
INVENTORS
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SUMMARY
The use of animal models for preclinical screening of potential new drugs for cognitive or other disorders requires that the models replicate to an acceptable degree the etiology, pathology and symptoms of the disorder. A number of transgenic mouse models exist for Alzheimer’s disease (AD); these models reproduce specific neurodegenerative lesions as well as cognitive and behavioral alterations that can be detected by behavioral testing. Behavioral testing in mice, however, is time consuming, expensive and tends to be highly variable and inconsistent between laboratories.

Using a well-characterized AD mouse model (hAPPJ20, a transgenic mouse expressing human amyloid precursor protein), Gladstone researchers discovered a strong correlation between behavioral tests indicative of cognitive deficit and the loss of a specific calcium-binding protein, Calbindin-D28K (CB) in granule cells of the dentate gyrus in the brain. The data show that CB may be used as a biomarker for cognitive function: measuring CB levels in brain sections provides a more quantitative, less variable parameter that can complement the commonly used behavioral tests. CB reductions have been subsequently identified in multiple AD mouse models, including hAPPJ9/FYN, hAPPArc48, EC-hAPP, hAPP/PS1, Tg2576, and ApoE4-272 mice. Gladstone researchers have developed standardized methods for reproducible and precise measurement of CB levels in immunostained mouse brain sections (US patent 7,297,836). The use of a reliable biomarker that reflects cognitive capacity in AD transgenic mouse models allows for more accurate and reproducible assessments, when these models are used in drug screening or preclinical trials. The technique can be automated for greater throughput and is a valuable tool for evaluating multiple drug candidates and aiding in selection of the optimal therapeutic. Moreover, the specific role of CB-related pathways in the development of cognitive deficiency in AD models is currently under investigation. Elucidation of key connections could provide new options for novel drug targets in the treatment of this devastating disease.

CB reductions in the dentate gyrus of hAPPJ20 mice and humans with AD.
Figure: Brain sections from non-transgenic (NTG) and hAPPJ20 mice were immunolabeled for CB or/and the neuronal marker, Neu-N. (A) Sagittal brain sections illustrating typical CB-immunoreactivity in the dentate gyrus (arrow) of a NTG mouse and a range of CB reductions in hAPPJ20 mice. (B) Double-labeling of sagittal vibratome sections for CB (green) and Neu-N (red) did not reveal obvious changes in the density of neuronal nuclei in the CB-depleted granular layer of hAPPJ20 mice. (C) Hippocampal sections from hAPPJ20 mice (upper) and AD cases (lower) were stained for CB. A comparable range of CB reductions was found in the granular layer of hAPPJ20 mice and AD cases. Numbers in parentheses indicate Blessed score, which increases with the severity of the dementia. Adapted from Palop et al., PNAS, 2003.

APPLICATIONS

- Improving the reliability of preclinical screening of lead compounds by utilizing validated biomarkers, like CB, to quantify changes in cognitive capacity over time (immunostaining in brain sections) and complement current behavioral testing in mouse AD models.
- Further development of the system to produce cheaper biosensor probes useful in CB imaging studies for the identification of AD patients at the early stage of disease, with only mild cognitive defects, who might respond to particular forms of new therapies.
- Further examination of the connections between CB regulation pathways and AD pathogenesis could help identify novel targets for developing potential new drugs for AD treatment.
- Including the quantitation of CB in analyte panels that are used to monitor patients in AD clinical trials. If samples of cerebrospinal fluid (CSF) are taken throughout the trial, CB can be measured alongside other specific analytes in the patient samples, and longitudinal changes of CB levels in the CSF samples can be related to AD progression.

ADVANTAGES

- CB quantitation provides a reliable and reproducible alternative to behavioral testing.
- Preclinical testing stringency is improved by incorporating calbindin-D28K biomarker readouts for cognitive function in animal experiments. The greater consistency and ease of execution of assays, also provides for much higher throughput. This opens up options for increasing test group sizes and utilizing more than one model if required. Improving the strength and reliability of preclinical testing, improves the chances that selected lead compounds survive clinical trials.
- Provides an additional analyte that can be measured alongside other known pathology indicators such as amyloid-β aggregates or Tau proteins. Notably, in one clinical study of 333 CSF samples from cognitively normal (Clinical Dementia Rating [CDR] 0), very mildly demented (CDR 0.5), and mildly demented (CDR 1) individuals, 190 analytes were measured. Significantly, the researchers found that the best panel of markers for predicting risk of developing cognitive impairment (CDR 0 to CDR>0 conversion) consisted of calbindin, Aβ42, and age. This panel had better predictive value than the second established panel consisting of tau, Aβ42, and age.

BACKGROUND OF INVENTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disease that causes a relentless decline in memory and other cognitive functions. This devastating disease affects 47% of those over 85 and one in eight over 65, but any patient benefits observed with current treatments are modest at best. New therapies based on alternative strategies for counteracting AD pathogenesis are in clinical trial pipelines. Basic research continues to uncover targets in key pathways that are thought to drive disease progression; the reliability of appropriate animal models used for preclinical screening is critical for identifying promising lead compounds. The less reliable the models, the greater the likelihood that selected candidates may fall out during later clinical trials. Early diagnosis is considered a major part of improving the success of medical intervention in AD. Studies suggest that initial pathogenesis could begin ~15 to 20 years before cognitive problems bring an individual to seek medical attention. Hence, the identification of biomarkers as reliable indicators for developing brain pathology and related cognition deficits has great value both for drug development and improving potential diagnostic testing that can detect disease at much earlier stages. The ability to more consistently detect the onset of AD at an early
stage of pathogenesis will assist in identifying new potential drug targets and therapies for AD patients. It may also provide opportunities for developing new strategies for intervention prior to significant loss of cognitive function. This is particularly important for those known to be at higher risk of disease because of identified genetic factors, e.g. apolipoprotein E4 carriers.

STAGE OF DEVELOPMENT

In order to measure hippocampal dysfunction in AD, Gladstone scientists have developed convenient surrogate measures that correlate with learning and memory deficit in mouse models. In contrast, amyloid plaque load does not correlate with cognitive deficiency in mice or humans. Impaired neuronal function, including disruption of normal synaptic transmission during AD, drives aberrant network activity. Over time, in models of AD or epilepsy, this causes depletion of specific calcium binding proteins such as CB and compensatory inhibitory remodeling of hippocampal circuits, with ectopic expression of neuropeptide Y. AD pathogenesis in mouse models can be assessed by measuring these biomarkers in specific brain regions. Levels of the markers expressed relate to the severity of cognitive impairment. Precise protocols were developed for quantifying CB (U.S. patent 7,297,836) and for additional biomarkers (Palop, Mucke & Roberson, 2011). Alterations in expression levels of the biomarkers in specific brain regions provide a read-out for disease severity in mouse models of AD. CB depletion in the dentate gyrus has also been documented in patients with AD (Palop et al. PNAS 2003).

LICENSING POTENTIAL

Gladstone seeks to license the use of patented methodologies for quantitation of calbindin-D28K by non-exclusive license agreement.

PATENT STATUS


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REFERENCES
