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Review

Modeling Alzheimer's disease with human iPS cells: advancements, lessons, and applications

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ABSTRACT

One in three people will develop Alzheimer's disease (AD) or another dementia and, despite intense research efforts, treatment options remain inadequate. Understanding the mechanisms of AD pathogenesis remains our principal hurdle to developing effective therapeutics to tackle this looming medical crisis. In light of recent discoveries from whole-genome sequencing and technical advances in humanized models, studying disease risk genes with induced human neural cells presents unprecedented advantages. Here, we first review the current knowledge of the proposed mechanisms underlying AD and focus on modern genetic insights to inform future studies. To highlight the utility of human pluripotent stem cell-based innovations, we then present an update on efforts in recapitulating the pathophysiology by induced neuronal, non-neuronal and a collection of brain cell types, departing from the neuron-centric convention. Lastly, we examine the translational potentials of such approaches, and provide our perspectives on the promise they offer to deepen our understanding of AD pathogenesis and to accelerate the development of intervention strategies for patients and risk carriers.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder, affecting up to one third of the population over age 85 (reviewed in Querfurth and LaFerla, 2010). Clinical symptoms include progressive dementia that occurs sporadically in the majority of cases, with no clear genetic inheritance or familial influence. Today, more than 100 years after the first diagnosis, there are still no effective treatments or prevention strategies (reviewed in Hardy, 2006). Studying the complex and lengthy pathogenesis of AD is challenging at all levels, ranging from molecules to behaviors, and is exacerbated in particular by the lack of reliable models that can capture human brain-specific processes. For one, the reliance of AD research on animal models limits our capability to faithfully recapitulate disease development in humans. The differences in rodent models and human neurons are reflected by the low success rates of clinical trials that were based on initial tests in mice (Gotz et al., 2018). Human brain cells are markedly different from rodent neurons (Choi et al., 2016; Wang et al., 2018a), astrocytes (Oberheim et al., 2009; Zhang et al., 2016) and microglia (Smith and Dragunow, 2014). Human-specific genotypes are often not completely captured by model organisms; even with well-characterized human AD mutations, transgenic animals fail to exhibit some core pathological features of AD, such as extensive neuronal loss or distinct neurofibrillary tangles (reviewed in Sala Frigerio and De Strooper, 2016).

In light of the recent breakthroughs in generating the major types of human brain cells by differentiating pluripotent stem cells or directly converting somatic cells, the *in vitro* models based on human induced cells have emerged as a precise and robust approach to studying AD pathogenesis in the context of human cell biology. Here, we first review the latest discoveries from pathological and genetic studies. These have led to several proposed mechanisms beyond the prevailing amyloid cascade hypothesis, such as neuroinflammation and vascular dysfunction (Fig. 1). We also summarize the knowledge and insights gained from recent studies employing induced human neurons, microglia, astrocytes, oligodendrocytes, and endothelial cells (Table 1). We then discuss the progress in high-throughput screens for therapeutic molecules and disease modifiers using human induced brain cells. Finally,

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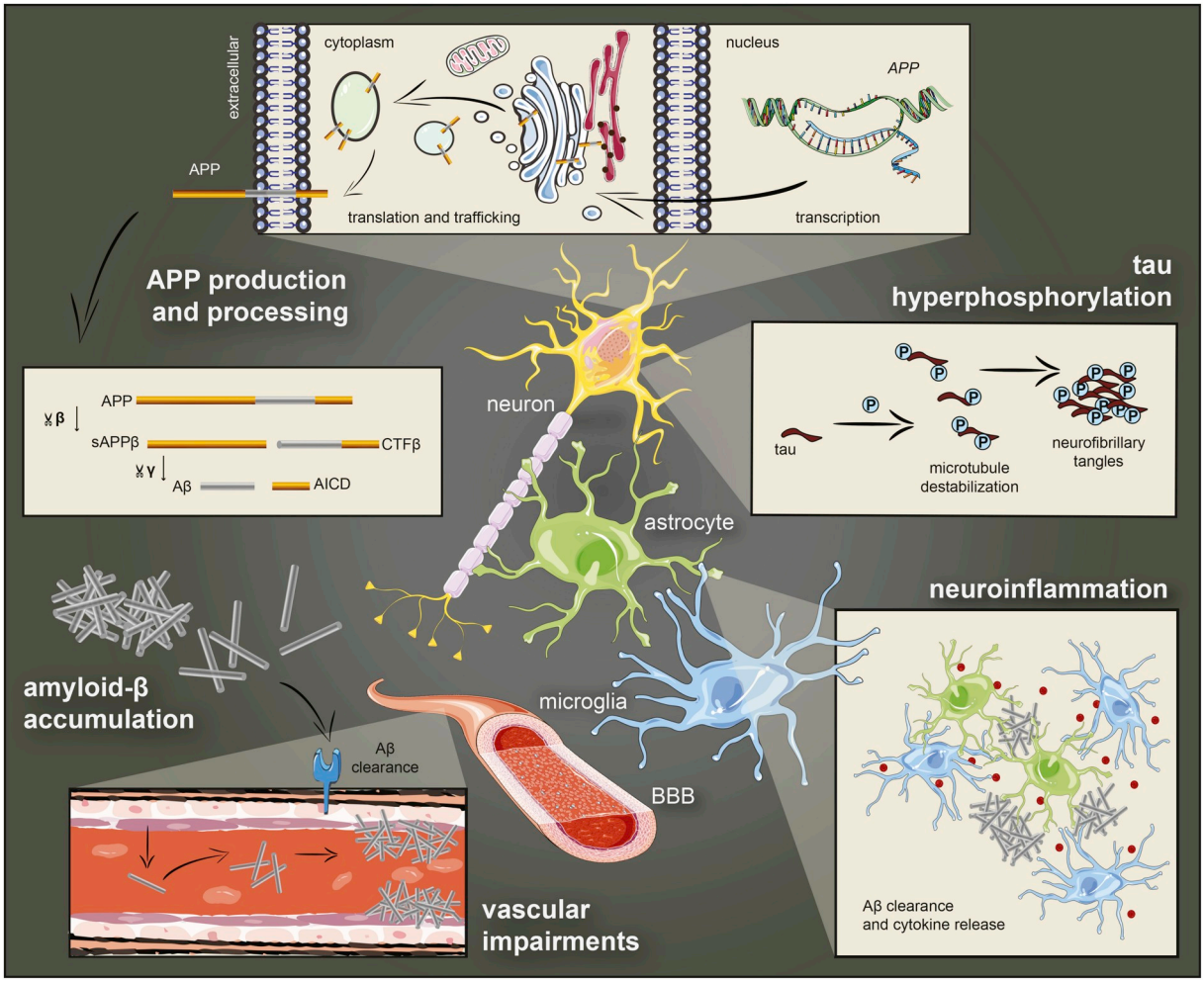

Fig. 1. Pathophysiology of Alzheimer's disease (AD)
The pathological hallmarks of AD are extracellular amyloid plaques and intraneuronal neurofibrillary tangles, whose building blocks are amyloid-β (Aβ) peptides and phosphorylated tau, respectively. Aβ is a proteolytic fragment of transmembrane amyloid precursor protein (APP) after cleavages by β- and γ-secretases. Tau is a brain-specific, axon-enriched microtubule-associated proteins and phosphorylated by an array of kinases. The other major pathological features, such as neuroinflammation and vascular dysfunction, contribute to and are reciprocally affected by the formation of plaques and tangles in AD development.
The major steps of Aβ biogenesis, including APP gene transcription, protein trafficking and processing on endosome membrane, have been heavily implicated in AD pathogenesis. The proposed mechanisms mediating Aβ clearance, including engulfment by brain immune cells microglia and uptake by receptors important for lipid metabolism, are found dysregulated in AD brains as well. Recently, several large-scale genome-wide searches for risk genes have substantially progressed our understanding about pathogenetic mechanisms for AD. The confirmed hits indeed support cellular functions tied to AD pathophysiology: 1) lipid metabolism, e.g. APOE, CLU and LRP1, 2) inflammation, e.g. TREM2, CD33 and CR1, and 3) endocytosis, e.g. PICALM, BIN1 and SORL1. The linkage of risk genes to pathophysiological features leads to a comprehensive view based on coordination among different brain cell types, going beyond the classic amyloid cascade hypothesis and a neuron-centric convention. Images are modified from Servier Medical Art by Servier under a Creative Commons Attribution 3.0 Unported License.

we provide our perspectives on how these conceptual and technical advances may be applied to build better "AD-in-a-dish" models and to inform AD pathogenesis and treatment.

