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TOWARDS PERSONALIZED THERAPIES FOR ALZHEIMER'S DISEASE: CHALLENGES FOR TRANSLATING MOLECULAR GENETICS AND SYSTEMS BIOLOGY INTO STRATEGIES TO PREVENT COMPLEX CHRONIC BRAIN DISORDERS

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ABSTRACT

The challenges for deciphering the etiologies and/or developing an intervention for the prevention of late-life complex chronic brain disorders, such as Alzheimer's disease (AD), stem from their (I) polygenic etiologies, (II) heterogeneity biological-behavioural signs and (III) prolonged degenerative processes beginning decadesbefore the onset of detectable symptoms. The crucial challenges for developing individualized therapies for AD dementia require the discovery, development, and validation of innovative technologies and complex algorithms, with a high degree of accuracy and/or prognostic certainty, for early detection of asymptomatic individuals in thegeneral population. The aim of this perspective is to discuss the merging evidence on a broad spectrum of putativerisk factors and outline the need for novel conceptual models or approaches regarding (I) research on thecomplexities of genetic-epigenetic-life style interactions in the expression of polygenic brain disorders, (II) theapplication of the systems biology framework to understand the emergence of AD and other related chronic braindisorders. Large population-scale analyses began to reveal an increasing number of genetic mutations andpolymorphisms, as well as a spectrum of putative epigenetic and lifestyle risk factors, which potentially play a central role in the expression of not only the rare inherited form of the early-onset familial type of AD but also the more prevalent late-onset sporadic form of the disease. A current working hypothesis speculates that the distinct endophenotypes of the sporadic form reflect the cascades of anomalous changes in molecular signalling pathways resulting from complex relations among several putative risks, e.g., gene-gene interactions, along with

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modulating influences of lifestyle, education, and the environment. The application of systems biology – using genomics and other omics sciences, such as metabolomics – along with advancing knowledge on genetic/epigenetic factors to test such hypothesis, is expected to yield new insights into the genesis and downstream pathophysiological mechanisms of AD and other complex brain disorders, therefore supporting the introduction of effective therapeutic strategies.

KEYWORDS: *Alzheimer's disease, brain disorder, deterioration, depression, Human Genome Project*

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive brain disorder that slowly destroys specific neural systems with the deterioration of some motor and brain functions. Symptoms include cognitive decline, behavioural problems including depression, delusions, agitation, and daily activities and self-care decline. The sporadic form of the disease, sporadic AD (sAD), is characterized by late onset, typically above 65 years of age, and accounts for about 95% of all cases.

The aetiology of this brain disorder is not entirely understood. However, growing evidence supports that the classic cluster of symptoms traditionally associated with the disease is not due to a single causative factor. Rather, it is the outcome of a complex combination of factors such as family history, genetics, comorbid conditions, and lifestyle. In addition, a growing body of evidence indicates that vascular risk factors for atherosclerosis, stroke and cardiac disease are significantly related to AD risk. These findings imply a central role in cerebrovascular dysfunction, which may promote neurodegenerative processes and precede the onset of sAD.

The relative importance of these putative risk factors in increasing the jeopardy or affecting the expression of the disease differs from individual to individual; progress in understanding the biology of such polygenic complex brain diseases leads to new debates about the clinical-biological phenotypes characterizing the disease. In general, it is widely known that:

(I) AD is highly heterogeneous regarding the onset of symptoms, patterns or a mix of clinical features, neuropathology, risk factors, genetics, biomarkers, and response to treatment.

(II) Gradual molecular alterations in the brain leading to the disease seem to begin decades before any symptoms can be detected.

(III) Genetic susceptibility and mutations are the most productive and promising (and also the most challenging) areas of AD exploration. In this regard, the Human Genome Project, following substantial technological developments, has led to a wealth of information on the genetics/epigenetics of AD (1,2).

There are promising prospects that advances in genetics/epigenetics and molecular biology will provide the critical breakthroughs needed to understand the disease pathophysiology and the knowledge required to develop effective therapies for AD and other chronic brain disorders.

The growing knowledge about the polygenic nature and the heterogeneity of AD requires a critical look at the role and influence of genetic and epigenetic factors. Such a careful reassessment of the genetics/epigenetics of complex brain disorders need to account for the distinct biological phenotypes and the heterogeneous behavioural-clinical phenotypes of AD. For instance, the challenge of discovering the causal relationships between various genetic mutations and the cascade of downstream biological (biochemical) events, including the expression of clinical phenotypes, needs to account for some unique features of the disease. From this standpoint, there are crucial aspects that need to be addressed, including:

(I) The means of translating accurate genetic and/or molecular information about a disease into effective treatments; for instance, the genetics of Huntington's disease has been well mapped; however, that knowledge has been difficult to translate into interventional approaches.

(II) Elucidating: (a) the specific mechanisms allowing various genetic mutations or susceptibility genes to explain the neuroanatomical specificity of the neurodegenerative processes and lesions as well as (b) the neurobiological mechanisms for the increased susceptibility of some neural structures for degeneration.

(III) Describing the specific combination of genetic/epigenetic factors that: (a) determine or affect the beginning and the rate of progression of the degenerative process and (b) account for the heterogeneity of clinical and neuropathologic features of AD.

(IV) The knowledge about the genetics of AD is based mainly on associations studies; in this regard, there is the need to develop the resources and technical capabilities for prospective validation of all putative genetic and/or epigenetic risk factors as prognostic tools for predicting the probability of disease onset.

(V) Evidence indicates that the disease's pathophysiological mechanisms begin decades before noticeable behavioural signs appear. Therefore, one of the crucial challenges for AD and other chronic brain disorders is to discover and, possibly, validate the optimal clusters of genetic/epigenetic risk factors for the accurate detection (and prediction) of individuals with an elevated risk of developing the disease in the pre-clinical asymptomatic stages of neurodegeneration.

