve the health of both women and men. Additionally, training the upcoming generation of biomedical

research scientists in best practices for SABV will be crucial to standardizing these concepts within the field.

Experts have already started to consider best practices models. Janine Clayton, M.D., Director of the NIH Office of Research on Women's Health, and Francis Collins, M.D., Ph.D., Director of the NIH, proposed initial guidelines for attention to SABV prior to implementation of the 2016 NIH policy.⁶ Their recommendations include taking care to ensure research outcomes are able to reveal sex-related differences in results when using subjects capable of sex-based differentiation and paying attention to the different implications of the terms "sex" and "gender." They suggest data should be presented disaggregated by sex (and gender where appropriate) and sex-based analyses should be reported regardless of outcome. If SABV was not included as a factor within study design, they propose including a detailed rationale for that decision. The NIH also plans for future releases of continuing education courses and materials on SABV, including *Bench to Bedside: Integrating Sex and Gender to Improve Human Health* and *Sex as a Biological Variable: A Primer*. Both are intended to help researchers gain a comprehensive understanding of how to incorporate SABV into clinical and preclinical studies.

Rich-Edwards et al. (2018) completed a more intensive review of proposed best practices within biomedical research study design.⁷ The authors suggest consideration of SABV at every stage of the methodological process: motivation, subject selection, randomization, sample size, data collection, and analysis and interpretation. Within the subject selection criteria, for example, the authors recommend consideration of sex-specific age incidence of the disease in question, as well as consideration of the reproductive stages of study subjects. Other recommendations include stratified randomization by sex and gender within studies with small samples sizes, consideration of sex and gender differences in disease presentation, and consideration of sex and gender dynamics in pharmacokinetics and pharmacodynamics. The variety of recommendations suggested by Rich-Edwards et al. provide an excellent foundation upon which Alzheimer's experts can begin to standardize field-specific best practices.

4 | CONCLUSIONS

For many years, optimism surrounding potential new Alzheimer's treatments has waxed and waned as drugs have shown promise but then failed within clinical trials. The research community's history of ignoring SABV—as it relates to inclusion of both sexes as research subjects, as well as to analyzing outcomes based on sex—is hindering progress. Incorporating SABV more consistently in preclinical research could enhance the probability of finding an effective treatment for many neurodegenerative diseases.⁸ Recent reports also offer evidence of sexually dimorphic disease-affected pathways in schizophrenia and bipolar disorders, which may suggest important pathways to finding therapeutic targets across other diseases, including Alzheimer's.⁹

Despite this potential for enhanced biomedical ingenuity, researchers still consistently fail to adequately examine SABV in preclinical research.^{3,4} There remains a need for strategic integration of SABV in preclinical trials.⁷ The SWHR strongly recommends a

standardized evaluation of SABV in AD and the adaptation of a best practices approach. Standardizing this practice across the field of Alzheimer's research may lead to significant discoveries and will certainly ensure that all patients benefit from Alzheimer's research, regardless of biological sex.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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