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Animal and cellular models of Alzheimer's disease: Progress, promise and future approaches

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Animal and cellular models of Alzheimer's disease: Progress, promise and future approaches

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Abstract

Alzheimer's disease (AD) is an incurable neurodegenerative disease affecting over 45 million people worldwide. Transgenic mouse models have made remarkable contributions towards clarifying the pathophysiological mechanisms behind the clinical manifestations of AD. However, the limited ability of these *in vivo* models to accurately replicate the biology of the human disease have precluded the translation of promising preclinical therapies to the clinic.

In this review, we highlight several major pathogenic mechanisms of AD that were discovered using transgenic mouse models. Moreover, we discuss the shortcomings of current animal models and the need to develop reliable models for the sporadic form of the disease, which accounts for the majority of AD cases, as well as human cellular models to improve success in translating results into human treatments.

Introduction

Alzheimer's disease (AD) is the leading cause of disability and dependency among the elderly. This fatal neurodegenerative condition causes a gradual decline in memory and cognitive abilities, ultimately leading to dementia. Today, over 45 million people worldwide are affected, and the number is projected to reach 131 million by 2050 (Nguyen 2018). Thus, AD is one of the foremost public health and social care challenges currently facing our aging society. Further research to better understand the underlying pathobiology of this disorder is crucial for developing novel and effective disease-modifying therapies, since approved treatments only provide symptomatic relief (Hebert and others 2013; Cummings and others 2020).

Transgenic (Tg) animal models of AD, especially rodents, have provided critical insights into the pathogenic mechanisms underlying the development and progression of this neurodegenerative condition, and these models have been by far the most commonly used preclinical testing tools (Sasaguri and others 2017; Dawson and others 2018). Overexpression of human amyloid precursor protein (APP) harboring familial AD (FAD) mutations has been widely used in the field, starting in 1995 with PDAPP mice, the intracellular amyloid-beta (Aβ) model created by LaFerla, followed shortly by the generation of Tg2576 and APP23 mice (reviewed in Sasaguri and others 2017; Dawson and others 2018). These models recapitulate several key hallmarks found in AD brains, such as Aβ plaque formation, dystrophic neurites and an associated inflammatory response, leading to cognitive impairments (reviewed in Sasaguri and others 2017; Dawson and others 2018). Since the first generation of AD animal models, other FAD mutations (including presenilin 1 and 2 mutations) have been introduced, generating models that develop more aggressive pathological phenotypes, and the microtubule-associated protein tau has also been incorporated, generating models that better mimic the human disease (reviewed in Sasaguri and others 2017; Dawson and others 2018; Martini and others 2018). Furthermore, the technological advances of the past few decades in terms of genetic manipulations have allowed the generation of novel mouse lines in which a gene is overexpressed, knocked out, knocked in, or conditionally expressed or repressed temporally or even in specific cell types, allowing researchers to uncover the role of these cells in the onset and progression of AD (Dawson and others 2018; Garcia-Leon and others 2019; Myers and McGonigle 2019). Although it is undeniable that the generation of animal models of AD has been one of the most

significant developments in the field and that the current Tg lines have offered useful insight into the pathobiology of AD, the success that has been achieved in preclinical research has not been translated into the clinic for numerous reasons, including some inherent limitations of these models (reviewed in LaFerla and Green 2012; Drummond and Wisniewski 2017). Thus, the field needs to generate mouse models that better reflect the more common form of the disease, namely, sporadic AD (sAD), with the expectation that these new models can achieve better concordance with human cases, which, in turn, will improve the likelihood of translating basic biological discoveries into safe and effective clinical applications (King 2018).

In this review, we provide an overview of the current state of animal and cellular models of AD, with a central focus on transgenic mice, highlighting some of the major pathological mechanisms discovered through these lines of research and discussing the factors that may account for the discordance between preclinical and clinical results. Finally, we propose new approaches and lines of research for AD modeling that may be more suitable for preclinical therapeutic development.

Animal models of AD: What have we learned from them?

The existing variety of transgenic models in AD research has allowed researchers to dissect key disease-associated cellular and molecular processes, such as amyloid formation, tau pathogenesis, glia activation/inflammation, neurodegeneration, synaptic deficits, metabolic alterations, and the implications of genetic and comorbid risk factors (Figure 1). Here, we highlight several of these relevant and complex pathological processes that are critical in the progression of this brain disorder.

One of the first predominant paradigms in the understanding of AD was the cholinergic hypothesis, which posited an association between cholinergic dysfunction (degeneration of cortically projecting cholinergic neurons in the basal forebrain) and cognitive decline in AD (Hampel and others 2018; Hampel and others 2019). Following this hypothesis, the first group of models developed were based on chemical lesions of cholinergic pathways, showing that acetylcholine (ACh) is an important neurotransmitter in learning and memory-related processes (including acquisition, encoding, consolidation, extinction and retrieval processes) (reviewed in H. Ferreira-Vieira and others 2016; Hampel and others 2018). In AD mouse models, the disruption of choline acetyltransferase (ChAT),

nicotinic (nAChRs) and muscarinic acetylcholine receptors (mAChRs), and levels of ACh is similar to what is observed in human cases (Gil-Bea and others 2005; Medeiros and others 2013; Lombardo and Maskos 2015). These studies have provided critical insights for a better understanding of the implications of cholinergic alterations and the progression of AD pathology. Moreover, they have also led to the development of cholinesterase inhibitor-based therapies, which are currently the most prescribed drugs to alleviate cognitive symptoms in mild to moderate stages of the disease, as well as the recent agonists design of nAChRs and mAChRs (Verma and others 2018; Moran and others 2019).

The initial studies of the amyloidogenic hypothesis were conducted by intracerebral injection of Aβ in wild-type (WT) rodents, providing an important proof of concept of the pathological role of the amyloid peptide, including, the generation of fibrillar aggregates (Soto and others 1998). Further studies have shown that this type of approach also produces severe cognitive and synaptic impairment, as well as changes in the inflammatory response, insulin resistance, oxidative stress, among other pathological consequences (Ferreira and others 2018; Goswami and others 2020; Wang and others 2020). With the development of new genetic modification techniques transgenic mice expressing familial AD mutations were also developed in the 1990s, bearing mutations in human *APP*, *PS1* and *PS2* genes (reviewed in Sasaguri and others 2017). These amyloidogenic models were key to the investigation of Aβ processing, aggregation, toxicity and propagation. As models for tau-related pathology, transgenic rodents were also generated by overexpressing WT human tau or introducing human mutations in the microtubule-associated protein tau (*MAPT)* gene, increasing the severity of neurodegeneration (Ramsden and others 2005; Yoshiyama and others 2007). These tau transgenic models, unlike APP and presenilin mutants, are not based on familial AD mutations, since none of the FAD mutations identified until now affects the *MAPT* gene (reviewed in (Götz and Ittner 2008). While models exhibiting either amyloid deposition or neurofibrillary pathology have been useful for studying these two processes separately, the combined model provides insight into the interaction between these two hallmarks of AD. In this regard, one of the most relevant models, recapitulating both Aβ plaques and neurofibrillary tangles (NFTs), is the triple-transgenic 3xTg-AD line (Oddo and others 2003). This model shows early Aβ accumulation, starting at 3-4 months of age, and develops plaques from 6 months of age (Belfiore and others 2019). NFTs appear later in