(VI) The task for personalized interventions for at-risk individuals in the disease's asymptomatic phase is to discover how AD progresses from pre-clinical (asymptomatic) stages to the first expression of symptoms and beyond. The best opportunity to develop strategies for early detection is to scrutinize and map out, in longitudinal studies, the patterns of modifications in various biological indices in individuals carrying AD mutations. Several longitudinal studies, especially clinical trials, have recently been conducted to provide helpful information for further investigations. A relevant example is represented by the Dominantly Inherited Alzheimer's Network (DIAN), consisting of a long-term observational study of individuals dominantly carrying inherited mutations causing AD (3). Another important study is the Alzheimer's Prevention Initiative (API), a therapeutic prevention trial conducted in cognitively healthy asymptomatic individuals belonging to a large kindred from the Colombian state of Antioquia who carry the inheritable "Paisa" *PSEN1* E280A mutation (https://www.alzforum.org/mutations/psen1-e280a-paisa), the most frequent cause of early-onset familial AD (EOFAD) (4,5). Another interesting prototype for the longitudinal study of disease progression is represented by Down Syndrome (DS). Interestingly, patients with DS carry three copies of the amyloid precursor protein (*APP*) gene and are also at risk for AD; hence, they should be considered for presymptomatic discoveries (6-8).

Overview of the genetics of Alzheimer's disease

Studies on the genetics of AD led to uncovering of three broad categories of mutations and genetic variations associated with: (I) early-onset forms of AD, (II) late-onset sporadic forms of AD, and (III) susceptibility for AD (9,10). Here, we will consider some of the findings in each category that may have some bearing on the discovery and subsequent development of technologies for the early identification of individuals at risk and/or the introduction of disease-modifying therapies.

Early-onset AD (EOAD)

Early-onset AD (EOAD) cases have an early age of onset (<60 years) and account for approximately 5% of all AD cases. In particular, 35-65% of EOADs are familial, and the remaining are sporadic (11,12). A fraction of EOFAD cases, around 10-15%, are autosomal-dominant AD (ADAD) that follow an autosomal dominant inheritance pattern (13) due to highly penetrant mutations in three genes: (a) *APP* on chromosome 21q21.2, (b) presenilin-1 (*PSEN1*) on chromosome 14q24.3, and (c) presenilin-2 (*PSEN2*) on chromosome 1q42.13. Roughly 90% of the remaining EOFAD individuals are assumed to be due to an autosomal recessive inheritance pattern (14).

As of December 2022, the database available at https://www.alzforum.org/mutations, consisting of a repository of variants in genes implicated in AD, contains 479 genetic variants: (a) 63 genetic variants for *APP*, (b) 339 genetic variants for *PSEN1*, and (c) 77 genetic variants for *PSEN2*. *APP* and PSEN mutations both drive the amyloidogenic process in ADAD patients through alterations in brain metabolism of *APP* and amyloid beta (Aβ) peptides that support the development of toxic species and pathogenic aggregates (15), which ultimately may play a key role in neuronal cell death and dementia. Although the evidence concerning *APP*, *PSEN1*, and *PSEN2* mutations provides insights into the probable molecular mechanisms underpinning EOFAD, this data is inadequate to explain all cases of AD dementia fully.

Sporadic AD (sAD) or late-onset AD (LOAD)

The vast majority of AD cases (>95%) are sporadic AD (sAD) or late-onset AD (LOAD) with the usual onset after the age of 65 years (9,10). Evolving data indicates that multiple genes contribute to susceptibility or risk for LOAD. Susceptibility genes are identified by genetic association studies in which allele frequencies for polymorphisms at or near a gene are compared between cases and controls. Susceptibility genes are revealed when case and control frequencies differ significantly. Early attempts to identify these susceptibility genes were driven by selecting candidate genes based on the existing knowledge of disease pathogenesis (16). In 1993, Hypothesis-driven association studies on candidate genes led to the discovery of the association between the *APOE* gene that encodes for the apolipoprotein E (ApoE) and is located on chromosome 19q13.2 and AD (17, 18). *APOE* exhibits three isoforms (*ε2, ε3*, and *ε4*) and six genotypes (*ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4*, and *ε4ε4*) resulting from a combination of two coding single-nucleotide polymorphisms (SNPs). The *APOE ε4* allele accounts for about one-third of the population-attributable risk for the disease (19). Despite efforts to elucidate the primary mechanisms, the same mode in which *APOE ε4* affects sAD onset and progression has yet to be verified. Current hypotheses on the pathogenic role of ApoE in AD revolve around the observation that Aβ accumulation is promoted in the brains of *APOE ε4* carriers and transgenic mice expressing the human *APOE ε4* allele

and mutant *APP* (20). ApoE does not modulate Aβ metabolism through direct binding to Aβ in solution but through its actions with other interacting receptors/transporters such as the low-density lipoprotein receptor-related protein 1 (LRP1)-dependent cellular uptake pathway in astrocytes (21,22). Moreover, ApoE participates in cholesterol transport and lipid metabolism and in addition to AD, the *APOE ε4* allele represents a confirmed risk factor in vascular disease, likely owing to its link to augmented plasma cholesterol concentrations (21).

In microglial cells, ApoE-induced intracellular Aβ degradation is mediated by the cholesterol efflux function of ApoE, which lowers cellular cholesterol levels and, subsequently, facilitates the intracellular trafficking of Aβ to lysosomes for degradation (21, 23).