2. Pathophysiology of Alzheimer's disease

The macroscopic changes of AD brains are characterized by parenchymal atrophy and enlarged ventricles, due to progressive neuronal loss beginning in and spreading from the entorhinal cortex in the medial temporal lobe (Convit et al., 1993; Gomez-Isla et al., 1996). These gross changes in relevant brain regions for memory and executive function correlate with AD symptoms (Delbeuck et al., 2003; reviewed in Vinters, 2015). However, the reasons why some brain regions are more vulnerable to neuronal loss compared to other regions remains unknown. Associated microscopic changes in neurons occur at the synaptic level. Accelerated loss of synapses relative to neuronal death and decreased ability to perform long-term potentiation are viewed as the pathological substrates of dementia (Jo et al., 2011; Kessels et al., 2010; reviewed in Sheng et al., 2012). Overall, the histopathological findings across affected brain regions in AD can be categorized into four central mechanisms that we will elaborate in the following sections: amyloid plaques (amyloid-β accumulation), neurofibrillary tangles (tau hyperphosphorylation), neuroinflammation, and vascular dysfunction. We also discuss recent studies that provided insight into AD pathology at the intracellular level, e.g. endocytic dysfunction, which may inform the design of promising *in vitro* models.

2.1. Amyloid plaques (amyloid-β accumulation)

The most recognized features of AD histopathology are extracellular spherical plaques composed of aggregated amyloid-β (Aβ) peptide and surrounded by atrophic neurites with decreased number of synapses (reviewed in O'Brien and Wong, 2011). The Aβ peptide is processed from amyloid precursor protein (APP) after sequential cleavages by the

Table 1
Summary of AD studies using induced neuronal (iN) cells derived from iPSCs.

Reference	Induction protocol	AD type	iPSC source	Mutant gene(s)	Phenotype						
					Aβ40	*Aβ42*	*Aβ42:Aβ40 Ratio*	*sAPP*	*P-Tau*	*Total Tau*	*Other phenotypes*
Yagi et al., 2011	SMAD inhibition to EB	Familial	Patient fibroblasts	PSEN1 (A246E), PSEN2 (N141I)	–	–	↑				
Yang et al., 2017	SMAD inhibition to EB	Familial	Patient fibroblasts	PSEN1 (A246E, S169del)	–	↑	↑		↑		Neuronal differentiation is affected
Kondo et al., 2013	SMAD inhibition to EB	Familial	Patient fibroblasts	APP (E693del)	↓	↓	–	–			ROS ↑
	SMAD inhibition to EB	Familial	Patient fibroblasts	APP (V717L)	–	↑	↑				
Muratore et al., 2014	SMAD inhibition to EB	Familial	Patient fibroblasts	APP (V717I)	–	↑	↑	↑	↑	↑	EEA1+ early endosomes ↑
Shirotani et al., 2017	SMAD inhibition to EB	Familial, Sporadic	Patient fibroblasts	APP (V717L, E693del)							Screen of AD biomarker candidates
Wang et al., 2018a	SMAD inhibition to EB	Sporadic	Patient fibroblasts	APOE4	↑	↑	↑	↑	↑		APOE ↓; Secreted APOE ↑
Sproul et al., 2014	SMAD inhibition (no EB)	Familial	Patient fibroblasts	PSEN1 (M146L)			↑				NLRP2 inflammasome ↑; ASB9↑; NDP↑
Armijo et al., 2017	SMAD inhibition (no EB)	Familial	Patient fibroblasts	PSEN1 (A246E)	–	↑	↑				Increased Abeta toxicity with Oligomer treatment
Moore et al., 2015	SMAD inhibition (no EB)	Familial	Patient fibroblasts	PSEN1 (intron4, M146I, Y115C)	↓	↑	↑		–	–	
	SMAD inhibition (no EB)	Familial	Patient fibroblasts	APP (duplication)	–	–	–		↑	↑	
	SMAD inhibition (no EB)	Familial	Patient fibroblasts	APP (V717L)	–	↑	↑		↑	↑	
Ochalek et al., 2017	SMAD inhibition (no EB)	Familial	Patient fibroblasts	PSEN1 (V89L, L150P)	↑	↑	↑		↑		Active GSK-3β ↑; Sensitivity to Ab ↑
Ortiz-Virumbrales et al., 2017	SMAD inhibition (no EB)	Familial	Patient fibroblasts	PSEN2 (N141I)	↑	↑	↑				NLRP2 inflammasome ↑; Firing frequency ↓
Garcia-Leon et al., 2018	SMAD inhibition (no EB)	Familial	Wild-type fibroblasts + CRISPR	MAPT (N279K, P301L, E10+16)					↑	↑	ROS ↑; Firing frequency ↑; Vm ↓
Paquet et al., 2016	SMAD inhibition (no EB)	Familial	Wild-type fibroblasts + CRISPR	APP (KM670/671NL), PSEN1 (M146V)							Describes neuronal differentiation methods
Brownjohn et al., 2017	SMAD inhibition (no EB)	Trisomy 21	Patient fibroblasts	APP (triplication)							
Shi et al., 2012	SMAD inhibition (no EB)	Trisomy 21	Patient fibroblasts	APP (triplication)	↑	↑		↑	↑	↑	
Muratore et al., 2017	SMAD inhibition to EB (to different cell lineages)	Familial	Patient fibroblasts	APP (V717I)				↑	↑(rostral)	↑(rostral)	Rostral but not caudal neuronal cells ↑ in Tau levels
Israel et al., 2012	NPC from neural rosettes	Familial, Sporadic	Patient fibroblasts	APP (duplication)	↑				↑		Active GSK-3β ↑; RAB5+ early endosomes ↑
Woodruff et al., 2013	NPC from neural rosettes	Familial	Isogenic iPSC	PSEN1 (ΔE9)	↓	↑	↑				APP C-terminal fragment ↑
Liu et al., 2014	NPC from neural rosettes	Sporadic	Patient fibroblasts	PSEN1(A246E, H163R, M146L)		↑	↑				Demonstrate that r-secretase modulator 4 can decrease Ab levers in human neuronal culture.
Young, 2015	NPC from neural rosettes	Sporadic	Patient fibroblasts	SORL1 (various SNPs)							The effect of BDNF on SORL1 expression is blocked without protective SORL1 genotype
Woodruff et al., 2016	NPC from neural rosettes	Familial	Isogenic iPSC	PSEN1 (ΔE9)							Soma and surface of APP ↑, axonal/soma Rab11 ↓, endocytosis and transcytosis of APP and LDL ↓, recycling of LRP1 is impaired
Fong et al., 2018	NPC from neural rosettes	Familial	Wild-type fibroblasts + CRISPR	APP (KM670/671NL, KO, V717F)	↑	↑	↑		↑	↑	

(*continued on next page*)

Table 1 (*continued*)

Reference	Induction protocol	AD type	iPSC source	Mutant gene(s)	Phenotype						
					Aβ40	*Aβ42*	*Aβ42:Aβ40 Ratio*	*sAPP*	*P-Tau*	*Total Tau*	*Other phenotypes*
Young et al., 2018	NPC from neural rosettes	Familial, Sporadic	Isogenic iPSC	APP (KM670/671NL)							Chaperone R55 decreased Aβ levels and Tau phosphorylation
Kondo et al., 2017	Ngn2 direct induction	Familial	Patient fibroblasts	PSEN1 (G384A)	↓	↑	↑				
Huang et al., 2017	Ngn2 direct induction	Sporadic	Isogenic H1	N/A (exogenous ApoE treatment)	↑	↑	–				Identify pathway linking APOE and APP
Birnbaum et al., 2018	Ngn2 direct induction	Sporadic	Patient fibroblasts	APOE3, APOE4	–	–	–	–	–		ROS ↑; MTOC1 protein level ↑
Lin et al., 2018	Ngn2 direct induction	Sporadic	APOE3 fibroblasts + CRISPR	APOE4	–	↑	↑				Synapse number ↑; EEA1 + early endosomes ↑
Huang et al., 2018	Ngn2 direct induction	Sporadic	Isogenic H1	N/A (exogenous ApoE treatment)							Synapse formation and activity ↑ with the range order of ApoE4 > ApoE3 > ApoE2
Van der Kant et al., 2019	NPC from neural rosettes	Sporadic	Patient fibroblasts	APP (duplication)					↑		Screen drug compounds with pTau/total-Tau as a readout

AD of familial (FAD), sporadic (SAD), and Trisomy 21 causes have been well modeled using iN cells derived from patient iPSCs, without or with CRISPR/Cas9 gene editing. Current protocols for inducing iPSCs into iN cells can be categorized into four principle methods: 1) SMAD inhibition with embryoid body (EB) formation, 2) SMAD inhibition without EB stage, 3) neural progenitor cells (NPCs) purified from neural rosettes by sorting, and 4) Neurogenin-2 (Ngn2) direct induction.