the pathological progression, at approximately 12 months of age, and suggest a direct effect of Aβ on the neurofibrillary pathology. A later study using an Aβ immunotherapy approach in 3xTg-AD mice also supports this idea and shows that early Aβ treatment can reduce the level of hyperphosphorylated tau (Oddo and others 2004). Similar evidence has been reported in other mouse models expressing mutant APP and tau proteins, such as the TAPP model, obtained by crossing Tg2576 mice (bearing the Swedish mutation KM670/671NL in APP) with JNPL3 mice (bearing a P301L mutation in tau) (Lewis 2001). TAPP mice develop amyloid plaques at the same age as the Tg2576 model; however, TAPP mice show more neurofibrillary pathology than JNPL3 mice, mostly in the limbic system and olfactory cortex (Lewis 2001). Further studies have identified several putative mechanisms by which Aβ can trigger tau pathology by altering the levels of the C-terminus of heat shock protein 79-interacting protein (CHIP) or by acting on interleukin-1β (IL-1β) signaling (Oddo and others 2008; Kitazawa and others 2011). Recent evidence has shown that tau is a downstream target of Aβ in the mechanisms of cognitive and synaptic impairments in AD (reviewed in Forner and others 2017). Further studies have demonstrated that Aβ binds to the cellular prion protein (PrP^C) in the membrane of neurons, triggering a cascade that activates the nonreceptor tyrosine kinase Fyn, which is implicated in the phosphorylation of tau and facilitates tau-dependent cognitive impairments (Ittner and others 2010; Larson and others 2012; Li and Götz 2017). These studies have resulted in the design of a novel and specific inhibitor of Fyn kinase as a promising therapeutic agent to alleviate cognitive decline in AD patients. All these evidences from APP/tau models support the amyloid cascade hypothesis, corroborating the idea that Aβ is a primary trigger and tau is a downstream target (Selkoe and Hardy 2016).

Given the importance of genetic variants of immune-related genes such as the microglial triggering receptor expressed in myeloid cells 2 (TREM2) in sporadic AD, this field of research is now intensely focused on the glial inflammatory response (reviewed in Heneka and others 2015; Heneka and others 2018; Pimenova and others 2018; Kunkle and others 2019). Both amyloidogenic and some tau models have shown strong microglia and astroglia activation, and although it is not yet clear whether this glial response exacerbates or attenuates AD pathology, these models are helping researchers to dissect the role of immune cells in disease progression. Activated microglia were once classified as M1 (cytotoxic) and M2 (protective); however, the phenotypic diversity of activated

microglia transcends this simple binary polarization (Ransohoff 2016; Tang and Le 2016). Thanks to transcriptional single-cell RNA-seq studies in amyloidogenic models, a disease-associated microglia (DAM) gene profile was identified (Keren-Shaul and others 2017). This microglial molecular signature is present not only in AD models but also in multiple sclerosis and amyotrophic lateral sclerosis models. DAM microglia are characterized by the upregulation of genes related to glial activation and phagocytosis (i.e, *Apoe*, *Trem2*, *Cst7*, *Csf1*, *MHC-I* (*H2-d1*) and *MHC-II* (*Cd74*)) and the downregulation of homeostatic genes (i.e, *Tmem119*, *P2ry12 and Cx3cr1*). Although DAM activation is not fully understood, it appears to be a two-step process dependent on the expression of *TREM2* and *APOE*, among others (Keren-Shaul and others 2017). In order to elucidate the role of DAM microglia in AD pathogenesis, new amyloidogenic models with TREM2 deficiency or overexpression have been developed, as have APOE-based models, providing a critical understanding of the role of microglial cells in disease progression (Ulland and others 2017; Gratuze and others 2018; Zhong and others 2019). Likewise, several studies in 5xFAD mice suggest that activated microglia might be protective, working not only to phagocytose Aβ but also to compact amyloid fibrils and isolate plaques to reduce their local toxicity. Active microglia might also participate in the formation and aggregation of amyloid plaques, sequestering highly toxic Aβ oligomers from the brain parenchyma. Experiments with sustained depletion of microglia in 5xFAD mice have shown a decrease in amyloid deposition, supporting the role of microglia in the generation of plaques (Sosna and others 2018; Spangenberg and others 2019). However, activated microglia can also be detrimental, since Aβ activates Toll-like receptors and the inflammasome, triggering the release of proinflammatory cytokines and causing neuronal damage; thus, the microglial response may differ depending on the stage of disease, the brain region and the stimulus received (reviewed in Heneka and others 2018). Microglia are also able to modulate tau pathology. Many studies in tau transgenic mice suggest that neurofibrillary pathology spreads through synapses, since tau aggregates propagate between synaptically connected regions (Wu and others 2016; Wang and others 2017). Tau also spreads between unconnected regions, and microglia may be a key contributor factor of this pathway. A study performed by Ikezu's lab has shown that phosphorylated tau colocalizes with microglial cells and that microglia are able to spread tau via exosome secretion (Asai and others 2015), suggesting that microglial cells participate in tau propagation between neurons mediated by phagocytosis and exocytosis processes.

On the other hand, reactive astrogliosis is also observed in AD models, allowing a better understanding of the role of this cell type in AD pathogenesis (Perez-Nievas and Serrano-Pozo 2018). In an APP/PS1 model, the deletion of *Gfap* and *vimentin* in astrocytes causes an increase in the number and sizes of amyloid plaques and dystrophic neurites (Kraft and others 2013). These findings support the notion that astrocyte activation limits plaque growth and toxicity. Moreover, reactive astrocytes surrounding amyloid plaques can phagocytose dystrophic presynaptic terminals, both in APP/PS1 models and in AD brains, contributing to clear dysfunctional synapses (Gomez-Arboledas and others 2018). Initial transcriptomic studies (Liddelow and others 2017) proposed two functional phenotypes for reactive astrocytes in acute central nervous system (CNS) injury conditions: "A1", or the LPS-induced neurotoxic profile, and "A2", or the stroke-induced neuroprotective profile. However, reactive astrogliosis induced by Aβ or tau pathology acquires transcriptional signatures that do not fit this simplistic binary classification (Das and others 2020), suggesting greater functional diversity of reactive astrocytes during chronic neurodegeneration.

AD is often referred to as type III diabetes since brain insulin resistance is frequently seen in patients with dementia. Insulin enters the brain through the blood–brain barrier and carries out several important functions, such as modulating synaptic plasticity and improving memory function (reviewed in de la Monte 2017; Lyra e Silva and others 2019). Multiple transgenic AD models such as 3xTg-AD, APP/PS1 and Tg2576 have demonstrated that Aβ oligomers cause profound alterations in insulin signaling in the brains of these models (reviewed in Lyra e Silva and others 2019). These studies suggest that Aβ interacts with the insulin receptor, either directly by causing its disruption or indirectly by modulating tumor necrosis factor alpha (TNFα), leading to brain insulin resistance (reviewed in Lyra e Silva and others 2019). Moreover, recent evidence from animal models has suggested that tau pathology is also a key trigger of impaired insulin sensitivity and secretion (Gonçalves and others 2019), indicating that multiple pathological mechanisms may be responsible for the alteration of the insulin pathway in the brain.

Clinical discordance and limitations of the current models

At present, the available treatments for AD are limited to agents (several cholinesterase inhibitors and one NMDA antagonist) that temporarily improve cognitive symptoms without altering disease progression. Since the latest of them (memantine) was approved in 2003, hundreds of promising treatment candidates, mostly targeting the amyloid cascade, have been identified in animal models; however, successful translation from preclinical testing to human clinical trials has failed (at a rate of more than 99%) (Cummings and others 2020). This continuing failure to develop an effective AD treatment can be attributed, at least in part, to inappropriate choice of mouse models, including premature translation of results obtained in animal models that mimic only specific pathological features of human AD (Figure 2). In this context, a better understanding of the pathogenic mechanisms and the improvement of existing animal models are urgently needed to provide critical insights for therapeutic development.