Interestingly, a milestone study by Rhinn and colleagues (2013) analyzed whole-transcriptome cerebral cortex gene expression data in unaffected *APOE ε4* carriers and LOAD patients. *APOE ε4* carrier status was associated with a consistent transcriptomic shift resembling the LOAD profile. Furthermore, differential co-expression correlation network analysis of *APOE ε4* and LOAD transcriptomic changes enabled the detection of a set of candidate regulatory gene mediators. Several of these genes, including the amyloid beta A4 precursor protein-binding family A member 2 (*APBA2*), integral membrane protein 2B (*ITM2B*), fibroblast yes related novel (*FYN*), ring finger protein 219 (*RNF219*), and synaptic vesicle protein 2A (*SV2A*), encode known or novel modulators of *APP* endocytosis and metabolism. In addition, a genetic variant within *RNF219*, namely rs2248663, has been reported to affect Aβ deposition in the human brain and LOAD age-of-onset. These findings established the existence of an *APOE ε4*-associated molecular pathway that promotes LOAD (24).

Susceptibility for AD

Susceptibility genes/loci for LOAD have been inspected. Until about 2005, only a few genes could be investigated due to technical limitations. With a few exceptions, most studies of this type could not be replicated, likely because of inadequately evaluated population substructure, sample sizes that were too small to detect meaningful associations, and *P*-value thresholds that were insufficiently stringent. However, subsequent progress in genomic tools, in conjunction with gathering large sample cohorts, resulted in the initiation of genome-wide association studies (GWAS) aimed at recognizing novel genetic risk factors (25). Advances in genotyping platforms permitted the cross-examination of most of the genome for disease-associated variation in a single experiment. This method resulted in an exponential improvement in susceptibility gene discovery for a large number of diseases, disease-related traits, and associated phenotypes. Since the original report of *APOE* as a genetic risk factor for sAD, several hundreds of genes were tested for association with AD, leading to thousands of studies reporting positive associations, lack of association, replications, and refutations. Given the incessantly growing amount of published AD genetic studies, it became challenging to follow and interpret such a vast volume of published data (9,10,26). In order to address this problem, the AlzGene online database (27) was introduced to provide a comprehensive, unbiased, and routinely updated online catalogue and meta-analyses for the increasing list of AD candidate genes.

GWAS enabled the discovery of several SNPs associated with clinical AD or AD biomarkers. Compared with *APOE*, these SNPs induce only minor risk effects, which, when taken in combination, might substantially contribute to AD pathogenesis. Historically, the first large-scale GWAS executed (28-33) was successful in documenting at least ten novel genomic loci, in addition to *APOE*, as low-risk factors for sAD the following were also documented:

BIN1 (bridging integrator protein-1), *CLU* (clusterin, also known as apolipoprotein J), *ABCA7* (ATP-binding cassette, sub-family A, member 7), *CR1* (complement receptor type 1), *PICALM* (phosphatidylinositol-binding clathrin assembly protein), *MS4A4E* (membrane-spanning 4-domains, subfamily A, member 4E), *MS4A6A* (membrane-spanning 4-domains, subfamily A, member 6A), *CD33* (CD33 antigen, also known as Siglec [sialic acid-binding Ig-like lectin]-3), *CD2AP* (CD2-associated protein), and *EPHA1* (ephrin receptor EphA1).

Besides the association with the amyloidogenic cascade, these genes are linked with some basic physiological and pathophysiological interacting molecular pathways and exhibit patterns of putative functional relationships in sAD, namely: (a) cholesterol and lipid metabolism (*CLU* and *ABCA7*), (b) immune and complement systems/inflammatory response (*CR1*, *CLU*, *BIN1*, *MS4A4E/MS4A6A*, *CD33*, *ABCA7*, *CD2AP*, and *EPHA1*), (c) endocytosis pathways (*BIN1*, *PICALM*, and *CD2AP*), and (d) cell adhesion (*EPHA1*) (16). Furthermore, in line with the breakthrough results of these initial larger-scale GWAS, a high number of gene variants, not reaching genome-wide significance in sAD, were reported to be associated with the same category of pathways, particularly with the immune system and lipid metabolism, thus emphasizing a crucial role of these mechanisms in sAD pathophysiology (34).

The need for large-scale meta-analyses of GWAS and much larger sample sizes to enhance the identification of additional loci prompted an international initiative, the International Genomics of Alzheimer's Project (IGAP). This collaborative effort links the resources of four consortia focused on AD genetics/genomics: the Alzheimer's Disease Genetic Consortium (ADGC), the Cohorts for Heart and Ageing Research in Genomic Epidemiology (CHARGE) consortium, the European Alzheimer's Disease Initiative (EADI), and the Genetic and Environmental Risk in Alzheimer's Disease (GERAD) consortium (9,10). Following a meta-analysis conducted in a total sample of 74,046 participants (25,580 cases and 48,466 controls), this international partnership determined 11 novel genome-wide significant LOAD susceptibility loci: *INPP5D, MEF2C, HLA-DRB5/DRB1, NME8/GPR141, ZCWPW1/PILRA, PTK2B, CELF1/SPI1, SORL1, FERMT2, SLC24A4/RIN3*, and *CASS4* (35). Some of these loci stressed the prominent role of pathways already known to be linked to an amplified risk for AD, such as lipid metabolism, immune response and inflammatory pathways, cell migration, and mechanisms of endocytosis. More important, other genes emphasized the occurrence of different novel pathways underpinning AD, including hippocampal synaptic activity, cytoskeletal activity, axonal transport, and microglial and myeloid cell function (35). Moreover, the IGAP then performed a meta- analysis on a clinically evaluated enlarged sample of 94,437 participants (35,272 cases and 59,163 controls) and revealed 25 GWS loci, including five new ones (*IQCK, ACE, ADAM10, ADAMTS1*, and *WWOX*). Pathway enrichment analysis revealed lipid metabolism, immunity, *APP*, and tau binding proteins metabolism, thus highlighting that gene variants affecting *APP* and Aβ pathways are related to EOAD (ADAD) and LOAD (36). A very recent GWAS meta-analysis that combined a large, new case-control study with previous GWASs, allowed for examination of the most elevated number of AD patients (111,326 cases) together with controls (677,633) and reported 75 risk loci, many of which (specifically 42) were new. Pathway enrichment analysis confirmed the involvement of *APP* /Aβ peptide and tau-binding proteins metabolism, innate immunity, microglial activation, and tumor necrosis factor-alpha (TNF-α) signaling pathway in ADD (37).

