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Perspective

Anti-amyloid: An antibody to cure Alzheimer's or an attitude

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SUMMARY

For more than a century, clinicians have been aware of the devastating neurological condition called Alzheimer's disease (AD). AD is characterized by the presence of abnormal amyloid protein plaques and tau tangles in the brain. The dominant hypothesis, termed the amyloid hypothesis, attributes AD development to excessive cleavage and accumulation of amyloid precursor protein (APP), leading to brain tissue atrophy. The amyloid hypothesis has greatly influenced AD research and therapeutic endeavors. However, despite significant attention, a complete understanding of amyloid and APP's roles in disease pathology, progression, and cognitive impairment remains elusive. Recent controversies and several unsuccessful drug trials have called into question whether amyloid is the only neuropathological factor for treatment. To accomplish disease amelioration, we argue that researchers and clinicians may need to take a compounding approach to target amyloid and other factors in the brain, including traditional pharmaceuticals and holistic therapies.

INTRODUCTION

Alzheimer's disease (AD) is a devasting neurodegenerative condition marked by progressive dementia. Presently, more than six million Americans are living with AD, and that number is estimated to reach 12.7 million by 2050, barring any medical breakthroughs.[1] In response to the growing number of affected individuals, significant resources have been dedicated to the study of AD and the development of therapeutic strategies. Remarkably, the Cure Alzheimer's Fund has documented the mortality rate significantly increasing for people with AD and decreasing in all other major diseases including stroke, cardiovascular disease, HIV, and some cancers.[2] The defining pathological features of AD are widely recognized as the presence of abnormal amyloid protein plaques and tau tangles in the brain. It is hypothesized that the accumulation of abnormal protein deposits of amyloid beta (Aβ) due to the excess cleavage of amyloid precursor protein (APP) leads to synapse and neuronal loss ultimately resulting in brain atrophy in AD. This hypothesis – termed the amyloid hypothesis – has dominated the scientific viewpoint on AD pathogenesis and therapeutic development for the past century.[3] While a significant portion of research and clinical trials have focused on targeting amyloid and APP proteins, the full scope of their roles in disease pathology, progression, and cognitive impairment is still not fully understood. We suggest that the time for a rapid drug intervention to expand the therapeutic toolbox used to treat AD is long overdue.

GENETICS SUPPORTS THE AMYLOID HYPOTHESIS

The popularity of the amyloid hypothesis is reflected in ongoing clinical trials and lead drug development candidates, many of which target either soluble or aggregated amyloid peptides. The central idea of this hypothesis is based on the premise that accumulation of the neurotoxic beta-amyloid (Aβ) peptide coupled with the ineffective clearance and degradation of Aβ clumps in the brain is the primary driver of the neurodegenerative processes in AD. While the hypothesis is not universally accepted among researchers, an important line of unbiased evidence confirms the importance of this peptide in AD – genetics. There are a variety of specific genes and gene mutations that increase the risk of developing AD (Table 1). The most well-established AD risk factor is *ApoE4*. Carriers of one allele of *ApoE4* have triple the likelihood of developing late-onset AD compared to non-carriers. Other risk genes beyond *ApoE4* have also been identified and include *BIN1*, *TREM2*, *ABCA7*, *INPP5D*, *CD33*, and others.[4] Interestingly, most of these risk factors function in pathways that control vesicle trafficking processes including phagocytosis,

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Table 1. Risk factor genes associated with Alzheimer's disease and their role within the brain

Gene	Role	AD Risk	Onset
APOE ε2	Lipoprotein synthesis	Reduced	N/A
APOE ε3	Lipoprotein synthesis (most common allele in general population)	Neutral	N/A
APOE ε4	Lipoprotein synthesis (AD risk gene with mutations found on chromosome 19)	Elevated	Early
ABCA7	Cholesterol processing	Elevated	Late
CLU	Regulates clearance of Aβ in the brain	Elevated when gene expression is deficient	Late
CR1	Chronic neuroinflammation	Elevated when gene expression is deficient	Late
PICALM	Neuronal communication		Late
PLD3	Unknown	Elevated	Late
TREM2	Regulation of neuroinflammation	Elevated due to rare variants	Late
SORL1	Odd variants known on chromosome 11	Elevated	Late
APP	Mutations on chromosome 21 cause overproduction of toxic Aβ peptide	Elevated	Early (inherited)
PSEN1	Mutations on chromosome 14 cause overproduction of toxic Aβ peptide	Elevated	Early (inherited)
PSEN2	Mutations on chromosome 1 cause overproduction of toxic Aβ peptide	Elevated	Early (inherited)