Over the years, most potential drug candidates have been tested in transgenic models that overexpress one or multiple mutated genes related to FAD; however, some limitations should be taken into account with overexpression-based mouse models, since their pathological changes do not develop in a physiological manner (reviewed in LaFerla and Green 2012; Götz and others 2018). Regarding the APP sequence, although mice and humans share 97% of the full protein sequence (695 isoform), overexpression of mutated human APP is necessary in most cases to generate Aβ plaques in mice. Considering that APP695 (the most utilized isoform), is endogenously expressed in neurons, the use of different neuron-specific exogenous promoters (hamster prion protein, PDGFB or mouse Thy) is also needed to control robust and persistent expression (reviewed in LaFerla and Green 2012; Sasaguri and others 2017). Therefore, the phenotype observed in many transgenic models strongly depends on their specific design (selected FAD mutations, hAPP, or promoter and background strain chosen, among others; see www.alzforum.org/research-models), challenging the interpretation of downstream response to Aβ pathology (Figure 2). Furthermore, several exogenous promoters have sex-regulatory transcription factors that may alter the expression of the inserted genes; this is the case of the 3xTg-AD mice, that showed differences in term of Aβ and tau onset and progression between male and female mice (Carroll and others 2010). With this regard, it is also important to consider that pathological changes might occur as an artifact caused by overexpression of FAD genes or by APP cleavage fragments other than Aβ (reviewed in Drummond and Wisniewski 2017). Moreover, one or multiple copies of the transgene construct can be randomly integrated into endogenous gene loci, leading to

unknown phenotypic consequences (reviewed in Götz and others 2018). Hence, some phenotypes might be due to transgene overexpression rather than normal disease pathology, which hinders interpretation of the results (Figure 2). In order to address part of these limitations, new AD mice have been developed using a Knock-in strategy approach to express the inserted genetic mutations in a more physiological manner (Sasaguri and others 2017).

Another disadvantage of traditional transgenic AD mice is the influence of the specific strain background on the behavioral and molecular phenotypes (Figure 2). Mouse strain backgrounds can affect both APP processing and the age of amyloid accumulation onset (reviewed in Jankowsky and Zheng 2017). Importantly, recent studies have supported previous evidence that genetic diversity is an important factor modulating AD pathogenesis (Neuner and others 2019; Onos and others 2019). More specifically, these authors showed remarkable differences regarding amyloid deposition and plaque-associated myeloid response when analyzing different genetic backgrounds on the well-established 5xFAD and APP/PS1 lines. Overall, these findings suggest that wild-derived strains are most promising lines to model AD.

The postmortem AD brain is characterized by the presence of amyloid-beta accumulation together with neurofibrillary pathology. However, there are very few AD models that develop both protein pathologies, and even if APP models display moderate levels of hyperphosphorylated tau, none of them develop NFTs (Figure 2). In addition, endogenous mouse tau is unable to produce NFTs; therefore, overexpression of the MAPT gene is necessary for NFTs development (Kampers and others 1999). It should be taken into account that mutations in the MAPT gene are not found in AD but, in some cases, may cause frontotemporal lobar degeneration (FTLD) (Iqbal and others 2016). Moreover, the development of mutated tau in a non-physiological manner might interact with Aβ in a different manner than in human patients (Busche and Hyman 2020).

The main limitation in most of the existing transgenic models is the lack of extensive neuronal loss and brain atrophy, two key neuropathological features observed in all AD patients (Wirths and Zampar 2020) (Figure 2). While some models have shown a substantial degree of neurodegeneration, it is manifested only in very specific brain regions and mostly in aged animals (reviewed in Onos and others 2016; Drummond and Wisniewski 2017; Jankowsky and Zheng 2017). Since dementia is a clinical manifestation of neurodegeneration, this shortcoming must be considered when selecting animal models.

Anatomical and physiological differences between mice and humans species are also difficult to monitor. Major AD-related proteins, such as Aβ, tau or ApoE, differ between rodents and humans in their molecular and biochemical properties (i.e., more SDS-soluble amyloid plaques in rodents), the isoforms they produce (i.e., only 4R tau in mice and only one ApoE isoform similar to ApoE4 in mice) and their pathogenicity (i.e., rodent Aβ and tau do not aggregate) (reviewed in Drummond and Wisniewski 2017). Moreover, amyloidogenic Tg mice have failed to reproduce the diversity and complexity of amyloid plaques of AD patients (Dickson and Vickers 2001; Thal and others 2015; Condello and others 2018; Boon and others 2020), since most of these models mainly develop fibrillar neuritic plaques (Drummond and Wisniewski 2017).

Therefore, these rodent proteins (and downstream molecular pathways) may respond and interact differently, supporting the idea that phenotype effects in transgenic animals could be different from those observed in humans. In addition, immune components and the inflammatory response are distinct between animal models and human patients (discussed below). Thus, these variables should be taken into consideration when testing preclinical AD therapeutics, such as amyloid-clearing drugs.

Another major consideration is the choice of an appropriate time point in the lifespan of the animal to reproduce a human neurodegenerative disease, given that age represents the main risk factor in AD (Figure 2). For instance, Aβ pathology in human patients appears approximately two decades before cognitive dysfunction arises (Long and Holtzman 2019). In contrast, plaque onset and behavioral abnormalities can occur before 6 months of age in most APP-transgenic models. Since aging involves several biological processes (immune system and mitochondrial dysfunction as well as proteasome dysregulation) and conditions (vascular changes and comorbid pathologies including hypertension and diabetes), the brain environment in which pathological changes are occurring should be critically studied (Sun and others 2020).

Tg mice are intended to reproduce the protein aggregation pathologies found in AD brains. However, targeting the amyloid cascade has failed in clinical translation for decades, revealing that AD still has unsolved complexities beyond plaques and tangles (Selkoe and Hardy 2016). In this sense, several genes involved in innate immunity (i.e., *TREM2*, *CD33*) have been identified in recent years as risk factors for sAD, indicating that glial cells have a critical role in disease progression (Scheiblich and others 2020; Sims and others 2020). The current scenario, based on the findings in amyloidogenic models, posits a cytotoxic/proinflammatory role for activated microglia along with a

strong inflammatory response to Aβ accumulation (Heneka and others 2015; Hansen and others 2018; Heneka and others 2018). However, emerging data from human samples, alongside the identification of sAD risk factors associated with microglial function (*TREM2, CD33, CR1*, etc.) (Takatori and others 2019), reveal the existence of a weak immune system in AD patients rather than overactivation. In this sense, compelling evidence of AD-associated degenerative microglia has been reported (Sanchez-Mejias and others 2016; Davies and others 2017; Streit and others 2020; Swanson and others 2020; Shahidehpour and others 2021). Thus, this disparity between transgenic models and patients' immune system activation could be a major critical factor that explain the lack of clinical translational meaning between rodent and humans.

Finally, it should be noted that FAD cases correspond to less than 1% of the total AD cases, with most clinical trials being conducted in sAD patients (Long and Holtzman 2019). Most Tg models used in preclinical studies harbor FAD and/or FTLD mutations; therefore, they do not specifically model sAD (Figure 2). Since genetic studies in human patients have revealed several heritable risk factors associated with mechanisms other than Aβ and tau, including the immune system, cholesterol metabolism and synaptic function (reviewed in Sims and others 2020), all these data highlight the need to consider specific targeting of sAD-related genes when designing preclinical AD models. In addition, a better understanding of the multiple pathological mechanisms underlying AD progression and its modulation will be necessary to guarantee successful clinical translation.