Transethnic GWAS, another GWAS-based approach for collecting results from multiethnic participants in existing analyses, was beneficial in revealing new loci for genetic traits and diseases (9). For instance, a recent transethnic GWAS meta-analysis, encompassing both Japanese (5,178 cases and 6,520 controls) and White participants from the 2019 IGAP stage 1 data, allowed the detection of the novel locus OR2B2 (38). Thus, transethnic GWAS can help identify several previously non-documented associations in investigations exclusively based on European participants (39,40). Therefore, upcoming transethnic GWAS for integrating much larger non-White cohorts are expected to support the identification of additional novel AD susceptibility loci (9).

In summary, AD is considered genetically heterogeneous, especially in late-onset forms showing polygenic risk inheritances. According to Kamboh (2022), the execution of large-scale GWAS and meta-analyses of GWASs allowed the disclosure of approximately 95 susceptibility loci for LOAD during the 2009-early 2021 period (9). In general, there is a clear indication for major cellular/molecular pathways dysregulation in AD, namely: (I) Aβ pathway progression, (II) inflammatory/immune response, (III) lipid homeostasis, (IV) modulation of endocytosis and vesicle-mediated transport, (V) regulation of cell cycle, (VI) oxidative stress response, and (VII) axon guidance (10,41).

Although GWAS represented a powerful method to discover risk loci in AD, a substantial proportion (up to 60%) of LOAD genetic variance remained unexplained (42). Rare or infrequent susceptibility genes are assumed to account for such a "missing heritability". The key platforms needed to identify rare genetic variants (population frequency <1%) with intermediate-to-high effect size in the disease are the next-generation sequencing (NGS) technologies, in the form of whole genome sequencing (WGS) and whole exome sequencing (WES) that can screen entire genomes and exomes, respectively. Large-scale NGS, such as WGS and WES, detected novel genes harbouring rare variants, usually missed by GWAS, associated with the disease (9,10,41). Notably, in order to progress the discovery of novel gene variants affecting AD risk, the National Institute on Aging (NIA) formed the Alzheimer's Disease Sequencing Project (ADSP) for sequencing and examining the genomes of a large number of well-characterized individuals to find an extensive range of AD risk (and protective) gene variants. The final aim is to accelerate the identification of new directions for AD pharmacological therapy research and prevention.

Benefits and pitfalls of pre-clinical models of Alzheimer's disease

Currently, no available animal model resembles all the cognitive, histopathological, biochemical, and behavioural alterations observed in AD patients. However, a partial reproduction of AD neuropathological changes and functional deficits have been obtained either with exogenous application of Aβ (43) or through genetically engineered mouse models expressing variants of APP, PSEN1, PSEN2, tau or ApoE proteins (44). These models provide excellent opportunities to examine the basis for the spatiotemporal evolution of the disease, as well as to identify novel targets for pharmacological intervention (45-47). Since memory loss is one of the major hallmarks of the disorder, the phenotypic characterization of these animals classically included electrophysiological studies to analyze synaptic transmission and long-term

potentiation (LTP) and behavioural tests to assess cognitive function. These are the most commonly used techniques to test animal disease models associated with cognitive decline and synaptic plasticity alterations (48-50). In addition, advances in histopathological and biochemical methods have been a powerful tool to address important questions about the pathogenic mechanisms of the disease. Most studies generally reported either inhibition of LTP or reduction in baseline fast excitatory transmission prior to plaque deposition. However, the relative importance of these changes and apparent discrepancies still needs to be resolved (51,52). Additionally, results from behavioural studies showed inconsistencies, possibly due to differences in the behavioral protocol, type of tests that were conducted, age of the animals, and the transgenic animals' genetic background (53).

So far, the amyloid cascade hypothesis has been the most important AD theory, postulating that the accumulation of Aβ species is the causative pathological event (54). Strong evidence that Aβ oligomers cause impairment of LTP was provided when naturally secreted soluble oligomers of human Aβ were injected intraventricularly into rats (55). These studies contributed to our knowledge of the cellular and molecular substrates involved in Aβ action. Understanding how Aβ accumulation and assembly compromise synaptic structure and function became the focus of therapeutically oriented research on the AD during the last two decades. Based on this hypothesis, interventions that reduce Aβ load in the brain would likely attenuate the neuropathological changes and functional deficits characterizing AD. Indeed, lowering the production of the peptide by inhibiting the enzymes responsible for Aβ generation, preventing the formation of Aβ aggregates, and increasing the rate of Aβ clearance from the brain have all proven successful in experimental models of AD (46).

In 2021, the U.S. Food and Drug Administration (FDA) approved aducanumab, a human IgG1 anti-Aβ monoclonal antibody, as the first disease-modifying treatment for AD. This regulatory decision raised controversies in the AD scientific community as it was based on a significant reduction of Aβ with no convincing data supporting clinical efficacy (56). For these reasons, the European Medicines Agency (EMA) and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) recently recommended the refusal of the marketing authorization for aducanumab. In addition, several other monoclonal antibodies targeting Aβ oligomers have been tested in clinical studies without producing encouraging results, thus raising concerns over the "amyloid cascade hypothesis" (57). Indeed, the development of transgenic AD mice carrying familial AD mutations has been useful in elucidating some of the mechanistic aspects of the disease, even though these models show poor predictive value (58). Several reasons might explain why these experimental studies failed to predict the outcome of clinical studies. First, transgenic mice carry familial AD mutations accounting for only 1-10% of all human AD cases. Also, while these models develop specific hallmarks of AD, they do not entirely recapitulate the complex human disease. For instance, it is questionable whether AD patients and transgenic mice share a similar temporal profile of disease progression. While in transgenic mice, cognitive impairment precedes plaque deposition, in AD patients, the latter is more likely to appear first. Also, determining the onset of memory deficits in AD transgenic mice is challenging since initial memory deficits are subtle, and memory tests may not be sensitive enough to detect early changes. Another weakness with transgenic mice is that only a few strains show evidence of significant cell death, different from the substantial neurodegeneration occurring in the human AD brain. Finally, differences between mouse/human species, including diversities in cerebrovascular anatomy, neuronal network physiology, disease susceptibility, and, most importantly, dynamics of drug-target interactions, all need to be considered in translation.

