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Review

The Rationale Behind the New Alzheimer's Disease Conceptualization: Lessons Learned During the Last Decades

José Luis Molinuevo[a,b,c,*], Carolina Minguillon[a], Lorena Rami[c] and Juan Domingo Gispert[a,d]
[a]*Barcelonaβeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain*
[b]*CIBER Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain*
[c]*Alzheimer's Disease and Other Cognitive Disorders Unit, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain*
[d]*Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain*

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Abstract. In the last decades, progress in neuroimaging techniques and cerebrospinal fluid assays has enabled the characterization of several Alzheimer's disease (AD) biomarkers. This knowledge has shifted the conceptualization of AD from a clinical-pathological construct, where its diagnosis required the presence of dementia with distinct pathologic features, toward a clinical-biological one that recognizes AD as a pathological continuum with a clinical picture that ranges from normal cognition to a dementia stage. Specifically, AD is now divided into three stages: preclinical (abnormal biomarkers and no or only subtle cognitive impairment), mild cognitive impairment or prodromal AD (abnormal pathophysiological biomarkers and episodic memory impairment), and dementia (abnormal biomarkers and clear cognitive and functional impairment). The possibility of assessing AD pathophysiology *in vivo* before the onset of clinical symptoms in the preclinical stage provides the unprecedented opportunity to intervene at earlier stages of the continuum in secondary prevention trials. Currently, large cohort studies of cognitively healthy participants are undergoing with the main aim of disentangling the natural history of AD to identify individuals with an increased risk of developing AD in the near future to be recruited in these clinical trials. In this paper, we review how the concept of AD has changed over the years as well as discuss the implications of this conceptual change.

Keywords: Alzheimer's disease, biomarkers, continuum, ethical challenges, preclinical, prevention

INTRODUCTION

This paper is part of the 20th anniversary issue of the *Journal of Alzheimer's Disease* and, following the Editorial Board suggestions, we will review how our work performed together with a great group of professionals has contributed to redefine Alzheimer's disease (AD). The objective of the present manuscript is to review how our view of AD has evolved as new biomarker knowledge has emerged during the last decades. In addition, the social and ethical implications of the new conceptualization of AD will be discussed.

LATE 1990S: GENETIC CHARACTERIZATION OF AD

More than 95% of affected individuals develop AD after the age of 65, which is known as late-onset AD,

*Correspondence to: José Luis Molinuevo, Barcelonaβeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain. Tel.: +34 93 3160990; E-mail: jlmolinuevo@fpmaragall.org.

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while from 1 to 5% develop what is referred to as early-onset AD (symptom onset in their mid-life). Early-onset AD is sometimes genetically determined due to autosomal dominant mutations whereas late-onset presents a complex etiology, being mostly of sporadic origin. Although of different etiology and with distinct genetic profiles, understanding the pathophysiology of autosomal dominant AD (ADAD) has contributed to the current understanding of the pathologic events that lead to the more common form of the disease.

In the late 1990s, the genetic characterization of AD and other dementias was an important topic. Mutations in three genes were identified, the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*), that lead to ADAD. At the time, the relative contribution of *APP* and *PSEN* mutations to ADAD was under debate. While a number of studies described that virtually all cases of ADAD could be explained by mutations in these three genes [1, 2], others suggested the involvement of other genes [3, 4].

ADAD research was mainly focused on the characterization of the clinical features and the genetic analysis of families with familial ADAD (e.g., [5–7]) since it was fundamental to understand the clinical characteristics associated with each mutation in order to offer proper genetic counseling. Clinical, pathological, and genetic overlap among different neurodegenerative disorders was also described [8] and mutations in the *MAPT*, *PGRN*,and *PRPN* genes that were associated with familial frontotemporal dementia, frontotemporal lobar degeneration (FTLD), and familial prion disease were identified [9–14].