Towards a new era of modeling: leaving behind classic FAD models

Knockins: A new generation of mouse models

Modeling AD using autosomal dominant familial mutations in Tg mice has been quite fruitful for an initial understanding of the basic pathogenic mechanisms involved in this neurodegenerative proteinopathy. Nevertheless, this approach has been insufficient for a complete clinical recapitulation of the predominant form of AD, the sporadic form. Consequently, these models have limited predictive value to test the effectiveness of novel treatments in patients. An illustrative example is the large number of compounds that successfully targeted Aβ pathology in preclinical assays but later failed in clinical validation phases. These unwelcome outcomes highlight the need to develop models that

more closely resemble the sporadic form of the disease (Cummings and others 2020) (Figure 3). A starting point to overcome the shortcomings of such an artificial and uncommon AD-like type was to develop mouse models expressing WT isoforms of human tau (htau mice) in a context free of endogenous tau (mTauKO) (Andorfer and others 2003; Andorfer and others 2005). However, these models generally showed a more limited tau pathology (late development of NFTs) than their mutated counterparts. Concerning β-amyloidosis, strains of *App* knockin (*App*-KI) mice were generated to drive humanized Aβ accumulation, aiming to avoid artifacts due to *App* overexpression. APP-KI mice, expressing human *App* with FAD Swedish (NL) and Iberian (F) mutations (App^{NL-F}) or with those two mutations as well as the Arctic mutation (App^{NL-G-F}), were conceived to better reproduce the slow progression of sAD, although this model contained multiple FAD mutations, making it a truthful model for familial disease. These mice show age-related memory impairment (at 18 months), Aβ42 overproduction, AD-like plaque formation including pyroglutamate Aβ, synaptic loss, gliosis and the expression of AD neuroinflammation-related genes (Saito and others 2014; Sasaguri and others 2017; Saito and Saido 2018). However, they do not reproduce neurodegeneration, so these mice may serve as models for preclinical stages. As a further step, De Strooper's laboratory has created several rodent models that express a humanized form of Aβ without any FAD mutation, resulting in a more representative model of sAD (Serneels and others 2020). Remarkably, there are virtually no signs of tauopathy in these *App*-KI models, which calls into question the reliability of the AD-like tau pathology exhibited by FAD models (Saito and others 2014; Serneels and others 2020). More recently, mice bearing the humanized *Mapt* gene (*MAPT*-KI) were crossed with the *App*-KI model. The resulting double-KI (dKI) mice showed neuronal loss together with an increase in phospho-tau levels compared to the single htau-KI (Saito and others 2019), reinforcing the idea about a close link between Aβ and tau pathologies in sAD (Figure 3).

Most recently, a new initiative created by the National Institute of Aging (NIA) has recognized the need to create novel animal models for this disorder and funded a new consortium, called Model Organism Development and Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD; www.model-ad.org), with the promise of enabling the generation, characterization and validation of the next generation of mouse models of late-onset Alzheimer's disease and stating general guidelines for preclinical testing and bringing together data and research results, among others. The rationale of this

consortium is to generate multiple humanized knockin lines to use as platforms to introduce several genetic risk variants commonly expressed in sporadic AD cases. It is hoped that this new program will help to fill some gaps in the standardization of modeling processes and increase the translatability of successful preclinical assays in the future.

Apolipoprotein E4 and genetic loci associated with sAD risk

Inheritance of ApoE4 is the strongest and most common genetic risk factor for sAD, increasing the odds up to 15-fold (in homozygosis) compared with the isoform ApoE3 (Serrano-Pozo and others 2021). Surprisingly, although the role of ApoE has been deeply investigated in classic FAD models, this pleiotropic molecule has received comparatively scarce attention as a therapeutic target. One of the reasons is the morphological and functional disparity between mouse (mApoE, one isoform) and human ApoE (hApoE, three isoforms). A proposal to overcome these drawbacks at modeling was the crossing of ApoE-KO or hApoE targeted replacement (ApoE-TR) mice with FAD- and tau-based mouse models, such as EFAD (5xFAD$^{-/+}$/ApoE-TR) or TE4 (P301S/hApoE4), respectively. The lack of mApoE in PDAPP mice reduces the Aβ burden, and the effect of hApoE on Aβ deposition seems to be isoform-dependent (Balu and others 2019). Specifically, among the ApoE isoforms, E4 is the strongest enhancer of pathological Aβ aggregation into oligomers/fibrils (E4>E3>E2) (Figure 3). Therefore, precluding or modulating the ApoE/Aβ interaction has potential significant therapeutic benefits. In addition, isotype loss of function seems to compromise Aβ clearance, as shown in PDAPP-ApoE4TR (Wisniewski and Drummond 2020). Concerning tau pathology, E4FAD mice showed an increase in phospho-tau, and further tauopathy and neurodegeneration were also reported in the TE4 model compared with TE2, TE3 and TEKO (P301S/ApoE-KO) mice (Balu and others 2019; Shi and others 2019) (Figure 3). However, a more recent work showed no direct impact of ApoE on tauopathy; rather, there was an indirect effect through the modulation of the microglial response (Shi and others 2019). Therefore, in contrast to Aβ pathology, more clarification is still needed on whether tau accumulation in AD is ApoE isoform-specific (Balu and others 2019; Yamazaki and others 2019).

Genetic polymorphisms underlying sAD are involved in a wide spectrum of functions, such as cholesterol/lipid metabolism (*CLU/APOJ*), endocytosis (*PICALM*), innate immunity and microglial function (*TREM2, CR1, CD33, ABCA7, etc.*) among others

(Andrews and others 2020; Sims and others 2020) and table 1. CLU, the second most common cerebral lipoprotein, seems to be involved in AD due to its metabolic and inflammatory role. Moreover, CLU deletion in PDAPP/ApoE-KO mice showed that this lipoprotein cooperates with ApoE in Aβ clearance (Yu and Tan 2012). However, a recent work using 5xFAD/CLU-KO mice reported that this protein might be involved in AD progression, increasing toxic Aβ pools, but only at the early stages (Oh and others 2019).

As mentioned above, the immune response has come to be recognized as one of the main elements of sAD risk; thus, immunomodulation appears to be a potential pharmacological target. Therefore, these genetic variants have been progressively incorporated or silenced in Tg mouse models. In fact, it was previously reported that homozygous loss-of-function mutations in TREM2 cause a rare form of dementia, Nasu-Hakola disease, pointing to a pivotal role of microglia in neurodegenerative conditions. Next, the TREM2 missense variant R47H was found to increase the risk for developing AD as much as the presence of the ApoE4 allele. Since then, other rare TREM2 variants conferring risk have been discovered, including R62H. It is not surprising that many groups quickly started to combine FAD-based mice with TREM2 variants/deletions to incorporate inflammatory dysfunction in an amyloidogenic context (Ulland and Colonna 2018). Deletion of TREM2 in the 5xFAD model increases amyloid burden, impairing microglial activation and survival. PS2APP/TREM2-KO mice exhibit an increase in amyloid and neuritic pathology (Meilandt and others 2020). In contrast, the upregulation of TREM2 in APP/PS1Δ9 ameliorates neuropathology and cognitive impairment (Jiang and others 2014). Hence, combining amyloidogenesis and TREM2 dysfunction helped to clarify that alterations in microglial activation (DAM) impair Aβ phagocytosis and plaque compaction (Ulland and Colonna 2018; Price and others 2020) (Figure 3). Regarding tau pathology, some groups reported that TREM2 silencing in the PS19 tauopathy model increased phospho-tau accumulation at early stages of the pathology, whereas others found no changes or reduced tau pathology and neurodegeneration in PS19 mice with the TREM2-KO or R47H variant (Leyns and Holtzman 2017; Gratuze and others 2020). Moreover, genetic variants of *LGALS3* (coding for Galectin-3 (Gal3), a ligand of TREM2) have recently emerged as another risk factor for AD. In fact, the 5xFAD/Gal3KO model shows a reduction in both Aβ pathology and behavior alterations (Boza-Serrano and others 2019). Interestingly, TREM2 interacts with lipoproteins such as ApoE and CLU (Yeh and others 2016). In turn, both ApoE and TREM2 interact with

and are involved in Aβ clearance. In this sense, ApoE was found to participate in the modulation of the microglial functional profile together with TREM2 (Wolfe and others 2019). As a recent example, APP/PS1Δ9/hApoE mice lacking TREM2 showed more severe alterations in microglial function within an ApoE4-isoform context (Fitz and others 2020) (Figure 3). Clearly, the combination of genetic sAD risk in murine FAD models yields very valuable information about the basic pathological mechanisms underlying these genetic risk factors. The new models generated from MODEL-AD using these genetic variants may provide more precise insight into the role of these genetic risks in the onset and progression of sAD pathology.