An Alzheimer's Association Business Consortium Think Tank was recently created to improve the translational predictability of such models (59). However, to better mimic the human condition, models or combinations of new models combining genetics with environmental interactions are needed. Also, the timing of disease development, heterogeneous mechanisms and pathways, comorbid conditions, and gender influence should be carefully considered.

Systems biology framework to Alzheimer's disease and complex polygenic brain disorders

In light of this, a systems biology framework for sporadic forms of AD consents to describing disease endophenotypes and examining commonalities among different neurodegenerative diseases (60,61). Indeed, systems biology is a holistic, integrative, and systems-level paradigm investigating how complex interactions among different molecular entities, including DNA-protein, transcript-protein, microRNA (miRNA)-protein, protein-protein, protein-metabolite interaction networks, occur across structurally/functionally organized networks and systems in both health and disease. Therefore, systems biology used at both the experimental and computational level (62,63) supports understanding the cellular/molecular pathways and the interactions involved in AD advancement (64-66).

Omics sciences, executed under the systems biology paradigm (67), are effective in illustrating and foreseeing the spatiotemporal trajectories of complex polygenic multi-factorial diseases, including cancer (68-70). A similar result is anticipated for AD (as well as other neurodegenerative diseases) and its pathomechanistic alterations (71,63). Hence, systems biology can support the incorporation of different diverging or converging molecular-cellular levels and time

phases of pathophysiological mechanisms, including inflammatory and immunological alterations, oxidative stress, protein misfolding, lipid dyshomeostasis, altered brain microvasculature, apoptosis, and autophagy (62,72).

Notably, among all the omics disciplines, comprehensive analyses focused on global metabolome profiling are gathering increasing consensus (71,73-75). Metabolomics designates the large-scale investigation of small molecules, usually known as metabolites, within biological fluids, cells, tissues, and organisms (71,73-75). Given that AD disrupts metabolic pathways, the long-term metabolic alterations produced can be reported in terms of metabolic signatures. Metabolome profiling can be effectively executed in peripheral tissues and biofluids, including CSF or, more recently, blood (plasma/serum), thus making this approach suitable for clinical applications. Various potential metabolic signatures have been documented for AD diagnosis (71,75) and are represented by numerous dysregulated metabolites whose levels fluctuate in the disease state or after drug exposure. The analysis of these metabolic patterns might offer significant information concerning disease pathophysiology (76,77).

In terms of technology, the advancement in analytical instrumentation associated with establishing standardized chemical fragmentation libraries and powerful data analysis algorithms made mass spectrometry-based metabolomics one of the most innovative platforms. Indeed, mass spectrometry is an excellent analytical platform, enabling the collection and simultaneous quantitation of femtomole amounts of cellular metabolites and their characterization, and exhibits an elevated sensibility and an extensive range of detectable metabolites. As a result, substantial improvement has been made in interpreting the major modifications occurring at both the mRNA, protein, and metabolite levels in tissues of AD organisms (73,74,78). Furthermore, as reviewed by Reveglia and colleagues (2021), biomarkers with different biological natures, including biogenic amines, oxylipins, lipid mediators, amino acids, oxidative stress markers, and metals, have been disclosed in AD research (73). Definitively, metabolomics will help effectively explore dynamic alterations in biological systems by providing appropriate information for clinical applications and translational medicine (73,74).

Genomics holds the promise of precision medicine in Alzheimer's disease

GWAS consortia enabled researchers to scrutinize several large datasets able to disclose genes that might have a subtler effect on AD risk. However, genetic clarification for a substantial portion of the heritability of AD pathology still needs to be revealed. Therefore, despite the relevant data attained from recent large-scale collaborative studies, novel strategies are required to identify novel risk loci. Optimistically, meta-analyses or pathway-based approaches will continue to discover new genes. To this aim, advances in high-throughput NGS technologies will continue to make WGS and WES approaches increasingly more affordable, thus allowing the opportunity to identify novel gene variants usually not documented in previously established GWAS marker panels. Furthermore, high-throughput NGS methods can potentially assess the effects of structural variations, such as copy number variations, and other large-scale structural rearrangements, which might help explicate the composite genetic architecture of AD (9,10,26,41).

Of note, besides SNPs results, researchers are developing and utilizing gene expression-based methods to increase the knowledge of AD pathogenesis (79). Ultimately, research in the area of AD epigenetics seems to be encouraging to explain the role of structural alterations in the neuronal DNA, due to DNA methylations or histone modifications, in supporting or delaying and/or preventing the expression of AD given an individual's genotype (80-82). Epigenetic processes are key regulators of interactions between the genome and the environment. Evidence suggests that sporadic forms of AD result from the combined effects of variation in several genes and environmental factors and from epigenetic aberrations (83,84). Notably, maps of epigenetic changes have been established to deliver more information on genetic variations and expression profiles for genes involved in AD. Therefore, targeting the epigenome has been thought helpful for establishing novel paths in AD therapy.