Altogether, the experience gathered during this period allowed us to establish a genetic counseling program for familial dementias: the PICOGEN program [15], which was based on the available experience and on the clinical practice guidelines on genetic counselling in Huntington's disease [16]. Briefly, PICOGEN offered genetic testing and counselling to patients that were suspected to carry disease-causing mutations for AD, FTLD, or prion disease. Additionally, asymptomatic subjects who decided to know their genetic status were evaluated within a structured protocol by a psychiatrist and psychologist prior to entering the program and followed up afterwards. PICOGEN participants stated that the main reason for their participation in the program was to receive early treatment when available in the future. Secondary reasons were to decrease anxiety, to decide family planning, and to inform their children. Although preliminary, PICOGEN also showed that predictive testing and disclosure is safe and may be of benefit when performed with a sensitive approach under strict pre-test counseling protocols and post-test follow-up programs. Although the diseases, their phenotype, and mutation characteristics were different, emotional reactions were largely similar [17].

PRE-BIOMARKER ERA CLINICAL CHARACTERIZATION OF EARLY AD STAGES

As mentioned earlier, the vast majority (>95%) of AD patients develop the disease after the age of 65 presenting a complex etiology distinct from ADAD, although both share common pathophysiological hallmarks. Historically, persons with cognitive impairment attending memory clinics, already had a full-blown dementia. Nevertheless, in the late 1990s, efforts from both patients' associations and public figures disclosing their condition as AD sufferers greatly contributed to increase disease awareness. This resulted in growing numbers of elderly individuals seeking medical advice when presenting with cognitive complaints that were not disabling (thus lacking the core feature of dementia). In this scenario, professionals aimed at characterizing the ranges between normal aging and dementia, and several terms have been proposed during the last decades, such as "Age-Associated Memory Impairment" (AAMI) [18] or "cognitive impairment no dementia" (CIND) [19]. From the plethora of terms proposed, "mild cognitive impairment" (MCI) has clearly gained widest acceptance. Nevertheless, the terms AAMI, CIND, and MCI have some subtle differences and thus may not necessarily represent identical populations.

As defined by Petersen and colleagues, MCI presented the following criteria: 1) subjective concern of a memory disturbance (preferably supported by an informant), 2) objective evidence of a memory deficit, 3) generally preserved cognitive functions, 4) intact activities of daily living, and 5) absence of dementia [20]. With these criteria, MCI still constituted a level of cognitive decline in which low-functioning normal older persons and high-functioning early dementia patients were hardly distinguishable. In this regard, research focused in developing sensitive psychometric tools as well as identifying both genetic and

neuroimaging features capturing MCI patients that progress to AD [21–27].

As initially described, MCI criteria included concern in the memory domain [20], which was accepted as a limitation of the concept, as other clinical symptoms leading to MCI and later on progressing to dementia were identified [27]. Nevertheless, studies still showed that while individuals with amnestic MCI (aMCI) had an increased risk to progress to AD, not all would [28]. Efforts were made to develop and/or include more specific neuropsychological as well as structural and functional neuroimaging measures to be able to identify patients with prodromal AD (patients with suspected AD pathology and a clinical picture with objective cognitive impairment not fulfilling dementia criteria). Prospective follow up of amnesic patients at risk for AD, whose features were intermediate between amnesic MCI and probable AD patients, termed prodromal AD, showed a significantly higher progression rate to probable AD than aMCI patients [29].

The concept of "subjective cognitive decline" (SCD) was first introduced by Reisberg and colleagues in their effort to define AD stages according to the Global Deterioration Scale (GDS): GDS stage 2 was characterized as subjective complaints of memory deficit in the absence of objectivized memory impairment [30]. In the last decade, the relevance of the identification of this group has been highlighted as a potential indicator of non-normative cognitive decline and eventual progression to dementia. SCD is currently defined as a "self-experienced persistent decline in cognitive capacity in comparison with a previously normal status that is not related to an acute event" and precludes the presence of MCI, the predementia stage characterized by objective cognitive impairment. Similar to the MCI status, SCD may be related to numerous conditions such as normal aging, personality traits, psychiatric conditions, neurologic and medical disorders, substance use, and medication. To develop a conceptual framework and research criteria for SCD, the Subjective Cognitive Decline Initiative (SCD-I) Working Group was recently established [31]. SCD increases the risk of cognitive decline, developing dementia and also the likelihood of being in the preclinical stage of AD (see below for the definition of the AD preclinical stage). The SCD-I has proposed a set of specific SCD features, under the name of SCDplus, associated with an increased likelihood of preclinical AD. These features are 1) subjective decline in memory, rather than other cognitive domains; 2) onset in the last 5 years; 3) age at onset $\geq$60 years; 4) concerns (worries) associated with SCD; 5) feeling of worse performance than others in the same age group; 6) confirmation of cognitive decline by an informant; and 7) presence of the *APOE ε4* allele [31, 32].