Making matters worse: comorbidities and dysbiosis

sAD is a very complex disease whose development and progression depend on many factors, such as comorbidities, diet, dysbiosis, lifestyle, and sex. This leads to the conceptual idea that the future treatment of AD may require customized multitarget therapy (Lee and others 2018; Saito and Saido 2018; Mauvais-Jarvis and others 2020). Therefore, it comes as no surprise that there is an increasing trend towards assays based on disease-modifying agents targeting pathways other than amyloid-β and tau, such as vascular damage and epigenetics (Cummings and others 2020). Therefore, there is also a need to develop reliable animal models able to (at least in part) reproduce such a variety of conditions for a better understanding of the implications of these risk factors in the progression of the disease.

Diabetes mellitus (DM) and related comorbidities such as obesity, hyper- or dyslipidemia and hypertension are important metabolic risk factors for dementia, including sAD (Dineley and others 2014; Baglietto-Vargas and others 2016; De Sousa and others 2020) (Figure 3). For example, obesity-dependent diabetes (db/db), streptozotocin (STZ)-induced diabetes, and high-fat diet-fed mice reproduce insulin resistance and AD-like neuropathology to some extent, including tau hyperphosphorylation (Bonds and others 2019; Kim and others 2019) without APP- or tau-based transgenesis. Combinations of type-1 or type-2 DM models with FAD lines have shown an increase in amyloidosis and/or tau hyperphosphorylation under DM conditions (Abbondante and others 2014; Mehla and others 2014; Guo and others 2016; Moreno-Gonzalez and others 2017; Trujillo-Estrada, Nguyen, and others 2019). Furthermore, several studies from our lab have reported that DM causes synaptic/memory impairment in a similar manner to AD

via a tau-dependent mechanism, suggesting a close relationship between these impactful diseases (Abbondante and others 2014; Trujillo-Estrada, Nguyen, and others 2019).

Another similarity between AD and DM is the activation of inflammatory pathways. The notion of neuroimmune communication, as the crosstalk between peripheral immunity and the neural system, is driving the scientific community to a more holistic conception of AD, conditioned by brain-body interactions, that is being translated to new paradigms of modeling and therapies. In this regard, the role of the "microbiota-gut-brain axis" is receiving increasing attention in the context of aging and neurodegenerative disorders (reviewed in Fang and others 2020) (Figure 3). Microbiota modifications in mice modulate the neuroimmune system, altering glial morphology and function. Importantly, metabolic risk factors and comorbidities such as DM, obesity or a high-fat diet can lead to dysbiosis, which, in turn, contributes to the perpetuation of these conditions. This has been convincingly demonstrated by the development of obesity in mice receiving microbial transplantation from obese patients, even as control mice remained healthy (Ridaura and others 2013; Levy and others 2017). Interestingly, amyloid deposition was modulated by the deleterious effect of antibiotics on the gut microbiome of the APP/PS1ΔE9 model. In addition, the colonization of germ-free APP-based mice with microbiota from APP mice leads to an increase in cerebral amyloidosis in comparison to those colonized with microbiota from WT mice. The P301L mouse model exhibits gut microbiome alterations with aging (Sun and others 2019). Moreover, a recent work with ApoE-TR mice showed evidence of an interrelation between this potent sAD risk factor and gut microbiome profiles (Fang and others 2020). Therefore, murine models are effective for proof-of-concept studies, although it seems there is still a long way to go in deciphering the underlying mechanisms connecting AD and dysbiotic microbiome configurations.

Many other medical (cerebrovascular disease, traumatic brain injury, infections), psychological (stress, anxiety or depression) and environmental conditions are important AD risk factors (reviewed in Edwards and others 2020; Moreno-Gonzalez and others 2020) but go beyond the aim of this review. Interestingly, not only are some hallmarks reproduced by FAD- and tau-based models, but they can also be modeled after being induced in Tg or WT mice to unravel the impact of all of them on AD progression (Baglietto-Vargas and others 2015; Kosel and others 2020).

Looking for more alternatives: Modeling across other mammalian species

In comparison to mice, rats have been less preferred for AD modeling mainly due to technical reasons, although they show several advantages, such as a larger brain size and greater resilience to stress. Since 2004, several Tg rat models of FAD have been created, reproducing intra- and extracellular Aβ buildup, tau hyperphosphorylation and cognitive impairment (reviewed in Götz and others 2018). The expression of truncated htau in rats led to the reproducibility of AD-like neurofibrillary pathology (Götz and others 2018). ApoE-KO rats are also available and are better models to mimic the speed progression of atherosclerosis than mice (Lee and others 2010).

Taking into consideration the contribution of longevity and aging, other long-lived vertebrates and nonhuman primates (NHP) can be considered natural models of AD (Gunn-Moore and others 2018). However, many mammal species usually fail at reproducing both plaques and tangles together with cognitive deficits (Devinsky and others 2018). For instance, aged dogs exhibit amyloid deposition, CAA and memory decline but not neurofibrillary changes (Youssef and others 2016). Interestingly, both plaques and tau pathology (NFT or related changes) have been reported to coexist in different cerebral regions from cheetah, aged striped dolphin (*Stenella coeroleoalba*), pinniped species (sea lion, seals and walrus) (Moreno-Gonzalez and Soto 2012; Gunn-Moore and others 2018; Takaichi and others 2021) and some NHP (Moreno-Gonzalez and Soto 2012; Devinsky and others 2018; Gunn-Moore and others 2018). In this sense, since the aminoacidic sequence of Aβ peptide is identical between marmosets (*Callithrix jacchus*) and humans, the recently generated PSEN1-ΔE9 marmoset might be a promising NHP model of FAD (Sato and others 2020). Finally, it is important to mention that all NHP are homozygous for ApoE4 that, on the other hand, functions similarly to ApoE3 because of a different amino acid at position 61 (Wisniewski and Drummond 2020). Further analysis regarding this point might yield new data concerning the role of ApoE in AD.

Beyond amyloid and tau. New approaches for modeling AD: use of human induced pluripotent stem cell-based models of AD

As discussed throughout the manuscript, animal models have notably contributed to decipher altered mechanisms in AD pathology. Nonetheless, the lack of translationality of therapies tested in preclinical studies to the clinical practice, indicate that important discordances between models and patients pathology exist, stressing the need of using

complementary models depending on the question to address. In this regard, the employment of cell culture lines have helped to elucidate, for example, how propagation of amyloids as Aβ or Tau occurs and the molecules and pathways implicated (Vaquer-Alicea and Diamond 2019).

Nevertheless, a reliable AD modeling requires the employment of complex systems able to recreate several aspects of the disease. In addition, notable biological and intrinsic differences exist between human and murine cells, which makes the first desirable for modeling human disease. Scarce access to human viable brain samples and limited expansion of brain cells *ex vivo* have strongly limited the development of AD patient-derived models to study the disease. However, this scenario changed with the discovery of human induced pluripotent stem cells (hiPSCs) in 2007, which opened a new paradigm in disease modeling, especially for neurodegenerative diseases. Human iPSCs are generated by the reprogramming of somatic cells, usually skin fibroblasts or blood leukocytes, towards a pluripotent stem cell (resembling embryonic stem cells) that can divide almost indefinitely and give rise to nearly any cell type in the body once subjected to proper molecular cues, similar to what occurs *in vivo* (Garcia-Leon and others 2019; Garcia-Leon and others 2020). Thus, over the past few years, several studies have used hiPSC-derived neurons and glial cells for disease modeling, assessment of pathogenic pathways implicated in AD and evaluation of candidate drugs in human-derived platforms in an effort to better assure the translation of the discoveries towards the cure of AD and reduce the intrinsic limitations that most AD models have (Figure 4).