Furthermore, thanks to recent methodological advances, it is possible to profile genome-wide DNA methylation(DNA methylome) and the state of chromatin in the whole human genome in a high-throughput manner (85,86).Remarkably, chromatin immunoprecipitation (ChIP) approaches, combined with DNA microarray analysis (ChIP-chip) or followed by high-throughput DNA sequencing methods (ChIP-seq), enabled the introduction of epigenome-wide association studies (EWAS), hence highlighting a new era of epigenomics research in AD. Furthermore, such technologies can help discover epigenetic modifications to elucidate further disease mechanisms (85, 86). Hence, given that GWAS scrutinizing gene variants has been accomplished, a new generation of EWAS is foreseen to deliver unique, relevant information to enrich the comprehension of AD pathophysiology and, possibly, to support the elaboration of effective therapeutic approaches (85).

CONCLUSIONS

Definitively, there is clear evidence supporting that sporadic LOAD is caused by a chronic, non-linear, dynamic pathophysiological cascade of events that commences early in life and drives converging and diverging mechanisms that

result in neurodegeneration and late-stage clinical dementia. Moreover, unlike EOFAD, because there is a network of genes and gene variants interacting in these sporadic forms, the genetics of sporadic LOAD appear particularly intricate and multifaceted and seem not to be dependent on a major single-gene effect. In this connection, high-throughput NGS technologies constantly support the identification of the still elusive common and rare DNA sequence variants, believed to account for the "missing heritability" in AD (9,10,41).

Genomics holds the promise of the precision medicine paradigm, "tailoring" (ex. adapting) the management of patients to every individual's genetic profile. This framework could be helpful in rationally developing and targeting drugs to genes, transcripts, protein pathways, metabolic pathways, and molecular networks that participate in AD pathogenesis. As a result, upcoming developments in the study of AD complex genetic heterogeneity will probably allow clinicians to provide more efficacious targeted pharmacological treatments that will be "customized", that is to say, directed to the definite genetic profiles of their AD patients.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Collins FS, Morgan N, Partinos A. The Human Genome Project: lessons from large-scale biology. *Science*. 2003;300(5617):286-90.
2. Manolio TA. Genome wide association studies and assessment of the risk of disease. *N Engl J Med*. 2010;363(2):166-76.
3. Bateman RJ, Aisen PS, De Strooper B, et al. Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimers Res Ther*. 2011;3(1):1.
4. Fuller JT, Cronin-Golomb A, Gatchel JR, et al. Biological and Cognitive Markers of Presenilin1 E280A Autosomal Dominant Alzheimer's Disease: A Comprehensive Review of the Colombian Kindred. *J Prev Alzheimers Dis*. 2019;6(2):112-20.
5. Tariot PN, Lopera F, Langbaum J, et al. The Alzheimer's Prevention Initiative Autosomal-Dominant Alzheimer's Disease Trial: A study of crenezumab versus placebo in pre-clinical PSEN1 E280A mutation carriers to evaluate efficacy and safety in the treatment of autosomal-dominant Alzheimer's disease, including a placebo-treated noncarrier cohort. *Alzheimers Dement (NY).* 2018;8(4):150-60.
6. Condello C, Maxwell AM, Castillo E, et al. Aβ and tau prions feature in the neuropathogenesis of Down syndrome. *Proc Natl Acad Sci USA.* 2022;119(46):e2212954119.
7. Iulita MF, Garzón Chavez D, Klitgaard Christensen M, et al. Association of Alzheimer Disease With Life Expectancy in People With Down Syndrome. *JAMA Netw Open*. 2022;5(5):e2212910.
8. Coppus AM, Schuur M, Vergeer J, Janssens AC, Oostra BA, Verbeek MM, van Duijn CM. Plasma β amyloid and the risk of Alzheimer's disease in Down syndrome. *Neurobiol Aging.* 2012;33(9):1988-94.
9. Kamboh MI. Genomics and Functional Genomics of Alzheimer's Disease. *Neurotherapeutics*. 2022;19(1):152-72.
10. Sims R, Hill M, Williams J. The multiplex model of the genetics of Alzheimer's disease. *Nat Neurosci*. 2020;23(3):311-22.
11. Cacace R, Sleegers K, Van Broeckhoven C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimers Dement*. 2016;12(6):733–48.
12. Zhu XC, Tan L, Wang HF, et al. rate of early onset Alzheimer's disease: a systematic review and meta-analysis. *Ann Transl Med*. 2015;3(3):38.
13. Jarmolowicz AI, Chen HY, Panegyres PK. The patterns of inheritance in early-onset dementia: Alzheimer's disease and frontotemporal dementia. *Am J Alzheimers Dis Other Demen*. 2015;30(3):299–306.
14. Wingo TS, Lah JJ, Levey AI, Cutler DJ. Autosomal recessive causes likely in early-onset Alzheimer disease. *Arch Neurol*. 2012;69(1):59–64.
15. Weggen S, Beher D. Molecular consequences of amyloid precursor protein and presenilin mutations causing autosomal-dominant Alzheimer's disease. *Alzheimers Res Ther.* 2012;4(2):9.
16. Schellenberg GD, Montine TJ. The genetics and neuropathology of Alzheimer's disease. *Acta Neuropathol*. 2012;124(3):305-23.

17. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA.* 1993;90(5):1977-81
18. Chartier-Harlin MC, Parfitt M, Legrain S, et al. Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. *Hum Mol Genet.* 1994;3(4):569-74.
19. Hardy J. A hundred years of Alzheimer's disease research. *Neuron*. 2006;52(1):3-13.
20. Vance JE, Hayashi H. Formation and function of apolipoprotein E-containing lipoproteins in the nervous system. *Biochim Biophys Acta*. 2010;1801(8):806-18.
21. Yamazaki Y, Zhao N, Caulfield TR, Liu CC, Bu G. Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol*. 2019;15(9):501-18.
22. Verghese PB, Castellano JM, Garai K, et al. ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. *Proc Natl Acad Sci USA.* 2013;110(19):1807-16.
23. Lee CY, Tse W, Smith JD, Landreth GE. Apolipoprotein E promotes β-amyloid trafficking and degradation by modulating microglial cholesterol levels. *J Biol Chem*. 2012;287(3):2032-44.
24. Rhinn H, Fujita R, Qiang L, Cheng R, Lee JH, Abeliovich A. Integrative genomics identifies APOE ε4 effectors in Alzheimer's disease. *Nature*. 2013;500(7460):45-50.
25. Gandhi S, Wood NW. Genome-wide association studies: the key to unlocking neurodegeneration? *Nat Neurosci*. 2010;13(7):789-94.
26. Pimenova AA, Raj T, Goate AM. Untangling Genetic Risk for Alzheimer's Disease. *Biol Psychiatry.* 2018;83(4):300-10.
27. Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: The Alz Gene database. *Nat Genet.* 2007;39(1):17-23.
28. Bertram L, Lange C, Mullin K, et al. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet.* 2008;83(5):623-32
29. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009;41(10):1088-93.
30. Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009;41(10=:1094-99.
31. Seshadri S, Fitzpatrick AL, Ikram MA, et al. CHARGE Consortium; GERAD1 Consortium; EADI1 Consortium. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA.* 2010;303(18):1832-40.
32. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet.* 2011;43(5):429-35.
33. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet.* 2011;43(5):436-41.
34. Jones L, Holmans PA, Hamshere ML, et al. Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS One.* 2010;5(11):e13950.
35. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet.* 2013;45(12):1452-58.
36. Kunkle BW, Grenier-Boley B, Sims R, et al. Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing. *Nat Genet.* 2019;51(9):414-30.
37. Bellenguez C, Küçükali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nat Genet*. 2022;54(4):412-36.
38. Shigemizu D, Mitsumori R, Akiyama S, et al. Ethnic and trans-ethnic genome-wide association studies identify new loci influencing Japanese Alzheimer's disease risk. *Transl Psychiatry.* 2021;11:5.
39. Bien SA, Wojcik GL, Hodonsky CJ, et al. The future of genomic studies must be globally representative: Perspectives from PAGE. *Annu Rev Genom Hum Genet.* 2019;20:181-200.

40. Wojcik GL, Graff M, Nishimura KK, et al. Genetic analyses of diverse populations improve discovery for complex traits. *Nature.* 2019;570:514-18.
41. Freudenberg-Hua Y, Li W, Davies P. The role of genetics in advancing precision medicine for Alzheimer's disease-a narrative review. *Front Med*. 2018;5:108.
42. Ridge PG, Mukherjee S, Crane PK, Kauwe, JSK. Alzheimer's disease: analyzing the missing heritability. *PLoS One*. 2013;8(11):e79771.
43. Chambon C, Wegener N, Gravius A, Danysz W. Behavioural and cellular effects of exogenous amyloid-β peptides in rodents. *Behav Brain Res*. 2011;225(2):623-41.
44. Myers A, McGonigle P. Overview of Transgenic Mouse Models for Alzheimer's Disease. *Current Protocols in Neurosc*. 2019;89(10):e81
45. La Rosa LR, Matrone C, Ferraina C, et al. Age-related changes of hippocampal synaptic plasticity in APP-null mice are restored by NGF through p75NTR. *J Alzheimers Dis*. 2013;33(1):265-72.
46. Nisticò R, Pignatelli M, Piccinin S, Mercuri NB, Collingridge G. Targeting Synaptic dysfunction in Alzheimer's disease therapy. *Mol Neurobiol*. 2012;46(3):572-87.
47. Balducci C, Mehdawy B, Mare L, et al. The gamma-secretase modulator CHF5074 restores memory and hippocampal synaptic plasticity in plaque-free Tg2576 mice. *J Alzheimers Dis*. 2011;24(4):799-816.
48. Mango D, Saidi A, Cisale GY, Feligioni M, Corbo M, Nisticò R. Targeting Synaptic Plasticity in Experimental Models of Alzheimer's Disease. *Front. Pharmacol*. 2019;10:778.
49. Nisticò R, Mango D, Mandolesi G, et al. Inflammation subverts hippocampal synaptic plasticity in experimental multiple sclerosis. *PLoS One*. 2013;8(1):e54666
50. Bonito-Oliva A, Pignatelli M, Spigolon G, et al. Cognitive Impairment and Dentate Gyrus Synaptic Dysfunction in ExperimentalParkinsonism. Biol Psychiatry 2013; 75(9):701-10.
51. Marchetti C, Marie H. Hippocampal synaptic plasticity in Alzheimer's disease: what have we learned so far from transgenic models? *Rev Neurosci*. 2011;22(4):373-402.
52. Middei S, Roberto A, Berretta N, et al. Learning discloses abnormal structural and functional plasticity at hippocampal synapses in the APP23 mouse model of Alzheimer's disease. *Learn Mem*. 2010;17(5):236-40.
53. Bryan KJ, Lee H, Perry G, Smith MA, Casadesus G. Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations. In Methods of Behavior Analysis in Neuroscience. 2nd edition. Buccafusco JJ. Boca Raton, 2009.
54. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science.* 1992;256(5054):184-85.
55. Walsh D, Klyubin I, Fadeeva J. et al. Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature.* 2002;416(6880):535–39.
56. Nisticò R, Borg JJ. Aducanumab for Alzheimer's disease: A regulatory perspective. *Pharmacol Res*. 2021;171:105754.
57. Panza F, Lozupone M, Logroscino G, Imbimbo BP. A critical appraisal of amyloid-β-targeting therapies for Alzheimer disease. *Nat Rev Neurol*. 2019;15(2):73–88.
58. Mckean NE, Handley RR, Snell RG. A Review of the Current Mammalian Models of Alzheimer's Disease and Challenges That Needto Be Overcome. *Int J Mol Sci*. 2021;22(23):13168.
59. Vitek, MP, Araujo, JA, Fossel, M, et al. Translational animal models for Alzheimer's disease: An Alzheimer's Association Business Consortium Think Tank. *Alzheimer's Dement*. 2020;6(1):e12114.
60. Lista S, Khachaturian ZS, Rujescu D, Garaci F, Dubois B, Hampel H. Application of Systems Theory in Longitudinal Studies on the Origin and Progression of Alzheimer's Disease. *Methods Mol Biol*. 2016;1303:49-67.
61. Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: the royal road to cutting the therapeutic Gordian Knot. *Alzheimers Dement*. 2012;8(4):312-36.
62. Castrillo JI, Lista S, Hampel H, Ritchie CW. Systems Biology Methods for Alzheimer's Disease Research Toward Molecular Signatures, Subtypes, and Stages and Precision Medicine: Application in Cohort Studies and Trials. *Methods Mol Biol.* 2018;1750:31–66.