Legend: "↑" indicates increased protein levels; "↓" indicates decreased protein levels; "–" indicates no change in protein levels; no symbols ("↑" / "↓" / "–") indicates information not available. SMAD is a family of signal transducers for the transforming growth factor beta (TGF-β) superfamily, whose signaling is of pivotal importance to stem cell differentiation. EBs are 3D aggregates of iPSCs cultured in suspension and can respond to external cues for normal embryogenesis and undergo differentiation and cell specification. Neural rosette is the signature structure organized by mainly NPCs differentiated from iPSCs (Tabar and Studer, 2014). Neurogenin-2 is a cell-fate-determining transcription factor that drives neurogenesis during development, and the protocols based on its ectopic expression in iPSCs are generally efficient (Zhang et al., 2013).

specialized proteases β- and γ-secretase (Haass et al., 1992). γ-Secretase is a complex consisting of four subunits: presenilins, nicastrin, anterior pharynx defective 1 (APH-1), and presenilin enhancer 2 (PEN-2). It cleaves the intramembranous domain of APP and generates multiple Aβ peptide isoforms with varied lengths; the most abundant isoform is Aβ40, and to a lesser extent Aβ42, which contains two more amino acids at its carboxyl terminus and has an increased propensity to aggregate (De Strooper, 2003). Aβ peptide monomers can aggregate and form oligomers, as well as larger plaques. Strikingly, these plaques are visible 10 to 25 years before the onset of cognitive decline in patients, highlighting the need to understand their role in early stages of AD (reviewed in Sheng et al., 2012).

The observation of amyloid plaques in post-mortem brain tissue from all AD patients, along with identifiable genetic mutations that favor Aβ formation and aggregation, led to the amyloid cascade hypothesis (Hardy and Higgins, 1992). This central theory in the AD field posits that Aβ accumulation leads to formation of oligomers and plaques, which contribute to other downstream pathological changes in AD. Self-propagation and accumulation of Aβ species may occur in a prion-like mechanism (Stohr et al., 2012; Condello and Stoehr, 2018). Aβ oligomers, rather than plaques, are thought to represent the more toxic form and better correlate with cognitive decline (Lue et al., 1999; Selkoe and Hardy, 2016).

Though Aβ deposits are defining features in AD pathogenesis (Hardy and Selkoe, 2002; reviewed in Selkoe and Hardy, 2016), they are not sufficient to cause clinical symptoms, as 20% of healthy seniors' brains contain Aβ plaques (Rodrigue et al., 2012). Moreover, the quantity of plaques does not correlate with symptom severity (reviewed in Haass et al., 2012). Antibody-based therapies targeting different Aβ forms for clearance have shown mixed results (reviewed in Cummings et al., 2018). Greater understanding is needed about the mechanisms by which monomeric, oligomeric, and multimeric forms of Aβ induce neuronal toxicity in AD, as well as elucidating how variants of the peptide differ in self-propagation ability.

2.2. Neurofibrillary tangles (tau hyperphosphorylation)

In post-mortem brain tissue from AD patients, intracellular neurofibrillary tangles in pyramidal neurons are another classic pathological feature (Goedert et al., 1989; reviewed in Lee et al., 2001; Matsuo et al., 1994). These neurofibrillary tangles are formed by the hyperphosphorylation of tau, a microtubule-associated protein normally enriched in axons (Hoffmann et al., 1997). In healthy neurons, tau is needed for axonal transport, neuronal polarity, and neurite outgrowth (reviewed in Zhang et al., 2016). In AD, however, tau becomes hyperphosphorylated and loses its ability to bind microtubules (reviewed in Duncan and Valenzuela, 2017). Kinases that are proposed to mediate tau hyperphosphorylation include glycogen synthase kinase 3 beta (GSK-3β), mitogen-activated protein (MAP) kinases, AKT serine/threonine kinases, protein kinase A (PKA), calcium/calmodulin dependent protein kinase II (CAMKII), and cyclin-dependent kinase-5 (CDK5) (Lovestone et al., 1996; Takashima et al., 1996; Noble et al., 2003). Hyperphosphorylated tau generates intermediates that become paired helical filaments, which are thought to be toxic and ultimately form neurofibrillary tangles that induce neuronal apoptosis (Andorfer et al., 2003; Khlistunova et al., 2006).

An active area of investigation is understanding how Aβ accumulation and/or tau hyperphosphorylation might initiate downstream pathological cascades in AD. As compared to amyloid plaques, neurofibrillary tangles correlate better with the severity of symptoms and cognitive impairment (Brier et al., 2016), suggesting that hyperphosphorylated tau plays a crucial role. However, several observations support the idea that Aβ acts upstream of tau to initiate pathological changes related to AD, as proposed by the amyloid hypothesis (Bloom, 2014; Selkoe and Hardy, 2016). Familial mutations in APP and subunits of γ-secretase, both of which affect Aβ-related pathways, lead to early-

onset AD (discussed in Section 3, below). Further, familial AD models of *APP* mutant mice have increased neurofibrillary tangles when crossed with tau mutant mice, suggesting that Aβ acts upstream of tau and potentiates tangles (Lewis et al., 2001). It is also worth noting that there is a distinct clinical entity called frontotemporal dementia (FTD), which is characterized by the presence of neurofibrillary tangles and the absence Aβ plaques. FTD involves behavioral changes and, at early stages, does not present with memory impairment as seen in AD. This finding further suggests that Aβ pathology is specifically necessary for the development of AD (reviewed in Hardy, 2006).

2.3. Neuroinflammation

Neuroinflammation has been recognized as an essential element of AD pathology (reviewed in Heppner et al., 2015). For example, inflammatory markers are present in the cerebrospinal fluid (CSF) as early as the onset of mild cognitive impairment, prior to AD symptoms (Brosseron et al., 2014). It is now understood that inflammatory responses are present in the early stages of AD, and worsen as the disease progresses (Sudduth et al., 2013).

Inflammatory responses in AD are mainly driven by glial cells in the brain (Liddelow et al., 2017). In AD, microglia and astrocytes become activated and cluster around Aβ plaques, where they secrete inflammatory signals and phagocytize the debris from plaques and injured cells (Eikelenboom and Stam, 1982; reviewed in Wyss-Coray, 2006). This process is distinct from classical neuroinflammatory diseases, such as multiple sclerosis and encephalitis, that are mediated by invading lymphocytes derived from the blood. In AD, markers of microglial activation also increase as the disease progresses (Suarez-Calvet et al., 2016). While microglia and astrocytes are thought to be the key effectors of neuroinflammation in AD, oligodendrocytes comprise the majority of non-neuronal cells in neocortex and contribute to inflammatory processes in AD development (reviewed in Ettle et al., 2016; Pelvig et al., 2008).

2.4. Vascular impairments

Vascular dysfunction has been clearly documented in AD pathology and manifests at a pre-clinical or early stage (de la Torre and Stefano, 2000). Over 90% of AD patients have mild cerebral amyloid angiopathy, in which Aβ forms deposits in the capillaries and deteriorates the blood-brain barrier (BBB), a key mediator of cerebral homeostasis (Abbott et al., 2010; Jellinger, 2002). The ensuing hypoperfusion leads to a chronic reduction in the supply of oxygen, glucose, and other nutrients to the brain, resulting in damage not only to the parenchyma but also to the BBB itself (Di Marco et al., 2015). Reciprocally, chronic cerebral hypoperfusion is reported to directly induce Aβ oligomerization and formation of neurofibrillary tangles (Wang et al., 2010), further supporting the early involvement of vascular dysfunction in AD pathogenesis. Indeed, in patients, the severity of vascular dysfunction and dementia are highly correlated. A vascular hypothesis for AD pathogenesis has thus emerged, proposing that the amyloid cascade and related neurodegenerative features are the sequelae of chronic vascular insults (Rius-Perez et al., 2018).

Multiple cell types residing in blood vessels contribute to pathological changes during AD. Aβ in the vasculature is principally produced by myocytes and endothelial cells (Van Nostrand et al., 1998), and is then cleared by perivascular macrophages or transported across the BBB via uptake by two cell-surface receptors: LDL receptor-related protein 1 (LRP1) and LDL receptor (LDLR) (Castellano et al., 2012; Shibata et al., 2000). In AD, Aβ deposition causes endothelial cells to secrete cytokines that increase inflammation and further impair the BBB (Carrano et al., 2011; Desai et al., 2007). The compromised BBB integrity may allow migration and invasion of circulating leukocytes into the brain parenchyma, aggravating the pro-inflammatory and cytotoxic events in AD pathology (Zenaro et al., 2017).