Here, we also describe whether the gene mutation increases or decreases the likelihood of developing AD, and on what timeline. The onset of Alzheimer's is typically defined as being early- or late-onset. Late-onset AD is much more common, generally being symptomatic after age 65. Early-onset AD is a relatively rare form of the disease usually diagnosed in individuals under the age of 65 – typically between 40 and 50 years of age.

endocytosis, and immune signaling, suggesting defects in these processes are in part a priming risk for AD pathogenesis.

In rarer cases, AD is driven by deterministic genes that guarantee development of a disease and are inherited in a familial autosomal dominant form. Research has identified multiple familial mutations in *APP*, *PSEN1*, and *PSEN2* that support the importance of Aβ peptide accumulation in the progression of AD.[5] Mutations in these genes form the catalytic component of the γ-secretase complex, which is responsible for cleaving APP into various Aβ oligomers, implying that aberrant APP metabolism is central to AD pathogenesis. However, it is estimated that only 1% of people with AD have one of these mutations. Individuals with Down Syndrome (DS) have three copies of chromosome 21, which is also the locus for the *APP* gene. Because of this, these individuals are significantly more likely to develop AD and do so at a younger age.

Interestingly, there are extremely rare familial mutations in these genes that reduce or even prevent the risk for developing Alzheimer's. For example, the Icelandic mutation reduces the cleavage of APP that would normally lead to abnormal amyloid processing, effectively preventing amyloid plaque accumulation and disease progression. Although these genetic and pathological studies point to the importance of amyloid in AD, it would be a great oversimplification to conclude that amyloid alone can explain the entirety of AD pathogenesis.

RESEARCH ALLEGATIONS CAST A SHADOW ON THE AMYLOID HYPOTHESIS

Despite well-established lines of scientific evidence, recent controversies driven by unsuccessful clinical trials and research publications have called into question whether amyloid is the pathological factor that initiates symptoms and drives AD pathogenesis, threatening the progress made in the field. Shockwaves were sent through the AD field after allegations that falsified images of $A\beta_{56}$ oligomers were used in seminal manuscripts from 2006.[6] The group claimed to have discovered a new oligomer, "amyloid beta star 56" ($A\beta_{56}$) that was isolated from a transgenic mouse model and injected into rats, which subsequently developed memory deficits – a hallmark of AD pathology. With over 2000 citations from this research alone, scientists believed an obvious target for treatment was discovered. To the field, the discovery of this additional oligomer made it seem obvious that amyloid accumulation resulted in a cascade of signaling and

neuronal damage, ultimately causing AD and related dementias. If this species was, in fact, the cause of memory loss, then inhibiting or degrading the protein would potentially prevent disease progression. However, confirmatory experiments have never been able to observe this oligomer species in mice or even human fluids or tissues. Furthermore, it was discovered that protein bands on the published western blots appeared to be duplicated.[7] The cleavage of APP does, in fact, lead to various oligomer species of amyloid, but the main forms studied in AD have been $A\beta_{40}$ and $A\beta_{42}$. While we were once excited by the possibility of this new finding, investigations into the lab's falsified images have led to increasing pressure on the validity of the amyloid hypothesis. Despite the allegations, there is still compelling evidence in post-mortem brain tissue and imaging analyses that amyloid is a major hallmark of the disease.[8] Has our desire to find a cure placed too much emphasis on amyloid at the expense of other putative targets? We would argue that amyloid is a critical determinant in AD, and we should consider a multimodal approach to treating AD.

It appears that brain amyloid accumulation may be related to AD as plasma cholesterol levels are to heart disease, or troponin is to myocardial infarction. They are critical determinants, occasionally adequate on their own, but not conclusive of the whole scope of treating the disease pathology. In fact, as amyloid burden increases in the AD brain, the key microtubule stabilizing protein tau, which is of critical importance in maintaining neuronal structure and function, becomes hyperphosphorylated.[9] The phospho-forms of tau subsequently aggregate within neurons forming structures known as tau tangles. These events lead to the loss of neuronal integrity, impaired signaling, and ultimately neuronal cell death. From a therapeutic viewpoint, modulating tau to treat AD is an important consideration.[10,11] Concomitantly, we must revolutionize how we approach targeting both proteins, and at a clinically relevant time window before disease progression.