THE EMERGENCE OF AD BIOMARKERS AND THE NEW CONCEPT OF AD

Several years ago, a "probable" AD diagnosis was defined as a clinical-pathological construct based on determining the presence of dementia and discarding other potential etiologies. Therefore, it was a syndromic diagnosis, that could only be confirmed post-mortem [33]. In the last decades, progress in neuroimaging [both magnetic resonance imaging (MRI) and positron emission tomography (PET)] techniques and cerebrospinal fluid (CSF) assays, has enabled the thorough characterization of several *in vivo* AD biomarkers. These include amyloid-β (Aβ) and tau concentration in CSF [34], hippocampal atrophy [35, 36], temporoparietal hypometabolism [37, 38], and cerebral amyloid and tau deposition measured by PET) [39, 40], among others. AD biomarkers have recently been divided into three binary categories (A, Amyloid biomarker; T, tau biomarker; N, neurodegeneration or neuronal injury biomarker) based on the nature of the pathophysiology that each measures [41].

The initial research criteria that incorporated AD biomarkers (see below) distinguished between the so called pathophysiological and topographical markers, but did not define which ones were more useful to define the disease. Subsequent studies showed which biomarkers presented optimal correlation with underlying AD pathology in post-mortem studies. In this respect, both CSF biomarkers and amyloid PET imaging showed the highest correlation, making them proxies of AD pathology [42–48]. In addition, several studies have contributed to define the diagnostic performance of the determination of $A\beta_{1\text{-}42}$, t-tau, and p-tau, establishing a molecular CSF biomarker signature of AD through autopsy-confirmed AD cohorts [49, 50].

The availability through MRI, PET, and CSF analyses of the characteristic and reliable biomarkers of AD discussed above, enabled the change of AD conceptualization from a clinical-pathological entity to a clinical-biological one. AD is currently defined as a pathologic continuum that can be divided into three stages: preclinical (abnormal biomarkers and no or

only subtle cognitive impairment), MCI or prodromal AD (abnormal pathophysiological biomarkers and episodic memory impairment), and dementia (abnormal biomarkers and clear cognitive and functional impairment). Furthermore, the possibility of assessing AD pathophysiology *in vivo* ensued a change in the research framework of AD. Two sets of criteria have recently been published, one by the International Working Group (IWG; [51]) that has later been revised (IWG-2; [52]) and the other by working groups assembled by the National Institute on Aging (NIA) and the Alzheimer's Association (AA) in the US (NIA-AA; [53]). Although both define AD as a pathological continuum, the NIA-AA outlines different clinical syndromes and the preclinical stage, which are diagnosed with their own specific algorithm, whereas a single diagnostic algorithm that may be applied at any stage of the clinic-biological continuum is proposed by the IWG. Both sets of criteria agree in the incorporation of core AD biomarkers in the diagnostic process and in the recognition of an asymptomatic preclinical stage that can be determined through these biomarkers. However, whereas biomarker abnormalities are required for diagnosis according to IWG, the NIA-AA uses biomarker information (if available) to assess the likelihood (high, intermediate, or unlikely) that a clinical syndrome is due to AD. Additional differences between these criteria reside on the fact that NIA-AA support the diagnosis of AD in asymptomatic individuals with biomarker evidence for Aβ accumulation, whereas for the IWG-2, these persons are considered to be in an at-risk state of the disease. Moreover, IWG criteria for typical AD require an objective impairment in episodic memory whereas a less strict approach is considered by NIA-AA criteria for the diagnosis of MCI due to AD.

One of the main drawbacks that prevented the clinical use of CSF biomarkers was the uncertainty concerning the lack of comparability of CSF measurement across different laboratories. Development of ratios and normalized indices, such as the AD CSF index, increased the diagnostic accuracy of CSF biomarkers. Importantly, the performance of the AD CSF index was very similar when using different analytical platforms [54]. In addition, collaborative efforts were fundamental to demonstrate the validity of the core AD CSF biomarkers for the differential diagnosis of AD dementia even with different pre-analytical conditions [55].