2.5. New directions and considerations

Recent advances have provided compelling evidence supporting alternative and complementary pathways to these four widely-accepted pathological mechanisms in the AD brain. In post-mortem tissue from AD patients, enlarged early endosomes are consistently observed and contain intracellular Aβ aggregates (Cataldo et al., 2000; Cataldo et al., 2004), suggesting that endosomal trafficking is impaired. In neurons, endosomes play critical roles at synaptic terminals during vesicle recycling and neurotransmitter packaging (Marquez-Sterling et al., 1997). Endosomes are also essential for recycling presynaptic and postsynaptic receptors; failure to endocytose glutamate receptors may contribute to higher levels of cell excitability in AD (Nieweg et al., 2015). Moreover, the endosomal pathway is linked to both APP processing and Aβ aggregation. During trafficking, APP, β- and γ-secretases are found on endosome membranes (Marquez-Sterling et al., 1997; Sannerud et al., 2016), an important site for Aβ production. Though normally released from endosomes into the extracellular space, Aβ peptides can disrupt endosomal trafficking and accumulate inside endosomes, further impairing their function (Treusch et al., 2011). Intracellular Aβ aggregation is reported at other membranous organelles, such as mitochondria (Lustbader et al., 2004). Post-mortem AD tissue also shows decreased mitochondrial size and increased presence of autophagosomes near these mitochondria (Hirai et al., 2001). Aβ is thought to dysregulate calcium homeostasis maintained by mitochondria, leading to the "calcium hypothesis" where increased cytoplasmic calcium levels in neurons contribute to AD pathology (Supnet and Bezprozvanny, 2010). While our understanding of endosomal and mitochondrial function in AD continues to evolve, there is much work to be done to clearly establish the roles played by these fundamental cell biology processes during disease pathogenesis.

3. Genetics of Alzheimer's disease

3.1. Hunting for candidates: the early-onset factors

Since the initial description of patient Auguste D. by Alois Alzheimer in 1906, researchers have identified many genetic risk factors that have provided significant insight into the underlying disease mechanisms. Breakthrough studies focused on patients with early-onset familial AD (FAD), which accounts for less than 5% of AD cases and presents before age 65. Guided by observation of the amyloid fibril neuropathology of Down syndrome patients, the first efforts to identify FAD genes focused on chromosome 21 (Glenner and Wong, 1984). Multi-locus linkage analysis of FAD families and screening of cDNA libraries lead to near-simultaneous discovery of the gene responsible for Aβ production in 1987 by multiple labs, later renamed *APP* (Goldgaber et al., 1987; Kang et al., 1987; St George-Hyslop et al., 1987; Tanzi et al., 1987a). Follow-up studies of these same pedigrees demonstrated that linked mutations were actually located on chromosome 14 within the presenilin 1 (*PSEN1)* gene (Sherrington et al., 1995; St George-Hyslop et al., 1992; Tanzi et al., 1987b; Van Broeckhoven et al., 1992). In 1990, the first conclusively pathogenic *APP* mutation was identified, and in 1995 a homolog of *PSEN1*, presenilin 2 (*PSEN2*), was described and shown to be mutated in one of the FAD families (Levy et al., 1990; Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). Hundreds of early-onset FAD mutations have been identified in *APP*, *PSEN1*, and *PSEN2*, and it is now appreciated that mutations in these three genes are responsible for most of the known cases of autosomal dominant early-onset AD, with mutations in *PSEN1* comprising the vast majority of these, followed by *APP* and *PSEN2* (http://www.molgen.ua.ac.be/ADMutations; reviewed in Guerreiro et al., 2012). These three risk factors are intimately connected at the cellular level, as PSEN1 and PSEN2 are two of the components of the multimeric γ-secretase complex that is responsible for the final step of cleavage of APP that generates Aβ fragments.

3.2. The search continues: late-onset risk genes

Late-onset sporadic AD (SAD) is the most common presentation of the disease, defined as those cases that occur over the age of 65, and is considered to be much more complex and heterogenous in etiology than early-onset FAD. In addition to the interaction of multiple possible genetic pathways, there are environmental, lifestyle, and normal aging factors that may contribute to an individual's likelihood of developing SAD. By far the most important genetic risk factor for SAD is apolipoprotein E (*APOE*), which was identified in 1993 by Strittmatter and colleagues in a study describing the increased association of a certain allelic variant of *APOE* (ε4) with familial late-onset AD (Strittmatter et al., 1993). Humans express three *APOE* allelic variants, ε2 (~8%), ε3 (~77%), and ε4 (~15%) (Utermann et al., 1980). Compared to the predominant ε3/ε3 genotype, an ε3/ε4 genotype increases the risk of AD approximately four-fold, homozygous ε4/ε4 genotypes confer an AD risk more than ten-fold increased, and the least-common allele ε2 is reported to lower AD risk (Corder et al., 1994). The three genetically-encoded variants of this protein (apoE2, apoE3, and apoE4) differ at just two amino acid residues: 112 and 158 (Weisgraber, 1994). These residues are both located within the N-terminal domain of the protein, just outside of the defined receptor-binding domain (Aggerbeck et al., 1988; Lalazar et al., 1988; Mahley et al., 1977; Weisgraber et al., 1983; Weisgraber et al., 1978; Wetterau et al., 1988). The composition of these residues has been shown to affect the interaction of apoE with lipids and receptors, as well as to modulate the intramolecular interaction of its two protein domains, but how these minimal structural differences translate to such tremendous disease risk is the subject of vigorous investigation (reviewed in Yu et al., 2014).

The apoE protein has been well characterized for its role in lipid transport and metabolism, but its role in the brain, particularly in the context of AD, is more complicated. Inside the brain, apoE is primarily secreted by glia and executes some signaling functions in development and synapse functions (Holtzman et al., 2012). Variant-dependent differences in apoE function have been described for its role in clearance of Aβ, as well as an association with smaller gray matter volumes and increased signatures of brain aging (reviewed in Yu et al., 2014). Significant evidence has been presented establishing that apoE impacts the abundance and activity of Aβ in the brain, not only by influencing clearance, but also by affecting aggregation and fibril formation of Aβ, and by directly interacting with Aβ (reviewed in Huynh et al., 2017). We have recently identified a role for apoE in stimulating the production of Aβ via transcription of the *APP* gene, with differential activity between the variants (Huang et al., 2017) (discussed in Section 4.2). Of note, *Homo sapiens* is the only known species that expresses multiple *APOE* allelic variants (McIntosh et al., 2012), and the mouse version shares only 40% homology in the promoter region with human *APOE* alleles, suggesting distinct gene expression control (Maloney et al., 2007). This highlights the need for human cell models to study AD.

A possible link has also been proposed between apoE and tau: it was observed that apoE2 and apoE3 are able to bind to tau protein *in vitro*, while apoE4 is not (Strittmatter et al., 1994). Tau itself has not been conclusively implicated as a genetic risk determinant for AD. Rare mutations in *MAPT*, the gene encoding tau, have been identified that cause a heritable form of FTD, a dementia clinically distinct from AD (Hutton et al., 1998; Spillantini and Goedert, 1998). There is also some evidence that a certain haplotype of *MAPT* can influence SAD risk by affecting tau expression in the brain (Allen et al., 2014).

One intriguing genetic risk factor identified recently is the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2), a surface receptor on immune cells that, within the brain, is exclusively expressed in microglia. A rare *TREM2* mutation, R47H, was shown to increase the risk of SAD on par with *APOE* ε4 (Guerreiro et al., 2013; Jonsson et al., 2013). Markedly, apolipoproteins including apoE and apoJ (also known as clusterin, another SAD risk gene discussed in the following section) were identified as ligands of TREM2 in an unbiased protein microarray screen (Yeh et al., 2016). Attempts to understand the AD-relevant role of TREM2 using mouse models have been inconclusive; a role for TREM2 in microgliosis has been convincingly emerging, but the exact mechanism by which TREM2 influences AD risk remains to be elucidated.

3.3. Spreading the net with new genomics technologies

In the last decade, exciting progress in AD research has been driven by advances in genomic techniques. Genome-Wide Association Study (GWAS), next generation whole exome (WES), and whole-genome sequencing (WGS) have identified both common and rare gene variants that are potential AD risk factors. More than 20 loci have now been identified, and though a thorough discussion of each of them is beyond the scope of this review, please refer to a recent summary provided by Carmona et al. (Carmona et al., 2018). Emergent themes of these newly-identified gene loci highlight the importance of the immune system (particularly neuroinflammation and activation of the complement cascade), cholesterol metabolism, and endocytic pathways (Fig. 1).