THE STATE OF ALZHEIMER'S CLINICAL TRIALS

In the relatively short time that clinical trials have been conducted on AD, almost none have proven to be clinically efficacious or demonstrate robust safety, with side effects ranging from meningoencephalitis, toxicity, and amyloid-related imaging abnormalities (ARIA) – such as brain edema, sulcal effusion, and hemorrhagic hemosiderin deposits.[12,13] Most of the drugs tested in clinical trials have been immunotherapy-based monoclonal antibodies (mAb) targeting one singular protein, primarily amyloid: with a failure rate of these drugs near 100%.[14] One recent breakthrough in developing amyloid therapeutics is aducanumab, sold under the brand name Aduhelm: a synthetic mAb that targets amyloid aggregates in both the oligomeric and fibrillar forms. Its efficacy is questionable though since the drug was approved solely for its ability to reduce amyloid while data ignored reporting on any significant symptomatic benefit for patients.[15] More recently, a second amyloid immunotherapy targeting soluble aggregated amyloid protofibrils, lecanemab was approved by the Food and Drug Administration (FDA). While 12-month primary endpoints were not met across doses in Phase II, new trials indicate a reduction in cognitive decline by 27% after 18 months.[16] To date, this is the most promising data generated from any AD trial. Out of the separate monoclonal antibodies that have been tested to target various aspects of the amyloid cascade pathway, lecanemab has demonstrated some efficacy (Figure 1A). More recently, donanemab is being studied for its effect in reducing amyloid plaques by binding to soluble or aggregated conformations of the $A\beta_{42}$ isomer.[17] Interestingly, Phase III trials and a press release noted that the antibody slowed cognitive and functional decline in AD by 33% compared to placebo participants, but also noting significant side effects are present.[18,19] This raises the question as to which amyloid species should be targeted. Amyloid plaques were long held as the neurotoxic structure, however newer studies and results from clinical trials suggest this may not be the case. Therapeutics like lecanemab and donanemab that target soluble amyloid appear to be much more effective than those that target insoluble fibrils and plaques (Table 2). These results correlate to studies in mice, where new data supports a more neurotoxic role for monomeric and oligomeric amyloid species compared to their fibrillary or dense-core plaque bound counterparts.[20,21]

As of now the question remains as to whether intervening with anti-amyloid therapy can even forestall clinical indicators and other downstream effects, such as cognitive and functional decline. Previous clinical trials have fallen short, in part because AD is a complex neuropathology with multiple mechanisms contributing to pathogenesis and progression. A more robust interventive approach using combinatorial targeting of key pathogenic mechanisms will likely be necessary. For example, inhibition of Aβ production by utilizing secretase inhibitors to block the cleavage of APP (BACE1), modulating the immune response to clear Aβ accumulation, active and passive vaccines, or utilizing natural intravenous immunoglobulin (IVIg)

