Pathobiology of Aging & Age-related Diseases

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EDITORIAL

Neuropathological assessment and validation of mouse models for Alzheimer's disease: applying NIA-AA guidelines

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Dozens of transgenic mouse models, generally based on mutations associated with familial Alzheimer's disease (AD), have been developed, in part, for preclinical testing of candidate AD therapies. However, none of these models has successfully predicted the clinical efficacy of drugs for treating AD patients. Therefore, development of more translationally relevant AD mouse models remains a critical unmet need in the field. A concept not previously implemented in AD preclinical drug testing is the use of mouse lines that have been validated for neuropathological features of human AD. Current thinking suggests that amyloid plaque and neurofibrillary tangle deposition is an essential component for accurate modeling of AD. Therefore, the AD translational paradigm would require pathologic Aβ and tau deposition, a disease-relevant distribution of plaques and tangles, and a pattern of disease progression of Aβ and tau isoforms similar to the neuropathological features found in the brains of AD patients. Additional parameters useful to evaluate parallels between AD and animal models would include 1) cerebrospinal fluid (CSF) AD biomarker changes with reduced Aβ and increased phospho-tau/tau; 2) structural and functional neuroimaging patterns including MRI hippocampal atrophy, fluorodeoxyglucose (FDG), and amyloid/tau PET alterations in activity and/or patterns of pathologic peptide deposition and distribution; and 3) cognitive impairment with emphasis on spatial learning and memory to distinguish presymptomatic and symptomatic mice at specific ages. A validated AD mouse model for drug testing would likely show tau-related neurofibrillary degeneration following Aβ deposition and demonstrate changes in pathology, CSF analysis, and neuroimaging that mirror human AD. Development of the ideal model would revolutionize the ability to establish the translational value of AD mouse models and serve as a platform for discussions about national phenotyping guidelines and standards for models of AD and other neurodegenerative disorders.

Keywords: *Alzheimer's disease*; *mouse models of Alzheimer's disease*; *neuropathological validation*; *NIA-AA guidelines*

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Received: 25 May 2016; Accepted: 25 May 2016; Published: 16 June 2016

Alzheimer's disease (AD) neuropathology is currently defined by the accumulation of pathological Aβ in the form of senile plaques and dystrophic neurites, and phosphorylated tau neurofibrillary tangles. Although there may be some evidence, based on genetic risk factors for sporadic AD, that could direct AD modeling away from amyloid- and tau-based models, current consensus suggests that amyloid plaque and neurofibrillary tangle deposition is an essential component for any model to present. Over the last several decades, there have been a series of standardized approaches to the diagnosis of AD, culminating recently in the publication of the National Institute on Aging- Alzheimer's Association (NIA-AA) guidelines for the neuropathological assessment of AD (1,2), which, for the first time, separated clinical from neuropathological parameters in the diagnostic algorithm. The NIA-AA guidelines have revolutionized the way we think about AD pathology by

Citation: Pathobiology of Aging & Age-related Diseases 2016, **6**: 32397 - http://dx.doi.org/10.3402/pba.v6.32397

emphasizing the fact that AD pathology may be present in the absence of clinical AD. A similar standardized approach for the neuropathological characterization and phenotyping of AD mouse models does not exist. We propose a standardized approach for the thorough characterization of AD mouse models with neuropathology adapted from the NIA-AA guidelines as the foundation for this approach.

The ABC scoring system

The NIA-AA guidelines were developed to provide a methodological framework for brain tissue sampling, staining, diagnostic analysis, and reporting, culminating in an ABC scoring system that is used to determine the level of AD neuropathological change. The ABC score is composed of summary measures of amyloid plaque distribution A0 to A3 (Thal phase), neurofibrillary tangle distribution B0 to B3 (Braak stage), and cortical neuritic plaque density C0 to C3 (CERAD score), and reflects the severity of the three critical pathologic processes of AD. The combined ABC score is then interpreted using tables published in the NIA-AA guidelines as 'not', 'low', 'intermediate', or 'high' AD neuropathological change (Table 1). These NIA-AA guidelines have been implemented in the routine diagnostic evaluation of all AD clinical and research autopsies across the nation and should be applied for the sampling and staining techniques in AD mouse models. This will ensure that an AD mouse model will be characterized according to the same standardized approach with maximal relevance to human disease. Certainly an attempt should be made to generate an ABC score for each mouse model in an age- and gender-dependent manner. The most valid mouse model of human AD would demonstrate similar lesions (neuritic plaques and neurofibrillary tangles) and an increasing density and distribution of lesions through lower stages of AD pathology to higher ones, resulting in an ABC score summary that correlates with 'not' or 'low' AD pathology at early ages, progressing to 'intermediate' and 'high' AD pathology per NIA-AA guidelines at older ages or in the presence of disease-modifying gene mutations or genetic backgrounds.