As an example of the utility of these recent studies, two large GWAS studies in 2009 (both involving more than 2,000 cases and more than 5,000 controls) lead to the identification of three new risk loci: *CR1*, encoding complement component (3b/4b) receptor 1; *PICALM*, encoding a clathrin assembly protein; and *CLU*, encoding clusterin, which was independently identified in both studies (Harold et al., 2009; Lambert et al., 2009). A three-stage GWAS study performed in 2011 by the Alzheimer's Disease Genetics Consortium validated the disease association of these three genes (Naj et al., 2014). Clusterin, also known as apoJ, has a number of intriguing functions (in addition to being a TREM2 ligand) that may be relevant to AD (reviewed in Guerreiro et al., 2012). For example, it has been shown to interact with Aβ and may influence its solubility. Picalm is involved in clathrin-mediated endocytosis and, in particular, plays a role in VAMP2 trafficking that affects the fusion of synaptic vesicles (Harel et al., 2008). Two other studies also pinpointed *BIN1* and *SORL1*, encoding proteins involved in endocytosis, and all of these results together strongly suggest that endocytic dysfunction may predispose to SAD (Miyashita et al., 2013; Seshadri et al., 2010). In light of earlier evidence showing a consistent pathology feature of enlarged early endosomes in postmortem AD brain tissues (Cataldo et al., 2000; Cataldo et al., 2004), the linkage of endocytic proteins to SAD now uncovered by GWAS studies has led to a renewed interest in the underappreciated role of endocytosis in AD pathogenesis.

Large studies demonstrate an amazing power to uncover rare genetic factors involved in AD that can elucidate previously unknown pathways or mechanistic details of the disease pathogenesis. Analyzing and processing these data, though, represents a significant challenge in the field. Databases such as AlzGene (http://www.alzgene.org) provide a central hub for the compilation of AD gene association data, and at the time of this writing, AlzGene housed data from 1395 published studies (Bertram et al., 2007). Increased understanding of the genetic components that determine AD risk also necessitate policymaking decisions about screening and genetic counseling of patients. A series of clinical studies, part of the REVEAL trial, has been conducted to investigate the psychological and behavioral impact of genetic screening results (Guan et al., 2017). Isolation of patient cell lines harboring some of the mutations discussed above has also opened the door to iPSC-based studies that show great promise for increasing our understanding of AD at a molecular level and enabling the development of therapeutics.

3.4. Somatic mutations and mosaicism

To date, genetic studies of AD have mostly searched for inherited mutations in large populations or family lineages. Disease-associated mutations are typically thought to be transmitted through the germ line

and to be present in all cells, except for most cancer mutations which arise postzygotically and somatically as a result of DNA replication errors or faulty DNA damage response. Increasingly, the role of somatic mosaicism outside of cancer is being investigated, including in neurodegenerative diseases and aging (Leija-Salazar et al., 2018; Poduri et al., 2013). Somatic mutations in humans are common and are enriched in the brain compared to other organs - up to 40% of single neurons from healthy adult frontal cortex contain at least one large insertion or deletion (McConnell et al., 2013). Somatic single-nucleotide variants (sSNVs) in human prefrontal and hippocampal neurons are found to increase linearly from age 4 months to 82 years and may be more abundant in neurodegenerative disease (Lodato et al., 2018). The sSNV mutation rate was estimated to be 1.3 mutations per division per cell during early embryogenesis and to increase during the stage of neurogenesis (Bae et al., 2018). Interestingly, iPSC-derived human neurons were shown to recapitulate *in vitro* the occurrence and accumulation of somatic genome abnormality observed in human brains (McConnell et al., 2013).

In precise relation to AD, a case of late-onset AD was attributed to a somatic mosaic mutation in the *PSEN1* gene, with the mosaicism at 8% in peripheral lymphocytes and 14% in cerebral cortex (Beck et al., 2004). The copy number of the *APP* gene can also be altered somatically, as shown in two postmortem human brain studies. Aberrant numbers of chromosome 21 (which contains *APP*) were identified in about 4% in neurons, much higher than the 0.6% rate in human lymphocytes (Rehen et al., 2005). Meanwhile, the prefrontal cortical neurons from SAD patients were found to contain on average two extra copies of the *APP* gene (Bushman et al., 2015). A recent article proposed a mechanism of DNA recombination for these somatic mutations, whereby *APP* mRNA could be reverse-transcribed into cDNA (potentially with mutations) that could then be reinserted in the genome (Lee et al., 2018). In addition to *PSEN1* and *APP*, somatic variants of other AD and neurodegenerative genes including *SORL1* have been identified in 50% of postmortem brains donated from patients and age-matched controls (Keogh et al., 2018). In terms of *in vivo* genetic effects, Mass and colleagues employed an elegant transgenic mouse model to introduce into microglia a somatic mosaicism of the *BRAF* gene mutation V600E, associated with a leukemic phenotype and late-onset neurodegeneration. They reported that somatic expression of BRAF(V600E) in just 10% of microglia in the brain was sufficient for a pronounced neurodegenerative feature with amyloidosis (Mass et al., 2017). This suggests that somatic genome variation may contribute to AD pathogenesis, and such a hypothesis is consistent with the possibility that AD pathology may be propagated in a prion-like fashion after an initial seeding event - only a small number of mutant cells would be required to trigger pathogenesis. As the detection of somatic mutations in human brain tissue requires state-of-the-art WGS equipment (Sala Frigerio et al., 2015), and a good amount of expertise and resources, the establishment of a reductionist method (such as induced neuronal cells) could provide a crucial framework to allow the study of somatic mosaicism in AD pathogenesis.

4. Induced neuronal cells

Many previous studies have been performed on transgenic mice engineered with a combination of mutations in *APP* and other AD-associated genes, and/or with overexpression of different apoE variants (Kitazawa et al., 2012; Morrissette et al., 2009; Elder et al., 2010). These well-established transgenic mice have served as informative models for AD, particularly in pathological and behavioral features. However, these transgenic mouse models also have limitations. For example, massive neuronal loss has for the most part not been observed in mouse models (Ebert et al.; 2012, Jucker, 2010; Yang et al., 2016; St Clair et al, 2017), probably due to the difference between human and mouse genetic backgrounds. Fortunately, in the last decade, the rapid development of iPSCs and cell reprogramming technologies has allowed researchers to study AD in a strictly isogenic context. In early protocols, direct conversion of human neuronal cells from embryonic stem cells (ESCs) or iPSCs was guided by principles of normal embryonic development. Neuronal differentiation processes were induced by extracellular signals such as soluble morphogens or physical contact with certain cell types (Thomson et al., 1998). Later, groundbreaking studies demonstrated that multiple cell types - even those from a different germ layer, such as fibroblasts - can be directly reprogrammed into human neuronal cells by expression of lineage-determining transcription factors (Ang and Wernig, 2014; Vierbuchen and Wernig, 2011). Now, the application of key neurogenic transcription factors to ESCs/iPSCs, with or without a combination of small molecules, allows for rapid and clean generation of human induced neuronal cells (Zhang et al., 2013). We here summarize the results and insights achieved by AD modeling with these human neuronal cells (referred as “iN cells”) (Table 1).

4.1. Familial Alzheimer’s disease and trisomy 21

Studies using iN cells derived from FAD patients have largely focused on *PSEN1*, *PSEN2*, and *APP* mutations. Yagi et al. were among the first groups to recapitulate the aberrant production of Aβ42 caused by *PSEN1* and *PSEN2* mutations with iN cells derived from multiple patients’ iPSC lines. (Yagi et al., 2011). This phenotype has been confirmed in later FAD studies based on iPSC-derived iN cells carrying various *PSEN1* point mutations (Armijo et al., 2017; Moore et al., 2015; Ochalek et al., 2017; Sproul et al., 2014; Yang et al., 2017). Most of the reports are in agreement with each other regarding increased Aβ42 levels and tau phosphorylation, although one showed unaltered levels of tau phosphorylation (Moore et al., 2015). In addition to these AD hallmarks, 14 other genes were found to be differentially expressed in neural progenitor cells derived from patients with *PSEN1* mutations, five of which were also expressed differentially in SAD brains (Sproul et al., 2014).

APP mutations in FAD patients have also been modeled by iPSC-derived iN cells. Most of the cell lines exhibited a consistent increase in Aβ production and tau phosphorylation (Israel et al., 2012; Kondo et al., 2013; Lin et al., 2018; Moore et al., 2015; Muratore et al., 2014), with an increase in total tau levels in some of these studies (Muratore et al., 2014; Moore et al., 2015). In a study of the *APP* Osaka mutation (E693Del, a microdeletion in the Aβ domain), phenotypic analysis of iPSC-derived iN cells revealed Aβ anomalies consistent with clinical and pre-clinical data: total levels were lower but oligomerization was greatly enhanced by the mutation (Kondo et al., 2013). In addition to the Aβ and tau abnormalities, the AD feature of enlarged early endosomes has been noted in iPSC-derived iN cells from patients with the *APP* London mutation (V717I) (Muratore et al., 2014) and *APP* duplication mutation (Israel et al., 2012). Recently-developed protocols for directed differentiation into more diverse neuronal cell types may expand our understanding of the impact of disease-associated genotypes beyond their effect on specific previously-characterized neuronal populations. For example, Muratore et al. differentiated iPSCs with the APP London mutation (V171I) into iN cells with rostral (forebrain, cerebrum) and caudal (hindbrain, cerebellum) fates. There was a marked difference in cellular AD pathology, with a lower Aβ42:40 ratio and less total and phosphorylated tau in caudal iN cells compared to iN cells derived from an isogenic corrected iPSC line (Muratore et al., 2017). The discrepancy in phenotypes between different types of neurons may reflect pathophysiological mechanisms underlying the selective vulnerability of certain brain regions in AD pathogenesis.