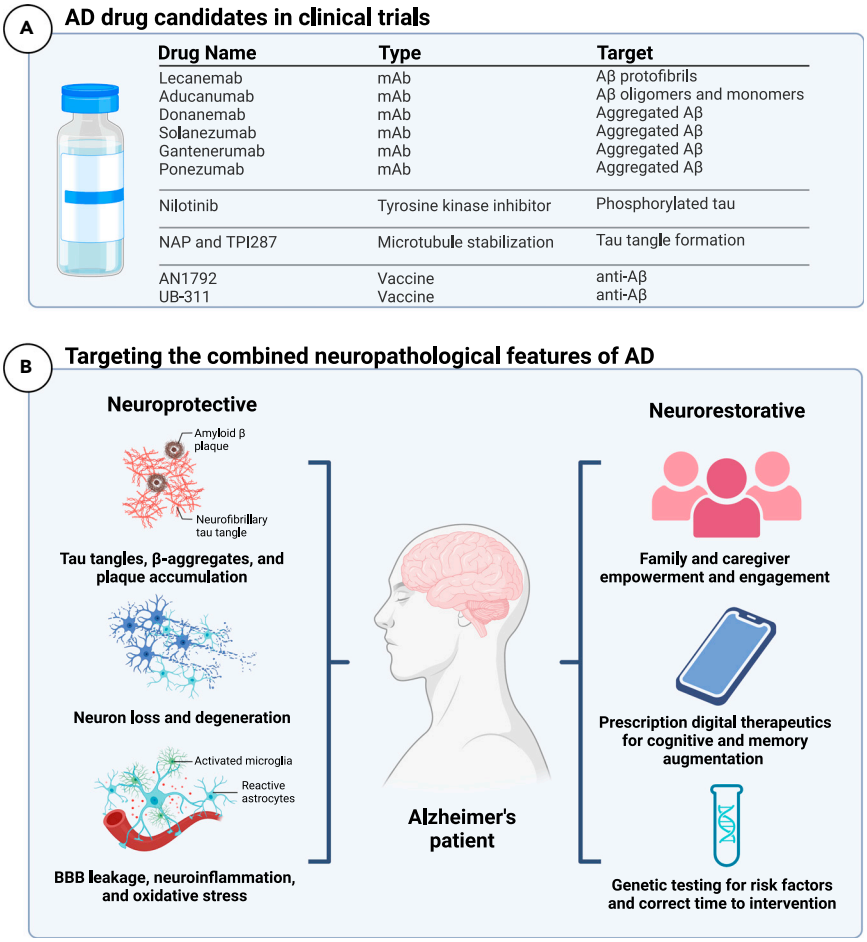

Figure 1. A new opportunity for combined and adjunct anti-Alzheimer's therapeutics

(A) A list of drug candidates has been developed and undergone clinical trials, with little to no patient efficacy. Most of the clinical drugs target a singular protein of AD, with close to 100% failure rates.

(B) To circumvent this singular drug target, we propose a therapeutic revolution where multiple neuropathological factors of the disease must be targeted. This can be done as a combined and adjunct therapeutic whereby neuroprotective mechanisms aim at targeting the molecular hallmarks of the diseases, and neurorestorative ways work to augment symptoms caused by AD and determine the correct time of intervention. Images made with BioRender. Aβ, amyloid-beta; AD, Alzheimer's disease; mAb, monoclonal antibody.

from human blood donors[49,50] would be holistically beneficial. However, attempts at targeting most of these areas have proven less effective or more difficult than hoped. Moreover, timing matters … interventions for the misprocessing of the APP cleavage pathway should be given at the right time, before extensive cognitive decline, to reduce amyloid accumulation in the brain and a clinically relevant decrease in symptoms. Of course, this requires the development and implementation of better diagnostic approaches and disease biomarkers. A significant number of resources and studies are now being directed at developing new testing methodologies and novel biomarkers to determine AD in its earliest stages.[51,52] These advances will afford for the ability to initiate therapy at the appropriate time and likely prior to cognitive decline.

EXPANDING THE THERAPEUTIC TOOLBOX: A NEW OPPORTUNITY FOR A COMBINATION ANTI-ALZHEIMER'S THERAPY

While we should acknowledge the promising early results observed from lecanemab therapy, it is important to recognize that it alone is not the ultimate solution for AD. Several pathways have been shown to be significantly affected in patients with AD. These pathways include druggable targets in mechanisms related to oxidative stress, blood vessel architecture, neuroinflammation, and neurogenesis that are thought of as

Table 2. Anti-amyloid and tau antibodies have been developed and commonly failed at curing the disease in previous clinical trials