A further step to consolidate the use of biomarkers in AD diagnosis was to assess their prognostic utility to predict the onset of dementia in predementia subjects. In this regard, we showed that, indeed, an abnormal AD CSF biomarker profile in predementia individuals was a powerful marker of risk for AD dementia. Only 15% of subjects with a pathological CSF ratio remained free of AD dementia at 5 years of follow-up and, conversely, all subjects who reverted to normal cognition presented a normal CSF profile at baseline [56]. In addition, we demonstrated that CSF biomarkers were useful in the differential diagnosis of early-onset cognitive impairment in clinical practice and increased the certainty of AD diagnosis to a high likelihood in most cases in both amnestic and non-amnestic presentations. Finally, AD CSF biomarkers also predicted subsequent impairment and progression to AD dementia with high accuracy in subjects with early-onset MCI [57].

RESEARCH IMPLICATIONS OF THE NEW CONCEPT OF AD

Disappointing results from clinical trials performed in AD dementia patients made it clear that modifying treatments for AD would require earlier diagnosis to optimize their potential benefits. In this scenario, the main application of the new AD diagnostic research criteria was to allow for an earlier and etiological diagnosis based on a biomarker profile [58] which, in turn, would enable earlier interventions in the prodromal stage and secondary prevention in the preclinical one. Non-demented AD populations emerged as potential targets for earlier interventions and, therefore, to characterize the relationships between their neuropsychological profile, CSF biomarker values, and neuroimaging characteristics stands out as fundamental (e.g., [59, 60] for the prodromal AD stage).

In this sense, the concept of preclinical AD rapidly gained attention and work performed by several groups rapidly verified the hypothesis that this stage of the disease presents distinct structural and functional imaging characteristics. As mentioned at the very beginning of this review, familial ADAD provides the opportunity to investigate brain changes even before symptom onset and we contributed to the topic in performing studies with AD mutation carrier participants recruited throughout the PICOGEN program [15]. This allowed us to evaluate cortical thickness, and water diffusivity indexes in presymptomatic and symptomatic *PSEN1* mutation carriers which were distinct: presymptomatic

carriers displayed increased cortical thickness (especially in precuneus and parietotemporal areas) and decreased mean diffusivity (which may correspond to brain swelling due to reactive neuronal hypertrophy and/or inflammatory response to amyloid deposition compared to healthy controls), while symptomatic subjects had decreased cortical thickness in the same areas and increased diffusivity possibly reflecting predominant neuronal loss, when compared to controls [61]. Longitudinal studies in presymptomatic carriers showed accelerated rates of decrease of thickness in the AD-related areas mentioned above, suggesting that brain structure in *PSEN1* mutation carriers follows nonlinear trajectories, with regional increases during the very early presymptomatic period [62]. Strikingly, we could replicate these findings in the context of sporadic AD when investigating the relationship between CSF Aβ values and cortical thickness in a group of cognitively preserved subjects. Briefly, an increment in cortical thickness in AD-vulnerable areas preceding cortical thinning that might be related to reactive neuronal hypertrophy and/or inflammation driven by amyloid in very early stages of the disease continuum, was also observed [63]. Similar nonlinear trajectories of AD-related brain atrophy in the AD continuum ranging from normal cognition to mild AD were found when AD-related pathology was tracked with the AD-CSF index. In addition, we showed that the impact of the *APOE ε4* genotype was most evident in the hippocampus and precuneus with carriers showing a steeper decline in gray matter volume after CSF-index values approaching the diagnostic threshold [64].