A clinical presentation of early-onset AD due to *APP* duplication occurs in Down syndrome, owing to the full or partial trisomy of chromosome 21 (TS21) containing the *APP* gene. Three copies of *APP* is sufficient to drive excessive production of Aβ and yield early-onset AD with high penetrance and morbidity; 100% of typical AD pathology features are found in postmortem Down syndrome brains as young as 40 years, and AD is the main cause of death in older Down syndrome

patients (reviewed in Hamlett et al., 2018). Owing to the striking homogeneity and accelerated pathology in this specific AD population, TS21 has been employed in disease modeling. In a study using human neuronal cells derived from TS21 iPSCs, significantly elevated Aβ levels were noted (Shi et al., 2012). The other core pathologies, such as increased Aβ aggregation and tau phosphorylation, were also observed within 2-3 months in the same study, again indicating that iPSC-derived neuronal cells with TS21 mimic *in vivo* AD pathologies of Down syndrome patients.

4.2. Sporadic Alzheimer's disease

Israel et al. (2012) published one of the first studies using iPSC-derived iN cells from SAD patients, in which they demonstrated the recapitulation of AD pathologies including increased Aβ, tau hyperphosphorylation, and enlarged early endosomes. They further revealed that tau phosphorylation and GSK-3β activation are regulated by the proteolytic processing of APP by β-secretase, but are independent of γ-secretase activity (Israel et al., 2012). These phenotypes were partially replicated in a separate study, where iN cells derived from both SAD and FAD patient iPSCs were also found to be more sensitive to Aβ toxicity and oxidative stress (Ochalek et al., 2017). Focusing on a key SAD gene, *SORL1*, Young (2015) examined the effects of multiple genetic variants and reported a novel phenotype involving the response of brain-derived neurotrophic factor (BDNF). The iN cells bearing disease-associated *SORL1* variants showed an impaired induction of SORL1 expression and APP processing by BDNF (Young, 2015). Further, some SAD risk genes may alter neuronal functions through aberrant pre-mRNA splicing contributed by disease-related polymorphisms, and such a proposal was recently demonstrated using iPSC-derived iN cells. Raj and colleagues cataloged splicing changes in the large postmortem RNA sequencing data from two major aging cohorts, identified genetic variations that influence splicing, and reproduced the mis-splicing of important genes including *APP*, *CLU*, and *PICALM* in iN cells expressing high levels of phosphorylated tau (Raj et al., 2018).

Studying the single greatest SAD genetic risk, *APOE* ε4, has been challenging due to the inherent complexity of the brain, as well as the inadequate sensitivity and species-specificity of conventional AD model systems. Further confounding *in vitro* modeling, astrocytes are the major source of apoE in the brain, with neurons expressing very low levels unless under stress (Wang and Eckel, 2014). Our group recently developed a novel approach to overcome these technical hurdles using iN cells. This methodology incorporated two critical experimental design strategies: 1) excluding glial cells, the major source of apoE in the brain, to eliminate the background "noise" and 2) using human wild-type ES/iPSC-derived iN cells for evolutionarily-consistent genomic context to boost the "signal." As a result, we have delineated a signaling pathway whereby apoE activates a non-canonical MAP kinase cascade that enhances Aβ production via transcriptional regulation. Specifically, we demonstrated that apoE, exogenously applied to mimic glial secretion, binds to its receptor(s) on neurons and activates dual leucine-zipper kinase (DLK), a MAP-kinase kinase kinase that then activates MKK7 and ERK1/2 MAP kinases. Activated ERK1/2 induces cFos phosphorylation, stimulating the transcription factor AP-1, which in turn enhances *APP* transcription and thereby increases Aβ levels. Importantly, this technique is sensitive enough to detect the differences between the three human apoE variants in activating this pathway, with the potency rank order mirroring their relative risk for AD: apoE4 > apoE3 > apoE2 (Huang et al., 2017). Recently, we have further revealed that this particular signaling pathway also governs the essential function of synapse formation in both human and mouse neurons, with the three apoE variants exhibiting the same disease-relevant rank order: apoE4 > apoE3 > apoE2 (Huang et al., 2018).

4.3. Induced neuronal cells from genome-edited iPSCs

With the recent emergence of gene-editing technologies, particularly CRISPR-Cas9, mutations can be introduced or corrected in ES/iPSCs in an isogenic fashion that allows analysis of neuronal phenotypes under a strictly-controlled genomic background.

To introduce FAD mutations by CRISPR/Cas9, Paquet and colleagues engineered iPSC lines bearing heterozygous and homozygous mutations of *PSEN1* (M146V) or *APP* (KM670/671NL, Swedish mutation) and derived iN cells, which displayed genotype-dependent disease-associated phenotypes accordingly (Paquet et al., 2016). Similarly, to study the role of Exon 9 of *PSEN1* in AD pathogenesis, Woodruff et al. generated hetero- and homo-isogenic iPSC lines with *PSEN1* Exon 9 in-frame deletion (ΔE9), and observed differences in pathology compared to *PSEN1*-null isogenic iPSC lines (Woodruff et al., 2013). With the same isogenic lines, they then proposed a neuron-specific impairment in endocytic axonal APP delivery, upon observing decreased endocytosis and transcytosis of APP and LDL at the axons (Woodruff et al., 2016). To investigate the cellular effects of abnormal tau phosphorylation, García-León et al. inserted triple *MAPT* mutations into a control iPSC line and compared the wild-type and mutant iN cells sharing an isogenic genome background. They reproduced the *in vivo* findings of tau aggregation, altered cortical differentiation, and electrophysiological activity; they also identified activation of pathways related to inflammation, oxidative stress, and neurodegeneration (Garcia-Leon et al., 2018). Moreover, a few attempts to correct or remove the mutations from FAD iPSC-derived iN cells have been reported, potentially informing the concept of gene therapy for FAD, which is still on the horizon. CRISPR/Cas9 gene editing was used in two studies to correct point mutations of *PSEN1* (G384A) or *PSEN2* (N141I) in FAD patient iPSC lines; both studies reported the phenotypic rescue of Aβ42 production and other AD features relative to the corresponding isogenic iN cells bearing the original mutation (Kondo et al., 2017; Ortiz-Virumbrales et al., 2017).

Gene editing has also been used to probe SAD genetics, with two recent studies using CRISPR/Cas9 to switch between the *APOE* ε3/ε3 and ε4/ε4 genotypes in patient-derived iPSCs in order to examine the detrimental effect of apoE4. Surprisingly, despite the low level of apoE expression in neurons, both groups were able to observe several phenotypes in apoE4- versus apoE3-expressing iN cells, including an increase in Aβ production and tau phosphorylation, which could be reversed by gene editing (Lin et al., 2018; Wang et al., 2018a). Wang et al. also suggested that the higher level of tau phosphorylation in apoE4 iN cells could be independent of the increased Aβ level. In the same study, iN cells were differentiated from an *APOE*-null patient iPSC line, in order to address whether the pathogenic effect of apoE4 in AD is a gain of toxic function or a loss of normal apoE3 function. They found that *APOE*-null and *APOE* ε3 iPSC-derived iN cells shared a very similar phenotype, and that the forced expression of apoE4 in *APOE*-null iNs cells by transfection could recapitulate the *APOE* ε4 phenotype. These findings argue for a toxic gain-of-function for the ε4 allele, at least in neurons. Mechanistically, this study demonstrated that, in a mixed culture of excitatory and inhibitory iN cells, GABAergic neurons are more vulnerable to the detrimental cell loss effect of the ε4/ε4 genotype (Wang et al., 2018a, 2018b). Interestingly, in the apoE4-expressing excitatory iN cells made by Lin et al., 25% more synapses were formed, indicating hyperexcitability may be a neuronal phenotype of *APOE* ε4 (Lin et al., 2018). Taken together, reduced inhibitory activity and enhanced excitatory activity may contribute to the excitation/inhibition imbalance in AD neural circuitry (Canter et al., 2016). Lin et al. also compared induced astrocytes and microglia derived from the same iPSC lines, and established a reference for human cell-type-specific changes associated with the apoE4 variant (discussed in sections 6.1. and 6.2.).

