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Modeling Alzheimer's disease: considerations for a better translational and replicable mouse model

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Alzheimer's disease (AD) mouse models have proven to be an invaluable tool for deepening our understanding of disease mechanisms and for developing therapeutics. However, one common frustration is the lack of replicability in behavioral findings. As we have discussed in our recent publication (Cho et al., 2021), in the htau mouse model, the cognitive impairment reported in the original study has not been consistently replicated by different labs over the past decade. This variability in behavioral findings seems to exist in many, if not all, AD mouse models that have been behaviorally evaluated.

Key factors that contribute to both the general lack of translatability and reproducibility include: genetic and physiological differences between mice and humans, issues specific to the development and use of modern inbred mouse strains (e.g., backgrounds used, breeding scheme, backcrossing history), and the methodological differences in behavioral assays. While this list is far from exhaustive it highlights challenges that the field faces.

Most conventional genetic mouse models of AD generally aim to incorporate modifications that lead to one or more of the following outcomes: increased beta-amyloid (Aβ) production through mutations in the amyloid precursor protein (APP) gene, modulation of secretases through mutations in presenilin genes (PSEN1 and PSEN2), and increases or modifications to tau protein through mutations in tau gene (MAPT) that lead to tau hyperphosphorylation and pathology. While these models exhibit many hallmarks of AD-related pathology, their potential translatability is limited due to the following factors: First of all, wild, non-laboratory modified, mice do not develop AD and the sequence, pathogenicity, and isoform prevalence of AD-related proteins such as Aβ, tau, and ApoE differ between mice and humans. Additionally, the lack of standardization in which murine ages are considered analogous to the average onset age of AD in humans has contributed to substantial mismatches in the stage of gross histopathological progression (i.e., plaques, neurofibrillary tangles, and neurodegeneration) seen between mouse models and human patients. In our study, we found that htau mice were most commonly tested at 12 months old, an age considered by The Jackson Laboratories to be "middle-aged" in C57BL6 mice and approximately equivalent to humans of 38–47 years old, an age group seldom impacted by AD, making it important to choose an age in mice that accurately correlates to an AD-relevant age in humans. In addition, other main features of AD, such as neuroinflammation or poor vascular health, are not often well recapitulated in AD mouse models. Thus, it is difficult to definitively say that mice can accurately recapitulate severe human AD.

AD mouse models also usually incorporate human transgenes from familial cases of AD which represent only 1–2% of all AD cases, leaving mechanisms of sporadic or late-onset AD (LOAD) poorly understood. Further, transgenes are commonly incorporated by injecting a transgene into an embryo at the pronuclear stage. This method in particular can introduce confounding factors, since the transgene can integrate into the genome at a random locus which, in turn, could alter the expression of this endogenous locus and confound the pathological effect of the transgene (Gamache et al., 2019) or contribute to the epigenetic silencing of the inserted transgene over time (Calero-Nieto et al., 2010). It is also common to pair a heterologous promoter to the transgene in order to drive its overexpression and distribution. Overexpressing a transgene could induce off-target or exaggerated physiological consequences that may confound the translatability of the model to human disease. Additionally, different promoters have different spatial expression patterns, creating another source of potential variability between mouse models.

The genetic background on which a mouse model is established can also have impacts on aspects such as sensorimotor functions, behavioral performance, and anxiety, introducing significant confounds. A common issue, before recent efforts to rectify it, was the use of several inbred strains harboring the recessive rd1 allele, e.g., C3H, CBA, FVB/N, and SJL which can result in retinal degeneration and blindness, seriously confounding the results of learning and memory tests that involve visual acuity. Thus, it is critical that investigators also understand non-AD-related genetic factors that can affect behavioral outcomes in the local colony.

While having a mouse that accurately models AD is much needed, the model should also be internally consistent, that is, produce replicable outcomes across different laboratories and cohorts. As discussed in our recent article (Cho et al., 2021), our review of the preclinical htau literature revealed that both the cognitive impairments in htau mice and the lack thereof, were equally represented in published studies. Notably, the age of impairment (if present) was not consistent across the reviewed studies. For example, in the Morris Water Maze (MWM) test where mice are tasked with finding and memorizing the location of a hidden platform inside of a pool, the original study described deficiencies in MWM at 12 months of age (Polydoro et al., 2009), whereas some later studies reported no impairment in the same test at ages ranging from 12–20 months. This lack of cross-study consistency and replicability in behavioral results is not unique to the htau model. In PS19 mouse, one of the most widely used tau pathology models, similar discrepancies in behavioral outcomes have also been reported. The original publication for behavioral assessment of the PS19 model reports impairment in MWM at 6 months old (Takeuchi et al., 2011), while others have reported no impairment in MWM at up to 9 months of age (Sun et al., 2020).