63. Castrillo JI, Oliver SG. Alzheimer's as a systems-level disease involving the interplay of multiple cellular networks. *Methods Mol Biol.* 2016;1303:3–48.
64. Seyfried NT, Dammer EB, Swarup V, et al. A Multi-network Approach Identifies Protein-Specific Co-expression in Asymptomatic and Symptomatic Alzheimer's Disease. *Cell Syst.* 2017;4(1):60–72.
65. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: Beyond biomarkers and towards mechanisms. *Nat Rev Mol Cell Biol*. 2016;17(7):451–59.
66. Civelek M, Lusis AJ. Systems genetics approaches to understand complex traits. *Nat Rev Genet.* 2014;15(1):34–48.
67. Hampel H, Nisticò R, Seyfried NT. Alzheimer Precision Medicine Initiative (APMI). Omics sciences for systems biology in Alzheimer's disease: State-of-the-art of the evidence. *Ageing Res Rev*. 2021;69:101346.
68. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat Rev Genet*. 2019;20(2):71–88.
69. Johansson HJ, Socciarelli F, Vacanti NM, et al. Breast cancer quantitative proteome and proteogenomic landscape. *Nat Commun*. 2019;10(1):1600.
70. Mertins P, Mani DR, Ruggles KV, et al. Proteogenomics connects somatic mutations to signalling in breast cancer. *Nature*. 2016;534(7605):55–62.
71. Wilkins JM, Trushina E. Application of metabolomics in Alzheimer's disease. *Front Neurol*. 2017;8:719.
72. Noorbakhsh F, Overall CM, Power C. Deciphering complex mechanisms in neurodegenerative diseases: the advent of systems biology. *Trends Neurosci*. 2009;32:88-100.
73. Reveglia P, Paolillo C, Ferretti G, et al. Challenges in LC-MS-based metabolomics for Alzheimer's disease early detection: targeted approaches versus untargeted approaches. *Metabolomics.* 2021;17(9):78.
74. González-Domínguez R, Sayago A, Fernández-Recamales Á. High-Throughput Direct Mass Spectrometry-Based Metabolomics to Characterize Metabolite Fingerprints Associated with Alzheimer's Disease Pathogenesis. *Metabolites*. 2018;8(3):52.
75. Trushina E, Mielke MM. Recent advances in the application of metabolomics to Alzheimer's Disease. *Biochim Biophys Acta*. 2013;1842(8):1232-9.
76. Batra R, Arnold M, Wörheide MA, et al. Alzheimer's Disease Metabolomics Consortium (ADMC). The landscape of metabolic brain alterations in Alzheimer's disease. *Alzheimers Dement*. 2022;1-19.
77. Horgusluoglu E, Neff R, Song WM, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI); Alzheimer Disease Metabolomics Consortium. Integrative metabolomics-genomics approach reveals key metabolic pathways and regulators of Alzheimer's disease. *Alzheimers Dement.* 2022;18(6):1260-78.
78. Mishur RJ, Rea SL. Applications of mass spectrometry to metabolomics and metabonomics: detection of biomarkers of aging and of age-related diseases. *Mass Spectrom Rev*. 2012;31:70-95.
79. Liang JW, Fang ZY, Huang Y, et al. Application of Weighted Gene Co-Expression Network Analysis to Explore the Key Genes in Alzheimer's Disease. *J Alzheimers Dis.* 2018;65(4):1353-64.
80. Nikolac Perkovic M, Videtic Paska A, Konjevod M, Kouter K, Svob Strac D, Nedic Erjavec G, Pivac N. Epigenetics of Alzheimer's Disease. *Biomolecules*. 2021;11(2):195.
81. Qazi TJ, Quan Z, Mir A, Qing H. Epigenetics in Alzheimer's Disease: Perspective of DNA Methylation. *Mol Neurobiol*. 2018;55(2):1026-44.
82. Balazs R, Vernon J, Hardy J. Epigenetic mechanisms in Alzheimer's disease: progress but much to do. *Neurobiol Aging*. 2011;32(7):1181-87.
83. Román GC, Mancera-Páez O, Bernal C. Epigenetic Factors in Late-Onset Alzheimer's Disease: MTHFR and CTH Gene Polymorphisms, Metabolic Transsulfuration and Methylation Pathways, and B Vitamins. *Int J Mol Sci*. 2019;20(2):319.
84. Bihaqi SW, Schumacher A, Maloney B, Lahiri DK, Zawia NH. Do epigenetic pathways initiate late onset Alzheimer disease (LOAD): towards a new paradigm. *Curr Alzheimer Res.* 2012;9(5):574-88.
85. Klein HU, Bennett DA, De Jager PL. The epigenome in Alzheimer's disease: current state and approaches for a new path to gene discovery and understanding disease mechanism. *Acta Neuropathol*. 2016;132:503–14.

86. Smith AR, Mill J, Smith RG, Lunnon K. Elucidating novel dysfunctional pathways in Alzheimer's disease by integrating loci identified in genetic and epigenetic studies. *Neuroepigenetics*. 2016;6:32–50.