4.4. Induced neuronal cells from other cell lineages

The generation of human iN cells can be achieved by direct conversion from other terminally-differentiated somatic cells, such as fibroblasts (Vierbuchen and Wernig, 2011), urine epithelial cells (Wang et al., 2013), and peripheral blood T cells (Tanabe et al., 2018), all of which are more readily available than patient iPSCs. This alternative path bypasses the pluripotential reprogramming, in which the epigenetic modifications accumulated during aging are erased from the iPSC genome. The iN cells directly converted from fibroblasts of aged donors were shown to preserve the age-dependent transcriptomic signatures (Mertens et al., 2015). In AD, genome-wide and locus-specific epigenetic alterations associated with DNA hypermethylation and histone deacetylation have been reported (Sanchez-Mut and Graff, 2015), suggesting a repressed chromatin state in general and a potential role for histone deacetylase (HDAC) inhibitors now in clinical trials (Kazantsev and Thompson, 2008). For key AD genes, histone lysine H3K27 acetylation (a marker for active enhancers and promoters) was found to be considerably altered in regions near *APP*, *PSEN1/2*, *MAPT*, and some SAD risk genes in a recent mapping of postmortem entorhinal cortex samples from AD donors and matched controls (Marzi et al., 2018)

As a proof of concept, there are a few published studies demonstrating this direct conversion technique with AD-linked mutations. For instance, Hu and colleagues developed a chemically-defined cocktail of seven small molecules that convert human fibroblasts into iN cells (Hu et al., 2015). The iN cells derived from fibroblasts of patients with *PSEN1* (S169Del) or *APP* London (V717I) mutations resembled iPSC-derived iN cells with respect to gene profiles and electrophysiological properties, and displayed the AD phenotypes of increased Aβ and phosphorylated tau levels. Because aging is the largest overall risk factor for AD, this approach could help us understand how normal aging or dysregulation of epigenetic mechanisms during aging contribute to AD pathogenesis.

4.5. Limitations of conventional iPSC-derived neuronal cells

The overall progress made by iPSC-derived neuronal cell-modeling (summarized in Table 1) proves the utility of this methodology for AD research, though it is not without limitations (reviewed in Choi et al., 2016). These cell models are typically cultured in monolayer, which is unable to recapitulate some aspects of Aβ metabolism, including its aggregation and the clearance by other cell types or CSF circulation. Aβ accumulation may be prevented by constant dilution and removal of Aβ during regular culture media changes, and this may partially explain the decreased amyloid pathology of *in vitro* models compared to animal models. Another limitation is the lack of cell-type diversity in neuronal cells derived from human iPSCs. Some of the induction methods, such as the protocols using transcription factor overexpression, generate a specific neuronal cell type with certain developmental and electrophysiological features. The lack of interaction between different neural cells within a brain-like microenvironment limits the ability of these systems to model the wide range of AD physiopathology. In addition, insufficient maturation and aging of iPSC-derived neuronal cells is a fundamental drawback for studying disease progression. To address these technical limitations, a transition from 2D to 3D culture systems has emerged lately, aiming to incorporate multiple cell types that mimic the *in vivo* milieu throughout brain development (reviewed in Poon et al., 2017).

5. 3D models: organoid, multi-culture, and brain-on-a-chip

5.1. Cerebral organoids

The cerebral organoid (also called neurospheroid) culture technology has the potential to solve the aforementioned issues with 2D iN culture, at least in part. Organoids are reduced organs that recreate physiological 3D tissue structure and cellular composition *in vitro*. Organoid tissue is architecturally more complex than 2D cultures, as it relies on self-organization of under-differentiated cells in an extracellular matrix-like environment (reviewed in Amin and Pasca, 2018). A few protocols are currently published, with a typical induction period of 8-12 weeks to allow better cell and network maturation (reviewed in Poon et al., 2017). The brain organoids were first introduced to probe neural progenitor dysfunction during early stages of CNS development, and demonstrated a microcephaly phenotype and progenitor abnormalities caused by Zika virus infections (Garcez et al., 2016; Lancaster et al., 2013). A recent improvement of adopting an air–liquid interface culture method allows growth of cerebral organoids exhibiting axon outgrowth with specific tract-like patterns (Giandomenico et al., 2019).

As for AD modeling, one team was able to recapitulate the major disease hallmarks of the disease, including the interplay between Aβ peptide accumulation and tau phosphorylation, generating brain organoids with iPSCs derived from FAD or Down syndrome patients (Gonzalez et al., 2018). In addition, the AD features of endosomal and lysosomal abnormalities were observed in FAD iPS organoids (Raja et al., 2016). Making a further translational step, one group generated large-scale arrays from neurospheroids uniform in size and cellular composition, and validated this method as a sensitive tool for long-term screening of compounds (Jorfi et al., 2018).

However, current organoid protocols have several technical limitations, including low reproducibility, incomplete cell type representation, and delayed maturation (Di Lullo and Kriegstein, 2017). The largest dataset to date of brain organoid single-cell mRNA sequencing indeed identified a considerable number of cells bearing a retinal signature, though a small but significant cluster was assigned to a mesodermal lineage. These data cast doubt on previous assumptions of pure, neuroectodermal patterning and emphasize the variability inherent in the available protocols (Quadrato et al., 2017). Accurately reflecting the complex and well-structured tissue organization of the brain with the current organoid models remains a challenge.

5.2. Multi-culture and brain-on-a-chip

The first attempt at developing a 3D culture model for AD was achieved by using an immortalized human neural progenitor line transduced to overexpress mutant APP and PS1 (Choi et al., 2014). Since then, several technical advances have improved 3D culture technology. For better-defined cell identities and incorporation of neuroinflammatory or vascular components, researchers have started to combine the modern technologies of induced non-neuronal brain cells, including microglia, astrocytes, oligodendrocytes, epithelial cells, and components of BBB (reviewed in the following sections). For instance, a tri-culture with separately-induced human neurons, astrocytes, and microglia grown in a wheel-shaped device was designed and demonstrated Aβ aggregation and tau phosphorylation, along with neuroinflammatory components such as chemokines, cytokines, neuronal loss, and recruitment of astrocytes by microglia (Park et al., 2018). To promote growth and maturation in the absence of vasculature, one team crafted a perfusable organ-on-a-chip system with mechanical fluid flow and noticed enhanced cortical development (Wang et al., 2018b). Based on these breakthroughs, additional optimization is expected to bring about a refined biomimetic brain microenvironment. For instance, the integration of induced oligodendrocytes into the aforementioned 3D tri-culture system would expand the ability of such models to simulate disease processes. Functioning microvessels can now be generated in tissue models, such as microtumors, by pre-patterning of channels, 3D printing, or by biology-directed angiogenesis (reviewed in Andrejecsk and Hughes, 2018). A vascularized organoid or multi-culture is an improved “brain-on-a-chip,” boosting the physiological relevance for modeling AD pathogenesis and potentially serving as a platform for high-throughput screening for disease modifiers.

6. Induced non-neuronal brain cells

The recent genetic and clinical data from individuals affected by AD, as well as knowledge from preclinical models, now substantiate the modern view that neuroinflammation and vascular dysfunction worsen and potentially drive AD pathology (reviewed in Heppner et al., 2015). Modeling the inflammatory and vascular aspects of AD pathogenesis would therefore generate better insight into disease mechanisms and possible interventions. Recent innovations in inducing human astrocytes, microglia, oligodendrocytes, epithelial cells, and cellular components of the BBB will help characterize the biology of and interplay between individual glial cell types.

6.1. Microglia

Microglia, the resident myeloid cells of the brain, have been thrust into the spotlight of AD research following the publication of independent large-scale GWAS studies that identified several AD-linked loci that are expressed abundantly or exclusively in microglia, including *APOE*, *TREM2*, and myeloid cell surface antigen *CD33* (Efthymiou and Goate, 2017; Villegas-Llerena et al., 2016). Microglia regulate neuroinflammation, remodel synapses, and support brain homeostasis in the face of changes in their microenvironments (e.g., infection or tissue damage) by migrating, proliferating, phagocytosing, and producing cytokines and/or neurotrophins (reviewed in Prokop et al., 2013; Salter and Stevens, 2017). Researchers are coming to appreciate the variety of microglial responses, and yet experimental efforts are greatly hampered by the difficulty of characterizing the rapid and dynamic changes of microglial cellular states *in vitro* or *in vivo*.