One contributing factor to this lack of behavioral replicability could be sex. In humans, the etiology, progression, and prognosis of AD differs between sexes. Women have a higher incidence of AD and experience a higher level of disability from the disease, but they also live longer, due to less comorbidities than men (Sinforiani et al., 2010). Additionally, one of the strongest genetic risk factor alleles for LOAD, ApoE4, was found to increase the risk of developing AD and mild cognitive impairment in women at earlier ages, and at higher rates, than in men (Neu et al., 2017). Sexual dimorphisms have also been illustrated in AD mouse models. Male PS19 mice were found to exhibit higher levels of tau hyperphosphorylation, decreased grip strength, more impaired MWM performance, and a lower survival rate when compared to female PS19 mice (Sun et al., 2020). Conversely, female 3xTg mice had greater Aβ burden and cognitive impairment than their male counterparts (Carroll et al., 2010). Another source of intra-model variability could also arise from the estrous status of female mice (Frick et al., 2000). Thus, it is imperative to consider the sexes, and sex-specific aspects, of the mice being tested when qualitatively comparing results from different studies that use the same mouse model.

In addition, the impact of genetic drift on the internal consistency within a model must also be considered. Particularly, we need to control for random mutations that can occur in isolated, inbred populations and confound the purported role of a transgene. Random, spontaneous mutations arise in genomic DNA constantly, and these random mutations can stabilize and alter the coding sequence of a gene as fast as 6-9 generations (Drake et al., 1998). In fact, after 20 consecutive inbred generations, the resulting mice are considered a substrain of the founder population. Thus, genetic drift can change a mouse line's phenotype and have an enormous impact on the results of an experiment and may explain discrepancies that are found within mouse behavioral data wherein one study reports a deficiency at a particular age while another may report no deficiency at the same age or even

older. Unfortunately, genetic drift cannot be completely prevented but its effects can be mitigated by maintaining and providing detailed information on the original source of the animals and their breeding history and refreshing the genetic background of a strain every 5–10 generations. These considerations are especially important when factoring in the possibility of genetic drift altering the incidence and severity of a transgene, which can result in variability with respect to molecular pathology and behavioral impairment across laboratories and cohorts.

Another main source for the discrepancies in behavioral results may come from procedural differences between studies. For example, in the htau mice literature, we found that procedural details such as the size ratio of platform-to-pool, the training regimen, and when and how the probe trial was run differed greatly across studies and could have influenced whether cognitive impairments were detected. Specifically, the size ratio of platform-to-pool was larger in the four studies that reported MWM impairments in htau mice, as compared to the four studies that did not find significant impairments (Cho et al., 2021). Additionally, it is important to note the type of analysis being done on the behavioral data to reach a conclusion of impairment or lack thereof. In terms of MWM, some researchers may analyze escape latency during the training phase while others may look at the percentage of time spent in the target quadrant or number of platform crossings during the probe trial. We also want to add that sample sizes should be obtained in as few cohorts as possible to avoid trial-to-trial variability. This is nowhere near providing a complete explanation on whether AD-like behavioral phenotypes are found, or absent, in an AD model, but it illustrates the importance of scrutinizing procedural details when qualitatively comparing outcomes from different studies. Since it is difficult to compare behavioral results across studies when tests are not always run in the same manner, wider standardization and transparency with respect to procedural details, statistical analyses, criteria for learning/memory tasks, age of mice, housing conditions, and criteria for excluding outliers/non-performing subjects would greatly improve replicability.

In this perspective, we have outlined potential pitfalls that can affect the translatability and replicability of AD mouse models, but there are many efforts to address these issues that should give the AD field reason to be optimistic. In terms of translatability, there is an increasing trend towards developing more high-precision knock-ins/outs of genes at the endogenous mouse loci of AD-related genes allowing for more accurate expression levels in the context of mouse physiology and mitigating any issues related to random integrations or overexpression that can lead to off- target effects. Many of these endogenous knock-in/out mice incorporate the aforementioned familial cases of AD genes but there have been increased efforts to introduce mutations/modifications to genes found to be linked to LOAD such as triggering receptor expressed on myeloid cells 2 (TREM2) variants that influence neuroinflammation and gliosis (Gratuze et al., 2018). In addition, genome-wide studies are being done to identify more genetic pathways and networks that underlie AD pathology and progression. One particularly promising consortium, MODEL-AD, aims to create new mouse models of LOAD by identifying AD-associated genetic variants from multiple computational analyses of large human datasets and incorporating them into mice (Oblak et al., 2020). MODEL-AD also seeks to characterize each new model through a standardized set of molecular, histological, and behavioral assessments that will be independently verified at multiple laboratories to ensure replicability.

The fact that preclinical results seldom translate to clinical breakthroughs may stem, in part, from the fact that behavioral readouts are seldom consistently robust. Fortunately, emerging technological advances have led to behavioral tests with potentially higher translational value. For example, the mouse version of the Paired Association Learning touchscreen assay, a cognitive test developed by the Cambridge Neuropsychological Test Automated Battery team to diagnose AD in humans, is conceptually and technically highly analogous to the human version, representing enormous translational potential.

It is our hope that implementing these measures will lead to more stable, translatable, and replicable mouse models. We also want to advocate for field-wide standardization of procedural details and protocols used for each behavioral test in order to allow for a more consistent cognitive profile of a particular mouse model; a well-curated and easily-accessible repository of these standardized details for different AD-relevant tests could be immensely beneficial in this regard. Since new ideas and hypotheses are built upon previous reports, we hope that, at the very least, the issues addressed in this perspective, while not fully comprehensive, can provide a solid foundation towards scrutinizing existing literature, profiling a chosen mouse model, and using that information to create an experimental workflow that can effectively utilize the model to assess therapeutic interventions or mechanistic questions related to AD.

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