hiPSC-derived neurons and glial cells for modeling AD

The amyloid hypothesis is still the most accepted thesis to explain the pathological cascade of AD (Selkoe and Hardy 2016), which focuses on neurons as the main cell type implicated in the disease. Several studies have generated hiPSC-derived neurons from familial AD cases to assess whether aberrant accumulation of Aβ can be reproduced *in vitro*, its functional consequences and/or the suitability of the platform for drug assessment. Most of these studies reported that mutant neurons secreted more long Aβ peptides ($A\beta_{40}$ and $A\beta_{42}$), presented increased activation of GSK3β and hyperphosphorylation of tau and showed a stressed phenotype, alterations that could be reversed after specific blockade of APP processing (Yagi and others 2011; Israel and

others 2012; Muratore and others 2014; Arber and others 2019; Lee and others 2019). On the other hand, few studies have evaluated the phenotypes shown by neurons derived from sporadic cases bearing *APOE4,* and they have reported an increased production of long Aβ peptides, a higher tau phosphorylation level and an altered neuronal maturation capacity (Meyer and others 2019) (Figure 4). Among glial cells, astrocytes are one of the most abundant cells present in the CNS of mammals and are essential for brain development and homeostasis (reviewed in Trujillo-Estrada and others 2019). Several groups have developed specific protocols for the generation of astrocytes from hPSCs, mimicking the *in vivo* ontogeny of these cells, which share a common progenitor with neurons (Krencik and others 2011; Shaltouki and others 2013; Santos and others 2017; TCW and others 2017). Human PSC-derived astrocytes from FAD patients presented increased expression of long Aβ peptides, which led to higher cellular stress and altered cytokine secretion after stimulation, with defective support to neurons (Oksanen and others 2017) (Figure 4). In astrocytes derived from sAD with the *APOE4* genotype, researchers have reported an altered phenotype, such as reduced cell volume and mislocalization of astrocytic markers and altered inflammatory response, suggesting that these astrocytes presented a stunted state, which led to poor neuronal support (Zhao and others 2017). Oligodendrocytes (OLs) are highly specialized glial cells that produce myelin within the CNS, allowing proper and fast conduction of electrical signals and providing metabolic support (Goldman and Kuypers 2015). The role that OLs play in AD was widely unknown until a recent study using spatial transcriptomics and in situ sequencing. De Strooper's laboratory reported that the expression of oligodendrocytic genes increases in the vicinity of Aβ plaques at the beginning of the pathology and dramatically decreases at advanced stages, responses that vary within different brain regions, partly because of differences in the Aβ load among them. In addition, they reported an oligodendrocyte Aβ-reactive state, suggesting that, together with activated astrocytes and microglia, OLs are clearly part of the multicellular inflammatory environment linked to Aβ plaques (Chen and others 2020) (Figure 4). These studies suggest that OLs may also play an important role in the inflammatory processes that occur in AD pathology, but we are far from a full understanding of the role that OLs play in AD pathology.

Microglia are innate immune cells of the brain that examine the brain parenchyma and react to damage to maintain brain homeostasis. In AD brains, activated microglia

accumulate around Aβ plaques, suggesting that they may have an important role in Aβ phagocytosis and in plaque compaction, and notably dysfunctional/degenerative microglia have been reported in human AD (Sanchez-Mejias and others 2016; Navarro and others 2018). Only recently has the developmental origin of microglia been revealed, which has facilitated the production of specific protocols to generate hiPSC-derived microglia. These protocols generate microglia-like cells that resemble human primary microglia able to phagocytose, secrete cytokines in response to stimuli and undergo calcium transients (Muffat and others 2016; Abud and others 2017a; Douvaras and others 2017; Haenseler and others 2017; Pandya and others 2017; Takata and others 2017; McQuade and others 2018). hiPSC-derived microglia are able to phagocytose both Aβ and tau oligomers both *in vitro* and *in vivo* and undergo important gene expression changes after exposure to these pathological insults (Abud and others 2017a; McQuade and others 2018) (Figure 4). In addition, *in vivo* matured hiPSC microglia expressed genes associated with AD, and when encountered with Aβ plaques, they acquired a distinctive phenotype resembling the DAM, with specific gene alterations that are unique to human microglia in contrast to murine microglia, suggesting that this system is appropriate for modeling AD (Hasselmann and others 2019). As the *TREM2 R47H* variant increases the risk of sporadic AD 3- to 4-fold, few studies have examined this using hPSC-derived microglia. Mutant microglia presented impaired survival and reduced phagocytic capacity of apoptotic bodies and Aβ plaques (Claes and others 2018). Overall, these previously described studies have been helpful for gaining insight into the role of neuronal and glial cells in AD pathology, and further studies in this field are needed to discover the novel underlying mechanisms by which these cells seem to play a critical role in AD.

hiPSC-derived 3D cultures and brain organoids for modeling AD

The generation of individual brain cell types from hPSCs has long been demonstrated. Nonetheless, as outlined above, the field lacks the ability to generate more complex structures reminiscent of the human brain to study neural mechanisms and disease modeling. In 2013, Lancaster and collaborators developed a 3D culture of neural cells that matured and formed a 3D structure, which served as a model for human-specific brain diseases poorly recapitulated by animal models (Lancaster and others 2013). This approach may be relevant for AD, as 3D cultures allow a higher maturation of neurons and glial cells and a higher retention of secreted molecules by the system, allowing more

credible Aβ and tau accumulation than monocultures. Using human neural progenitors overexpressing FAD mutations and cultured in 3D, the Tanzi laboratory reported the generation of Aβ plaques in culture, which led to the presence of tau aggregates (Choi and others 2014). Treatment with gamma- or beta-secretase-specific inhibitors reduced the levels of Aβ and, remarkably, attenuated tau pathology. On the other hand, a reduction in tau phosphorylation had no effect on Aβ accumulation (Kwak and others 2020). The group further optimized the system by including hPSC-derived microglia to introduce the inflammatory component into the system. They found that microglia migrated to the core of the culture formed by neurons and astrocytes and acquired a reactive and inflammatory phenotype only in cultures overexpressing FAD *APP* variants, which led to neuronal toxicity and astrogliosis. Their study is, to date, the only study using a 3D *in vitro* model able to reproduce the main pathological hallmarks AD, such as Aβ and tau pathologies and neuroinflammation (Park and others 2018). All these studies performed using brain organoids suggest that 3D culture may be a better system to reproduce AD *in vitro*, as it supports further cell maturation and allows a retention within the matrix of secreted proteins, enhancing the aggregation of Aβ and tau, better reflecting the *in vivo* situation and allowing the study of the relationship between Aβ and tau pathologies (Figure 4). However, this tool requires further optimization to reduce variability and cell composition, as most of the generated brain organoids lack microglia and oligodendrocytes, which makes them less than an optimal system for modeling the human brain.

hiPSC-derived neuron and glial cell xenotransplants in AD models

AD is characterized by a complex and still understood pathology in which all brain cell types participate. Therefore, in order better assess the functionality and features of hPSC-derived cells, their interactions with the other brain cell types should be tested in the context of AD. In addition, hPSC-derived brain cells are characterized by presenting mostly a fetal/immature phenotype, which impedes the full assessment of the mature functionality of generated cells. For these reasons, some groups have performed transplantation of hPSC-derived neuronal and glial cells into immunodeficient mouse brain parenchyma presenting AD pathology. De Strooper's group first described the transplantation of hPSC-derived neurons into the brains of *APP*/*PS1* mutant NOD-SCID mice, finding that transplanted neurons expressed 3R and mature 4R tau isoforms,

presented tau hyperphosphorylation and associated neurodegeneration, interacted with endogenous Aβ plaques and associated with reactive astrocytes and microglia, helping to decipher the AD-associated mechanisms affecting neuronal functioning (Espuny-Camacho and others 2017).