Together with the studies performed to characterize distinct structural and functional imaging characteristics of the AD preclinical stage, the search and validation of novel AD biomarkers that may be useful to further characterize the AD continuum, understand the pathological mechanisms of the disease, and be used routinely in clinical practice appeared as equally relevant (see a recent meta-analysis of CSF and blood biomarkers in [65]). In this line, we contributed characterizing two factors that may be involved in the neuroinflammatory mechanisms of the brain triggered by microglia and astroglia in response to pathological aggregates of amyloid and tau. These are the secreted 40 kDa glycoprotein YKL-40 (also known as Chitinase 3-like 1) mostly expressed by astrocytes, and the innate immune receptor expressed at the surface of microglia TREM2. We showed that CSF YKL-40 protein levels were higher in preclinical AD, although this increase lost significance when controlled for age, and significantly correlated with t-tau and p-tau levels, as well as with meaningful neuropsychological tests. Hence, YKL-40 appeared as a possible marker of early pathophysiological changes, potentially linked to an astrocytic inflammatory process, as well as an early marker to take into consideration in the pathophysiology of the disease to potentially measure its progression [66]. Furthermore, we showed that at the earliest stages of AD-related cognitive decline, CSF YKL-40 was related to a cerebral structural signature that was distinct to that associated with p-tau neurodegeneration, supporting the presence of concomitant neuroinflammatory and neurodegenerative processes at the initial stages of cognitive impairment [67]. Similar to what we found with CSF YKL-40, CSF levels of the soluble fragment of the innate immune receptor TREM2, sTREM, changed dynamically: they are slightly elevated in preclinical AD, peak in MCI due to AD and, in the dementia stage are found reduced versus MCI. We also found that CSF sTREM2 levels were associated with markers of neuronal injury and tau pathology [68]. In addition, higher CSF sTREM2 values were associated with increased gray matter volume in MCI patients in brain areas vulnerable to AD which typically present with atrophy in early AD in association with increased CSF p-tau levels, suggesting a microglial activation and enhanced TREM2 expression in response to incipient neurodegeneration [69]. Finally, we showed that *APOE ε4* allele carriers had increased CSF YKL-40 levels particularly at the preclinical and MCI stages, and showed an inverse association between gray-matter volume and CSF YKL-40 levels, whereas noncarriers displayed a positive one. Taking into account that the *APOE* apolipoprotein is mostly produced in the brain by astrocytes, these results suggested an increased astroglial activation in *APOE ε4* carriers. In contrast, significant differences between *APOE ε4* allele carriers and noncarriers were not found with respect to CSF sTREM2 [70].

IDENTIFICATION OF CANDIDATES FOR SECONDARY PREVENTION TRIALS

The setup of preventive studies requires the identification of individuals with an increased risk of developing AD in the near future that are suitable to be recruited as asymptomatic subjects in clinical

trials [71]. In this context, and aiming at increasing our knowledge of the pathophysiology and pathogenic factors emerging at early preclinical AD stages, we established the ALFA (for ALzheimer and FAmilies) program for the prospective follow-up of a cohort of cognitively normal subjects, most of which are the offspring of AD patients [72]. Within this program, the ALFA parent cohort is composed of 2,743 cognitively normal participants, most of them first-degree descendants of AD patients, aged between 45 and 74 years, who have been thoroughly characterized from a sociodemographic, clinical, lifestyle, and cognitive point of view. In addition, participants' *APOE* haplotype has been also determined. The ALFA parent cohort will serve as the basis for the establishment of research protocols and studies, both observational and interventional, of preclinical participants at risk of cognitive impairment due to AD, such as the ALFA+ cohort study. On top of a similar characterization as in the ALFA parent cohort (neuropsychological, clinical, etc.), it entails the acquisition of both wet (CSF and blood sample collection) and imaging (MRI and PET) biomarkers. The ALFA+ study started in October 2016, will include 500 participants, and complete follow up visits will be performed every three years. In brief, the ALFA+ study will serve to untangle the natural history of the disease and to model the preclinical stages in order to develop successful trials [72].

A fundamental aspect of preclinical AD research resides on the need for collaborative efforts among research groups and stakeholders involved (funding agencies, study participants, regulatory agencies, etc.). This is apparent in the recent design of international initiatives such as the European Prevention of Alzheimer's Dementia (EPAD; Europe) and sister programs upcoming in the US and Canada in which public-private consortiums are created to speed up the development of better and safer medicines for AD prevention. Similar to the ALFA project, these studies are based on research platforms of participants from which specific sub-studies are implemented. The EPAD project, funded by the Innovative Medicines Initiative, aims to deliver a standing, adaptive, multi-arm proof of concept study for early and accurate decisions on a candidate compound's (or combination of compounds') ongoing development for the prevention of AD dementia. It also contains an observational cohort major component (EPAD Longitudinal Cohort Study) of 6,000 preclinical participants for trial readiness, run-in data and improved disease modelling [73]. The more recently established Amyloid imaging to prevent Alzheimer's Disease (AMYPAD) project will contribute to define the role of amyloid imaging both in modelling the preclinical stage of the disease and its value and efficiency during the diagnostic process.