Drug	Type	Target	Result
Lecanemab	mAb	Aβ protofibrils	Phase III trial underway, slows progression[22] Phase III trial underway, slows progression[22]
Donanemab	mAb	Established Aβ plaques	Shows signs of benefit in phase II trial[23] Shows signs of benefit in phase II trial[23]
Crenezumab	mAb	Binds to Aβ oligomers and monomers	Stopped at Phase III trials, not likely to meet endpoints[24,25]
Aducanumab	mAb	Aggregated Aβ	Decrease Aβ and slow cognitive decline as of Phase II trials[12,26,27]
Bapineuzumab	mAb	Aggregated Aβ	Failed to decrease cognitive decline[28] Failed to decrease cognitive decline[28]
Verubecestat	BACE1 Inhibitor	Enzyme cleavage of APP	Failed to decrease cognitive decline and had treatment-related adverse events in Phase III trials[29,30]
Atabecestat	BACE1 Inhibitor	Enzyme cleavage of APP	Dose-related cognitive worsening and neuropsychiatric adverse events (AEs) in Phase III trials[31,32]
CNP520	BACE1 Inhibitor	Enzyme cleavage of APP	Lack of efficacy and worsens cognition in Phase III trials[33,34]
Lanabecestat	BACE1 Inhibitor	Enzyme cleavage of APP	Lack of efficacy and did not slow cognitive decline in Phase III trials[35] Lack of efficacy and did not slow cognitive decline in Phase III trials[35]
Solanezumab	mAb	Aggregated Aβ	Failed to decrease cognitive decline in Phase III trials[36,37]
Gantenerumab	mAb	Aggregated Aβ	Failed to decrease cognitive decline[38,39]
Ponezumab	mAb	Aggregated Aβ	Abandoned after phase II trials[40,41]
Tideglusib	(GSK-3β) protein kinase inhibitor	Phosphorylated tau	No significant clinical benefit in phase II trials[42]
trx0014 and lmtm	Methylene blue dye derivative	Inhibit tau aggregation	Technique and efficacy in slowing cognitive decline are controversial[43,44]
AN1792	Vaccine	Anti-Aβ	Discontinued, 6% of participants developed meningoencephalitis, with no significance in Aβ antibody development[45–47]
UB-311	Vaccine	Anti-Aβ	Latest clinical trial drug in Phase II, shown to be well-tolerated but safety profile like placebo[48]

Here, we describe some of the most reported therapeutics targeting AD, their drug classification, specific target in the Alzheimer's pathway, and a summarized result of the human clinical trial. Most of the drugs tested in clinical trials have been immunotherapy-based monoclonal antibodies (mAb) targeting one singular protein, primarily amyloid: with a failure rate of these drugs near 100%.

potential neuroprotective approaches.[53,54] To elucidate these pathways, researchers are beginning to understand the molecular and cellular mechanisms that underlie AD to develop effective combination treatments, including developing and targeting drugs to cross the blood-brain barrier without causing general toxicity. Beyond amyloid, tau targeted therapies have been the second highlight area for new treatment opportunities.[10] Tau is an important protein also closely associated with AD as it is important for microtubule stabilization to support neuronal structure. In AD, tau often becomes hyperphosphorylated leading to microtubule destabilization and eventual neuronal dysfunction. A variety of targets surrounding tau exist, including the kinases that phosphorylate tau and the ability for it to aggregate into tangles.[55] These kinases are closely linked to the amyloid cascade pathway, specifically APP, therefore targeting them may prove effective in both protein pathways.[56] One hurdle in developing therapeutic targets for these kinase inhibitors is the high specificity that is needed. Kinase inhibitors including the tyrosine kinase inhibitor Nilotinib are currently being evaluated in AD.[57] Other therapies to stabilize microtubules in neurons including NAP

and TPI287 are in early clinical studies.[58–60] As with amyloid targeted, the majority of clinical or near clinical drugs targeting tau are immunotherapies and directed at preventing tau aggregation and tangle formation. The tau aggregation inhibitors methylthioninium chloride (MTC) and a stabilized-derivative LTMX, showed initial promise, but failed to slow cognitive decline.[61] However, the study did provide hope in that a subset of trial participates, approximately 15% did show significant clinical benefit, suggesting refining tau inhibitors may be of value in moving tau targeting forward. Importantly, tauopathies in combination with amyloid are two significant pathological features in Alzheimer's that should be dually targeted, in contrast to the traditional mono-therapeutic approach, albeit few have succeeded due to toxicity or low efficacy.

Two plausible targets of high therapeutic value outside of amyloid and tau include neuroinflammation and mitochondrial dysfunction. Activation of neuroinflammatory pathways including the production of cell death inducing cytokines such as IL-1β and TNFα as well as increased fragmentation of mitochondrial and reactive oxygen species (ROS) production are well-defined components of human AD pathology. New brain-penetrant therapeutics are being developed to target IL-1β production by inhibiting the NLPR3 inflammasome as well as key cleavage enzymes including caspase-1.[62,63] Another route of modulation exists by targeting mitochondrial dynamics and ROS production. At the pharmacological level these therapeutics provide promise for potentially the first disease modifying drugs for AD and possibly other neurodegenerative diseases. As amyloid is not the only determinant of AD, it is plausible that if anti-amyloid therapies are successful, they will need to work in concert with other treatment targets to have the maximum effect, like combined antiretroviral therapy (cART) to treat HIV infection.[64] Combined anti-Alzheimer's therapy can include an anti-amyloid drug, an anti-tau drug, and even a neuroprotective or anti-inflammatory drug.