As hPSC-derived microglia present an immature phenotype when generated *in vitro* and play a key role in AD pathology, few groups have evaluated whether increased microglial maturity can be achieved in an *in vivo* environment and how they react to AD pathology. The Blurton-Jones group first transplanted hPSC-derived microglia into immunocompromised AD mice, finding that transplanted cells presented a similar phenotype and functioning as endogenous microglia, as they were able to respond to Aβ plaques (Abud and others 2017b; McQuade and others 2018). Next, they further characterized the transcriptome and functionality of hPSC-derived microglia when transplanted into AD mice, reporting that transplanted cells better resembled primary human microglia compared to *in vitro* cultured cells, performed homeostatic functions, responded to injury, and reacted to Aβ plaques with a differential transcriptome signature compared to murine microglia, suggesting that these procedures could be better used for assessing the role of human microglia in AD (Hasselmann and others 2019). Recently, they evaluated the responses of both WT and *TREM2* KO hPSC-derived microglia in an *in vivo* environment and found that *TREM2* deletion reduces the survival, phagocytosis and chemotaxis of microglia, resulting in an impaired response to Aβ plaques *in vivo*, with a loss of DAM responses (McQuade and others 2020). Similarly, Mancuso and colleagues reported that hPSC-derived microglia survive and integrate in the mouse brain and mimic primary human cells at the transcriptome level, with hPSC-derived and host mouse microglia displaying a divergent response to oligomeric Aβ (Mancuso and others 2019). These emerging studies highlight that transplantation of hPSC-derived neural cells into an *in vivo* AD environment may better reveal pathways and functions altered in AD patients, but further studies are needed to accurately address this.

Concluding remarks

Current Tg AD models have provided critical mechanistic insights shedding light on the ways in which multiple pathological processes occur in this devastating illness.

Moreover, these models have been used to decode the role and involvement of multiple risk factors in the onset and progression of AD. However, they present multiple limitations, reducing their similarity to human cases and the likelihood of developing effective clinical therapeutic interventions. We hope that the shortcomings of the existing models can be addressed with the new lines generated by the MODEL-AD consortium and that these humanized platforms provide more predictable outcomes for the sporadic form of this neurodegenerative disorder. The combination of these new animal lines with hiPSCs, organoids and chimeric models (xenotransplants of hiPSCs in AD models) may provide better consistency with AD cases, and promising clinical success may be achieved for this disease in the near future.

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Figure legends

Figure 1. Discovery of the main pathological processes underlying AD by using transgenic rodents. Rodent models have been used to shed light on many pathways and

mechanisms implicated in AD pathogenesis, such as Aβ and tau pathology, inflammatory processes, cell loss, synaptic dysfunction, metabolic alterations and the implications of comorbid and genetic factors.

Figure 2. Main pathological differences between AD patients and classical transgenic models. The high failure rate in clinical translation may be due to the inherent limitations of overexpression-based mice, the most important of which are listed in this illustration.

Figure 3. Old and new ways of modeling of AD. The brains of sAD patients present amyloid neuritic plaques, NFTs, synaptic/neuronal loss and gliosis. AD development has also been related to age-associated microglial dysfunction/degeneration. Classical APP-based FAD models have usually exhibited excessive amyloid deposition and disproportionate glial response compared to AD patients. Even though these mice accumulate hyperphosphorylated tau (phospho-tau) within dystrophic neurites, they do not generate NFTs. Only Tg mice bearing human MAPT with AD-unrelated mutations (Mtau-Tg) reproduce tangle formation. Plaques and NFTs are only found when FAD and MAPT mutations are combined in the same animal. With the aim of better imitating sAD, multiple combinations of mouse models are emerging. Knockin (KI, green) models consist of mice expressing humanized Aβ (*APP*-KI), tau (*MAPT*-KI) or both (dKI). AD and *APP/MAPT*-dKI deposits are usually more similar than plaques shown by FAD-based mice. Moreover, dKI mice exhibit a higher accumulation of phospho-tau than single *MAPT*-KI mice. However, MAPT expression does not affect plaque burden. The severity of both amyloid and tau pathology is differentially affected by the expression of human ApoE isoforms (orange; Epo4>E3>E2), involving hampered Aβ clearance. TREM2 silencing (blue) led to alterations in microglial phagocytosis and plaque-compaction capacity, giving rise to a higher neuritic and amyloid burden within an FAD context. The glial response is downregulated in Mtau-Tg/TREM2KO mice, but the role of TREM2 in tauopathy remains unclear due to conflicting results. Finally, plaque size increases earlier in the FAD background when TREM2-KO and hApoE4 expression are combined (brown), and the expression of DAM genes is differentially influenced by the ApoE isoform. In addition, a higher likelihood of developing sAD is associated with physiological and psychological comorbidities as well as lifestyle. All of these risk factors can be reproduced using models of diabetes, a high-fat diet or dysbiosis (among others), which, in turn, can be crossed with all the previous ones, enabling the study of both the basic mechanisms and customized novel therapies.

Figure 4. Summary of the methodology employed for the derivation of neural cells and brain organoids from hPSCs. The main phenotypes encountered in neural cells and brain organoids derived from iPSCs of AD patients are depicted. *hPSCs: human pluripotent stem cells; iPSCs: induced pluripotent stem cells; NPCs: neural precursor cells; OPCs: oligodendrocyte precursor cells; MPs: myeloid precursor cells; OPs: organoid precursors; SB: SB431542; LDN: LDN193189; CNTF: Ciliary Neurotrophic Factor; LIF: Leukemia inhibitory factor; RA: Retinoic acid; Shh: Sonic hedgehog; PDGFa: platelet-derived growth factor alpha; T3: triiodothyronine; NT-3: neurotrophin 3; FGF2: fibroblast growth factor 2; IL-3: interleukin 3; Il-6: interleukin 6; IL-34: interleukin 34; TGFb: Transforming Growth Factor beta; CX3CL1: chemokine (C-X3-C motif) ligand 1; Aβ: amyloid-beta; AD: Alzheimer´s disease.*

Table 1. Validated AD genomic risk factors. Gene nomenclature, common name, expression pattern and gene function for the most frequent GWAS related to AD. For review see (Cuyvers and Sleegers 2016; Robinson and others 2017; Andrews and others 2020).

Gene nomenclature	Common name	Expression pattern	Gene function	Reference
ABCA7	ATP-binding cassette sub-family A member 7	Microglia, neurons	Immune system, lipid metabolism	(Hollingworth and others 2011; Steinberg and others 2015; Bellenguez and others 2017)
APOE4	Apolipoprotein E4	Astrocytes, microglia	Cholesterol/ lipid metabolism	(Strittmatter and others 1993)
BIN1	Bridging integrator 1	Neurons, astrocytes, microglía	Synaptic function, endocytosis, trafficking, immune response, calcium homeostasis, apoptosis.	(Chapuis and others 2013; Tan and others 2013; Taga and others 2020)
CD33	Sialic acid-binding immunoglobulin-like family (siglec-3)	Myeloid cell receptor expressed in microglia and macrophages	Immune system, synaptic function, Aβ clearance	(Ulyanova and others 1999; Hollingworth and others 2011)
CLU	Clusterin or Apolipoprotein J	Neurons, astrocytes	Cholesterol/lipid metabolism, immune system	(Bell and others 2007; Harold and others 2009; Lambert and others 2009; Schürmann and others 2011; Herring and others 2019)
CR1	Complement receptor type 1	Microglia, astrocytes	Immune system	(Lambert and others 2009; Fonseca and others 2016)
PICALM	Phosphatidyl inositol binding clathrin assembly protein	Neurons, astrocytes	Synaptic function, endocytosis	(Lambert and others 2009; Parikh and others 2014)
SORL1	Sortilin-related receptor 1	Neurons	Intracellular vesicular sorting of amyloid precursor protein	(Böhm and others 2006; Rogaeva and others 2007; Pottier and others 2012; Bellenguez and others 2017)

TREM2	Triggering receptor expressed on myeloid cells 2	Microglia	Immune system	(Guerreiro and others 2013; Jonsson and others 2013; Bellenguez and others 2017)