ETHICAL IMPLICATIONS OF AD NEW CONCEPT

As mentioned before, one of the most relevant applications of the new concept of AD is to allow for secondary prevention strategies in asymptomatic individuals in the preclinical stage, which gives rise to a variety of novel ethical challenges. In clinical research, these ethical issues are mainly related to determining appropriate risk/benefit ratios and whether or not to disclose information about biomarker status [74].

With regards to risk/benefit ratios, the earlier in the disease continuum, the longer clinical trials aimed at detecting change will have to last and, therefore, asymptomatic participants will be exposed to pharmacological agents for an extended period. These trials should therefore be designed to ensure that the potential benefits justify participant's procedural burden and associated risks. Improving the participant's understanding of the relevant issues, such as the probabilistic over deterministic nature of biomarkers, will be necessary for them to determine the acceptable risk-benefit ratio. In relation to this, one benefit of conducting trials in preclinical, asymptomatic, individuals is that they are in a better position to protect their own welfare and express their values regarding what risk is acceptable in providing informed consent. A perceived benefit for the participants in clinical trials is the possibility of receiving an efficacious therapeutic agent and, hence, individuals enroll in research because they consider it may be of benefit to their own health and believe that this outweighs the possible risks. Furthermore, it has been shown that altruism (potential benefit to their relatives, future sufferers or society) also motivates participating in a clinical trial [75]. In addition, indirect benefits for clinical trial participation may also be perceived. For example, participation may yield positive psychological impact on self-confidence, self-worth and the perceived benefit of providing societal value [76].

With regards to disclosure of biomarker status, the main risks include placing a cloud of uncertainty over participants that may affect their daily lives and/or performance in specific procedures,

and the complexity of conveying clinically non-relevant biomarker status of uncertain prognosis. Main benefits include the protection of biomarker-negative individuals from risks and harms related to the trial, and the positive impact that this information may have on people's lives. We recently pointed at the relevance of differentiating between study types (observational versus interventional) to favor disclosure (transparent enrollment) or not (blinded enrollment) [74]. Briefly, when considering the prospect of long-term studies, to avoid the impact of knowing on participants' performance, together with disclosing clinically non-relevant biomarker or genetic status of uncertain prognosis, blinded enrolment was recommended for observational studies. By contrast, transparent enrollment was favored for interventional studies, since protecting the subjects that are biomarker negative from risks and harms related to the intervention prevails over the motivations noted above to support blinded enrollment. An additional argument for the transparent design is that it better reflects future clinical practice of drug prescription to those who learn that they have an altered AD biomarker which, in turn, would provide information about the success of this potential future clinical practice. New trials currently undergoing, such as the Generation S1 with *APOE ε4* homozygotes, will be disclosing *APOE* status, through a harmonized genetic counseling protocol (NCT02565511). In this scenario, investigators in transparent research designs can further protect individuals by assessing if potential participants are emotionally capable of enrolling. Data from the REVEAL study showed that those who exhibited a high degree of emotional stress before undergoing genetic testing were more likely to have emotional difficulties after disclosure [77]. For those included, continuous counseling has been shown to have a direct positive effect on stress and anxiety [78].

CONCLUSION

The last decade has witnessed an outstanding change in the conception of AD, from a clinical-pathological construct to a clinical-biological one, in which the disease can be identified and staged through biomarkers. The possibility of assessing AD pathophysiology *in vivo* before the onset of clinical symptoms in the preclinical stage provides the unprecedented opportunity to intervene at earlier stages of the continuum through secondary prevention trials. To identify individuals with an increased risk of developing AD in the near future to be recruited in these clinical trials, a number of cohort studies and international collaborative efforts are being established. In addition, the ethical implications of performing clinical research in asymptomatic individuals are being elucidated and addressed. In this scenario, one has reason to feel cautiously optimistic about the future of AD research.

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