In terms of next steps for AD treatment, a more holistic approach is needed that addresses the underlying neuropathological co-morbidities in addition to the pathology of the disease itself (Figure 1B). There are two branches of therapeutic approaches to be utilized: neuroprotective and neurorestorative. While the holy grail for AD would be a truly disease modifying curative therapy, neurorestorative therapies that target the consequences of the disease, including cognitive and memory augmentation provide promising hope in the interim. Neurorestorative therapeutics in this regard act to slow the progression of memory loss, but not completely circumvent it. Activities such as being mentally active, socialization, and physical activity have been shown to prevent, or at least, delay the onset and progression of AD.[65–67] The thought behind these activities is that mental stimulation supports the growth of new nerve cells and stimulates nerve cells to communicate with one another. Nevertheless, delaying symptom onset by using more neuroprotective therapies in combination with augmenting any suffered memory loss as a restorative effect would be beneficial for the overall management of AD and decrease its incidence and prevalence. Another approach to neurorestorative methods is to implement enhanced neurorehabilitative programs for patients and caregivers. Non-drug interventions have proven to be helpful for patients in long-term recovery efforts toward cognitive function and relieving disease symptoms, particularly when caregivers and families are empowered and engaged.[68] This can ultimately affect patient-caregiver relationships, patient anxiety, and overall patient quality of life. In an exciting advance, prescription digital therapeutics (PDT) are becoming more well studied and even approved by the FDA, which act as a new adjunct therapy to oral drugs.[69] This digital therapy can be utilized to assist treatment or be used prior to disease onset to intervene before symptomatic presentation. The advantage of promoting memory augmentation through a digital health platform, or software as a medical device (SaMD), is that ease of access and time to intervention are significantly expedited. Digital therapies have the potential to help patients long-term through noninvasive means that do not pose any negative pharmacokinetic effects on other drugs and improve the patient-caregiver dynamic. The importance of studies that also focus on symptomatic recovery cannot be understated when it comes to improving a patient's life. There is no doubt that there is room to improve the therapeutic toolbox for patients who suffer from not only AD but other neurological disorders, and adjunct therapies are just a start to ameliorating chronic symptoms.

Conclusion

Despite substantial funding allocated to Alzheimer's disease and related dementias (ADRD) research, the success rate in developing effective drug candidates has been limited. The vast amount of money spent on research has yielded few effective therapeutic options, highlighting the urgent need for a paradigm shift in how we approach research and therapeutic development. It is increasingly evident that initiating treatment

before the onset of symptoms is likely to be the most effective approach. The failure of certain drugs in clinical trials may be simply due to mistiming of intervention and target validity. Until we gain a better understanding of the multifaceted nature of the disease, achieving a definitive treatment for AD may remain elusive, making it one of the most crucial discoveries in modern medicine. Alzheimer's needs a therapeutic revolution. Exploring innovative and combinatorial methods to accelerate the discovery and development of breakthrough therapies for Alzheimer's is crucial to maximizing the impact of the resources invested in this field.

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AUTHOR CONTRIBUTIONS

O.M.O., O.N., and M.T. conceptualized the paper and contributed to the writing of the manuscript. D.M.D. contributed to the discussions on the genetics of AD and the amyloid hypothesis. B.L.H. contributed to the discussions about novel therapeutic strategies. O.M.O. and O.N. created the tables and figures.

DECLARATION OF INTERESTS

O.M.O. is the founder & CEO at NeurOn Therapeutics, a biotechnology company that develops therapies for the treatment of disorders of the central nervous system through the application of digital medical device technologies. B.L.H. is the co-founder & CSO at Asha Therapeutics, a biopharmaceutical company that focuses on therapeutics targeting neurodegenerative diseases such as AD. He also consults for Ventus Therapeutics, Venn Therapeutics, and USA Prime Biotech. The authors declare no other competing interests.

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in their field of research or within their geographical location. One or more of the authors of this paper received support from a program designed to increase minority representation in their field of research.

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