Table 1. The ABC scoring system developed by NIA-AA can be used to help assess and validate the neuropathological features of Alzheimer's disease in mouse lines that are potential preclinical models for AD drug testing

Assessment	NIA-AA scoring
A beta plaques	A0 (not)
	A1 (low)
	A2 (intermediate)
	A3 (high)
Neurofibrillary tangles, including pretangles and threads	B0 (not)
	B1 (low)
	B2 (intermediate)
	B3 (high)
Neuritic and diffuse plaque density	C0 (none)
	C1 (sparse)
	C2 (moderate)
	C3 (frequent)

A standardized approach is essential for successful histopathology readout. The NIA-AA guidelines specify a standard sampling protocol and staged approach to tissue evaluation. This includes examination of multiple regions of cerebral cortex as well as hippocampus, basal ganglia, brainstem, and cerebellum for Aβ and paired helical filament (PHF) tau pathology to enable calculation of Thal, Braak, and CERAD scores. Similar assessments can be implemented in mice. In our group, at the time of euthanasia, mice are first perfused with ice-cold PBS and one hemisphere dissected and flash frozen for potential neuroanatomic, biochemical, and molecular studies, and the other half fixed in paraformaldehyde according to standard diagnostic protocols. The fixed hemisphere is then serially sectioned in the parasagittal plane and three levels stained for hematoxylin and eosin combined with Luxol fast blue for standard diagnostic neuropathology, Congo-red or thioflavin S stains for amyloid plaques and cerebral amyloid angiopathy assessments, Bielschowsky silver stain for neuritic plaques and neurofibrillary tangles, anti Aβ immunohistochemistry (6E10) for plaque distribution and density assessments, and anti-PHF-tau (AT8) for neurofibrillary tangle distribution.

The ABC score is generated as follows.

1. Aβ plaques. Scoring of diffuse Aβ plaques is assessed in cerebral cortex, hippocampus, striatum, midbrain, brainstem, and cerebellum according to protocols established by Thal (3) resulting in a Thal phase 0–5 which is translated into the NIA-AA score of A0–A3 (2).
2. Neurofibrillary tangles. Scores for neurofibrillary tangles are determined in trans-entorhinal cortex, corpora ammonis, fronto-parietal cortex, and primary visual cortex to generate a Braak stage (4), which is translated into the NIA-AA score of B0–B3. Most existing mouse models do not generate neurofibrillary tangles, so we have developed a modified B score for phospho-tau pathology including distribution of cytoplasmic neuronal tau such as pretangles and threads.
3. Neuritic plaques. Most existing mouse models do not form neuritic plaques, so a C score for CERAD (5) neuritic plaque density (none, sparse, moderate, or frequent) and a modified C score for diffuse plaque density are generated from frontal and parietal cortex from each mouse.

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Existing AD mouse models have not been characterized according to NIA-AA guidelines, and based on our experience and the published literature, we expect that virtually all existing models would be classified as 'not' AD, since the combination of human-like patterns of neuritic plaques and neurofibrillary tangles distribution does not exist in any published mouse model. Put simply, using this approach, existing mouse models would fail to even modestly recapitulate human disease when human diagnostic criteria are applied, which may explain consistent failure of clinical applications of AD model–derived diagnostic and therapeutic approaches. This will be very important to test and prove, and will raise the standard by which all AD models are judged. While we strongly feel that AD models should accurately reflect all aspects of human disease, we also recognize the challenges in developing a model with all the pathologic features of human AD. Therefore, mice can be judged on separate scores of A (plaque distribution – Thal), B (tangle distribution – Braak), and C (neuritic plaque density – CERAD), in addition to the summary score.

Correlative assessment procedures

Neurodegenerative diseases including AD have been well-characterized biochemically. Specifically, AD is characterized by deposition of fibrillar Aβ in the form of plaques and tau in the form of tangles, but the composition of fibrillar and soluble forms of Aβ and tau is complex. In AD, $A\beta_{1-40}$ and $A\beta_{1-42}$ isoforms are the most common with $A\beta_{1-42}$ being the more toxic. Frozen tissues from the hemispheres contralateral to the ones used for histopathology are used for sequential protein extractions and to quantify soluble and insoluble levels of cortical and hippocampal $A\beta_{1-40}$ and $A\beta_{1-42}$ (6). Luminex immunoassays are used to establish a baseline for potential preclinical studies and to understand any differences with respect to age and neuroanatomy in comparison with human pathology. These extracts are also interrogated for phospho-tau levels and, if appropriate, tau isoforms using immunoassays including Luminex and possibly western blots.