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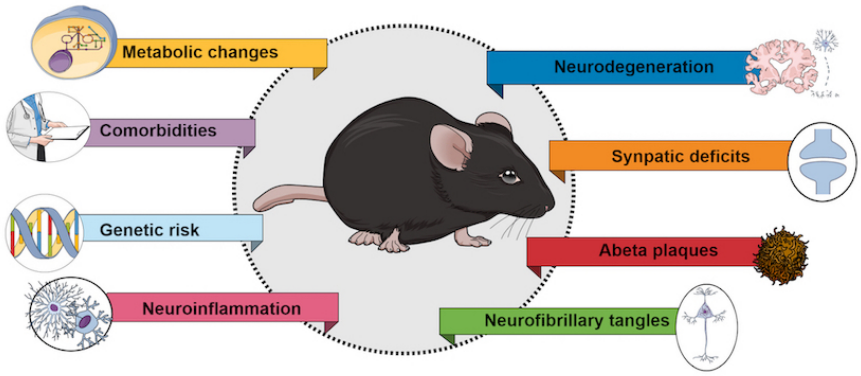

Discovery of the main pathological processes underlying AD by using transgenic rodents

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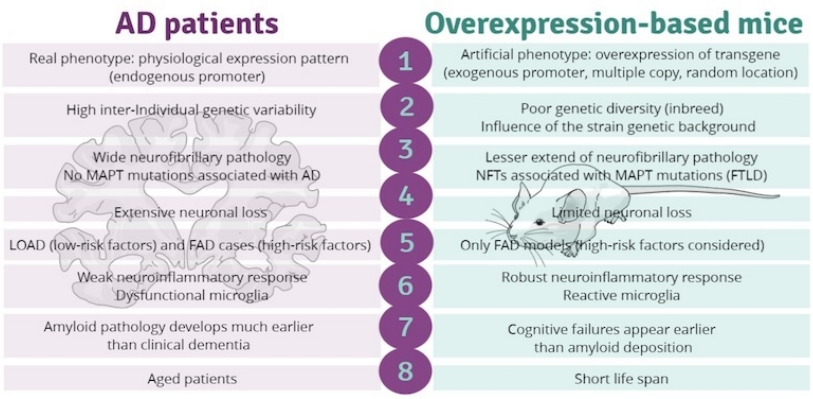

Main pathological differences between AD patients and classical transgenic models

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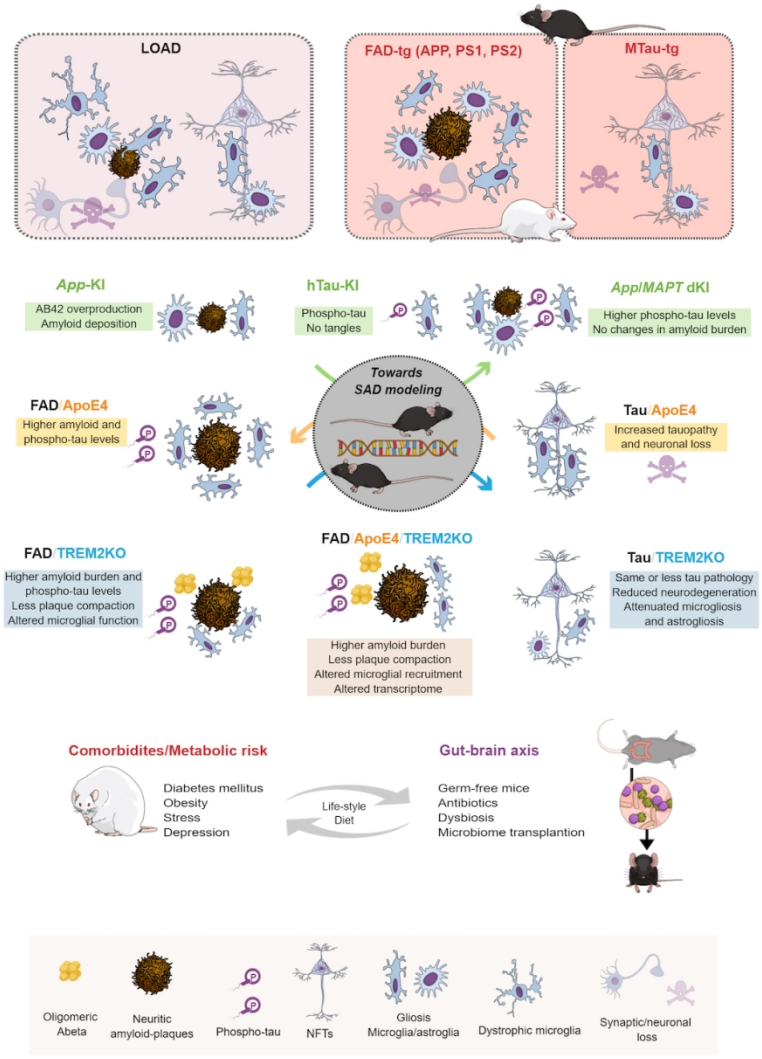

Old and new ways of modeling of AD

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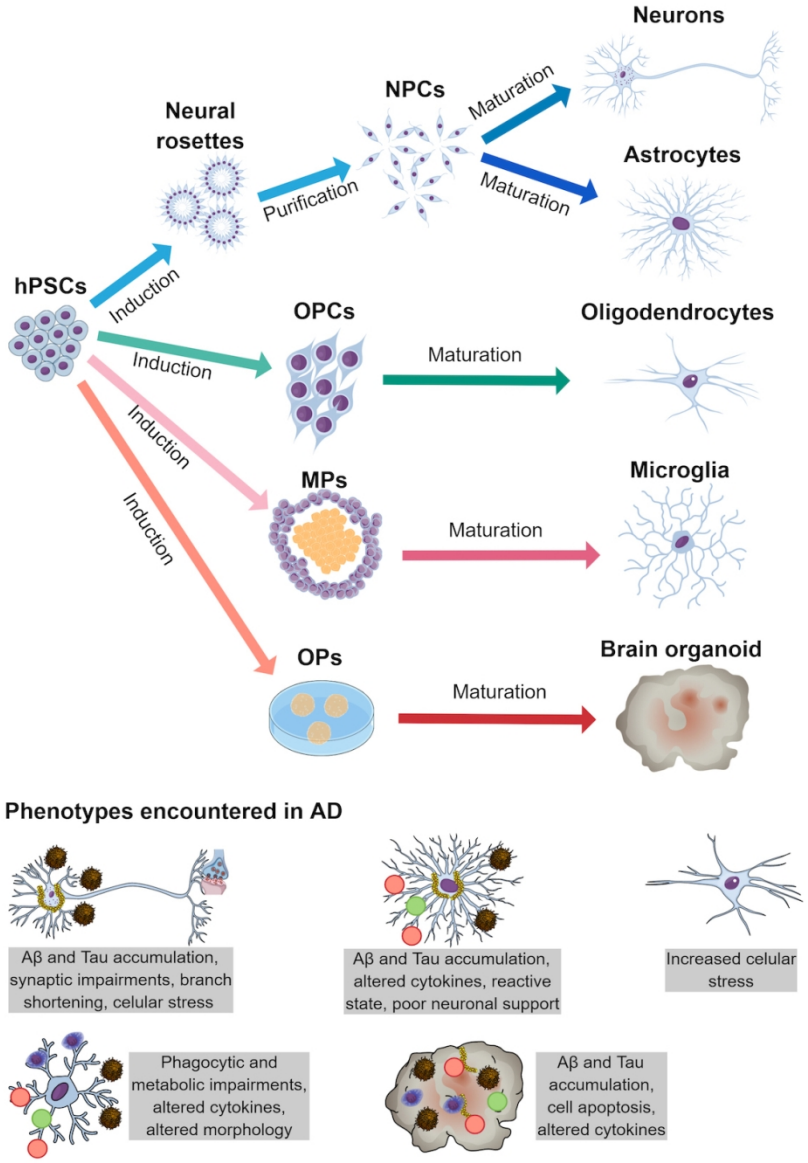

Summary of the methodology employed for the derivation of neural cells and brain organoids from hPSCs

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