Cerebrospinal fluid (CSF) should be collected from every mouse euthanized for neuropathology and pooled in order to perform triple assays of $A\beta_{1-42}$, total tau, and p-tau^{181} using the Innogenetics AlzBio3 kit that is standard for CSF biomarker assessment in virtually all AD clinical trials (7). However, there are severe limitations to CSF analysis in mice associated with the very small volumes available to be collected. The AlzBio3 kit optimally requires 100 µl of CSF, whereas only 1–2 µl are available in any given mouse. Ideally, this assay would be directly applied in mouse models of AD, but interspecies differences in Aβ sensitivity and specificity, and extremely low volumes of CSF available in mice, may necessitate adaptation/development of alternative multiplex CSF immunoassays or mass spectrometry–based approaches. We are currently working on enhancing the sensitivity of the assay on pooled mouse samples, recognizing that CSF biomarkers along with neuroimaging are the two most widely used and most promising pre-diagnostic assays for AD, so any CSF biomarker data to correlate AD models with human disease would be extremely valuable.

Neuroimaging is a fundamental and priority component of the neuropathological validation of AD mouse models, given its current and future critical importance in clinical AD diagnosis and management. It is used to assess progressive brain changes such as reduced glucose metabolism and temporal lobe and hippocampal atrophy. Imaging analysis correlated with neuropathology will play a major role in determining the suitability of a mouse model to be validated and considered for drug testing or other preclinical research studies. There are two main imaging modalities currently in use to clinically evaluate structural changes such as cortical atrophy in AD patients, and these are FDG-PET and amyloid imaging. Therefore, both will be major staging parameters for helping to validate AD mouse models. MRI procedures such as diffusion tensor imaging and possibly arterial spin labeling are also of much interest as is tau PET imaging.

Because data generated from behavioral tests in mice are poorly reproducible and challenging to translate to human disease, memory testing is not a validation criteria or a drug testing endpoint. However, as with virtually all studies of novel genetic mouse models of AD, it is useful to determine whether mice are symptomatic or asymptomatic in association with progression of AD pathology and biomarkers. Therefore, memory testing should be performed during the validation process so correlations can be made with neuropathological features. There are several options for selection of a specific procedure including Morris water maze, fear conditioning, and radial water tread maze. In terms of simplicity and efficiency in measuring contextual learning and memory, the radial water tread maze can easily be performed using standardized protocols thereby allowing enhanced reproducibility (8).

Summary and conclusions

Using our AD translational paradigm, existing and novel mouse lines that do not show pathologic Aβ and tau deposition reminiscent of human disease would not be considered for further or comprehensive characterization. But if this criterion is met, detailed assessment would then be performed that specifically evaluated neuropathology in relation to human AD. This includes plaque and tangle lesion severity, the pattern of disease spread through the brain, and the biochemical characterization of Aβ and tau isoforms. Additional assessment procedures would then be useful to further correlate the pathology phenotype with human AD, including changes

in CSF, neuroimaging, and cognition. The challenge for developing a highly validated mouse model showing tau-related neurofibrillary degeneration following Aβ deposition with changes that mirror human AD is daunting, but given the current high priority by the National Institutes of Health and other funding agencies for gathering the resources and expertise, it is certainly doable. The proof will come when the next-generation mouse models will be able to accurately and reliably predict positive drug responses in clinical trials.

Conflict of interest and funding

The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

References

1. Serrano-Pozo A, Qian J, Monsell SE, Frosch MP, Betensky RA, Hyman BT. Examination of the clinicopathologic continuum of Alzheimer disease in the autopsy cohort of the National Alzheimer Coordinating Center. J Neuropathol Exp Neurol. 2013;72(12):1182–92.
2. Montine TJ, Koroshetz WJ, Babcock D, Dickson DW, Galpern WR, Glymour MM, et al. Recommendations of the Alzheimer's disease-related dementias conference. Neurology. 2014;83(9): 851–60.
3. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002;58:1791–800.
4. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 1991;82:239–59.
5. Mirra SS, Heyman A, McKeel D, Suma SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology. 1991;41:479–86.
6. Yang Y, Cudaback E, Jorstad NL, Hemingway JF, Hagan CE, Melief EJ, et al. APOE3, but not APOE4, bone marrow transplantation mitigates behavioral and pathological changes in a mouse model of Alzheimer disease. Am J Pathol. 2013; 183(3):905–17.
7. Kang JH, Korecka M, Figurski MJ, Toledo JB, Blennow K, Zetterberg H, et al. The Alzheimer's disease neuroimaging initiative 2 biomarker core: a review of progress and plans. Alzheimers Dement. 2015;11(7):772–91.
8. Wiley JC, Pettan-Brewer C, Ladiges WC. Phenylbutyric acid reduces amyloid plaques and rescues cognitive behavior in AD transgenic mice. Aging Cell. 2011;10(3):418–28.

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