In the past few years, several protocols for the generation of human iPSC-derived microglia-like cells have been published (Abud et al., 2017; Douvaras et al., 2017; Haenseler et al., 2017; Muffat et al., 2016; Pandya et al., 2017). These methods, based largely on cellular ontogeny of the hematopoietic and myeloid lineage, are all capable of generating a large and pure population of cells that are phenotypically similar to their *in vivo* counterparts. For example, the response to lipopolysaccharides (from bacterial infection) and ADP (released by injured cells), phagocytosis of Aβ, and migration to an injured site (in 3D culture) all resemble *in vivo* microglia (Douvaras et al., 2017; Lin et al., 2018; Muffat et al., 2016). While generating truly physiological microglial phenotypes from iPSCs is still an evolving technique, two independent studies have employed existing approaches and reported consistent findings on the biology of TREM2, one of the strongest risk factors for AD and other forms of dementia. In particular, they both prepared induced microglia-like cells from patient-derived iPSCs harboring mutations of Nasu-Hakola disease (an early-onset dementia) and FTD, and noticed some specific deficits in TREM2 protein surface expression and phagocytosis, but not in response to lipopolysaccharide exposure (Brownjohn et al., 2018; Garcia-Reitboeck et al., 2018). Furthermore, another study was able to compare and contrast the transcriptome profiles of *APOE* ε3/ ε3 and *APOE* ε4/ ε4 microglia in an isogenic context by gene editing, and suggested that the *APOE* ε4 allele may slow Aβ uptake and clearance (Lin et al., 2018). Mechanisms of other AD risk genotypes in microglia, like the TREM2 R47H variant and the CD33 gene, can be further studied using iPSC methodology. Overall, this *in vitro* model uniquely allows the study of microglia under well-defined conditions and provides a promising framework to elucidate microglial genetics and interactions with other brain cells in AD pathogenesis.

6.2. Astrocytes

Similar to microglia, astrocytes in the AD brain become reactive and form increased processes around Aβ plaques. These processes form a fibrous network that is different from glial scar tissue seen in other central nervous system diseases (reviewed in Sofroniew and Vinters, 2010). Due to their versatile and fundamental functions, astrocytes are thought to play a central role in almost every proposed AD etiology, from the classical amyloid cascade to the growing inflammatory and vascular hypotheses. Astrocytes can help degrade Aβ deposits, or can succumb to Aβ toxicity (Olabarria et al., 2010; Wyss-Coray et al., 2003). They can also take up Aβ, forming intracellular deposits that contribute to clearance (Nagele et al., 2003; Thal et al., 2000). Additionally, apoE, the greatest AD risk factor, is predominantly synthesized and supplied by astrocytes in the brain to regulate lipid metabolism and associated signaling functions involved in AD pathogenesis (Huang et al., 2017; reviewed in Huynh et al., 2017). A reliable *in vitro* model of human astrocytes would help to elucidate the previously underappreciated biology of astrocytes in AD pathogenesis.

Functional astrocytes from human stem cells or fibroblasts can now be derived using multiple protocols, with a wide range of efficiency and efficacy in different aspects of differentiation. In general, the induced astrocytes described in the literature can recapitulate well the key functional properties of those *in vivo*, including the presence of glycogen granules (energy storage and supply), glutamate (neurotransmitter) uptake, cytokine stimulation (inflammation), and support for neuronal survival and synaptogenesis (Canals et al., 2018). One study utilizing induced astrocytes derived from both SAD and FAD patients reported a pronounced pathological phenotype in the patient-derived lines, with significantly less-complex morphological appearance, overall atrophic profiles, and abnormal expression pattern of some functional markers (Jones et al., 2017). Another group specifically investigated the astroglial effects of apoE variants by reprogramming human skin fibroblasts from cognitively normal individuals carrying *APOE* ε3/ε3 or ε4/ε4 genotype to iPSCs and then differentiating them into astrocytes. Their results revealed *APOE* genotype-dependent effects on neurotrophic functions, with *APOE* ε4/ε4 being less potent than the *APOE* ε3/ε3 genotype (Zhao et al., 2017). More recently, Lin et al. analyzed induced astrocytes derived from CRISPR/Cas9-engineered iPSCs bearing *APOE* ε4/ε4 or *APOE* ε3/ε3 genotypes, and found that apoE4-expressing astrocytes displayed impaired Aβ uptake and cholesterol accumulation (Lin et al., 2018). Additionally, Fong et al. used CRISPR/Cas9 gene editing to generate isogenic iPSCs of *APP* knockout, Swedish, and V717F mutations, and revealed that full-length APP may be required for proper cholesterol homeostasis and Aβ clearance in human induced astrocytes (Fong et al., 2018). Aside from lipid metabolism and neuronal support, the regulatory role of astrocytes in BBB maintenance during neuroinflammation has been demonstrated by utilizing human induced astrocytes in an *in vitro* BBB model (Mantle and Lee, 2018). Results from human induced astrocytes are a proof of concept for modeling astroglial contributions in AD pathogenesis.

6.3. Oligodendrocytes

Virtually ignored in the AD research field are the alterations and consequences of oligodendrocyte biology in the development of disease. Oligodendrocytes constitute a major group of non-neuronal cells (up to 75% by one stereological counting study on postmortem human brains), and the population size shrinks substantially during aging (Pelvig et al., 2008). They are best known for their functions in producing myelin to facilitate neuronal transmission and offering a critical buffer capacity to maintain the equilibrium of ions, metabolites, and inflammatory reactions for axonal health (reviewed in De Strooper and Karran, 2016). There is significant evidence linking myelin vulnerability and breakdown to early stages of AD pathology, which argues that myelin sheath loss may be an early pathogenic event prior to appearance of Aβ aggregation and tau hyperphosphorylation (reviewed in Ettle et al., 2016). Aβ deposition and formation of neurofibrillary tangles cause focal demyelination, possibly via inflammation and oxidative stress as the common pathophysiological processes; this kind of damage can normally be repaired by local regeneration of oligodendrocyte precursor cells (OPCs) (Nasrabady et al., 2018). However, this

protective renewal of oligodendrocytes was found to be defective in the AD mouse brain (Behrendt et al., 2013). From a clinical perspective, compelling evidence comes from an MRI cohort study, in which the age-associated myelin breakdown was found in individuals over age 50, and these white matter changes were aggravated in AD patients and *APOE* ε4 allele carriers (Bartzokis, 2011). The focus of AD research on oligodendrocyte biology may increase to better understand disease mechanisms and therapeutic intervention options.

To date, there are a few published protocols that result in successful generation of human induced oligodendrocytes from human stem cells or fibroblasts (reviewed in Lee and Park, 2017). The resultant induced OPCs were shown to possess a morphology and gene expression signature resembling their primary counterparts and gave rise to mature oligodendrocytes that could ensheath multiple host axons introduced by co-culturing or engrafting *in vivo*. These induced OPCs were able to rescue the histological and behavioral phenotypes in animal models of demyelinating diseases, such as multiple sclerosis and shiverer-mutated mice (Douvaras et al., 2014; Ehrlich et al., 2017; Rodrigues et al., 2017; Yang et al., 2013). The use of induced OPCs has not yet been applied to investigate how oligodendrocytes respond to and affect AD pathogenesis, and represents an exciting opportunity to shed light on the underpinnings of fundamental CNS functions and dysfunctions in which oligodendrocytes play an active part.

6.4. Endothelial cells and blood-brain barrier components

Vascular impairments in AD patients are currently considered exacerbating factors for disease progression, yet substantial evidence suggests that Aβ deposition and neuroinflammation can actually damage the capillary structure and distort BBB functions, leading to a vicious cycle during AD pathogenesis (Zenaro et al., 2017; Burgmans et al., 2013). The reciprocal interactions between vascular dysfunction and neurodegeneration have thus emerged as an important focus for therapeutic and research efforts. Nevertheless, the lack of robust *in vitro* models hinders the progress of this field.

With recent advances in iPSC differentiation technology, the cellular components of the vasculature, including endothelial cells, pericytes, and vascular smooth muscle cells, can now be generated with high efficiency and reliability from human stem cells (Klein, 2018). By coupling the techniques of physiological scaffolds, 3D cultures, and microfluidic chips, the functions and dysfunctions of the BBB can be modeled *in vitro* by neurovascular units (Campisi et al., 2018; Cochrane et al., 2018). For example, a human iPSC-based BBB model demonstrated inflammatory barrier damage (measured by permeability and electric resistance) triggered by cytokines TNF-α and IL-6, as can be observed in AD pathogenesis (Mantle and Lee, 2018). The recent development of a three-organs-in-a-chip model, which can mimic the influx and outflux across the BBB and brain parenchyma, has characterized the metabolic coupling between endothelial cells and neurons (Maoz et al., 2018). These technical advances would be very advantageous in determining the causes and effects of vascular dysfunction in AD pathogenesis, and have the potential to expand AD modeling systems beyond our current focus on neuronal physiology.

7. Translational potential of induced brain cells

With these high-throughput approaches using iPSCs, induced brain cells can be supplied continually and easily prepared on a large scale for drug screening with pharmaceutical compound libraries. Human neurons and astrocytes induced from FAD and SAD iPS cells were employed to identify drug targets at the endoplasmic reticulum (ER) along the oxidative stress pathway, and specifically were used to test the therapeutic effect of docosahexaenoic acid (DHA) with promising results (Kondo et al., 2013). A few years later, iPSC-derived iN cells served as a high-throughput platform to model AD drug responsiveness and to screen a library of existing drugs for Aβ-lowering agents. The search resulted in 27 hits and three were combined to formulate an anti-Aβ cocktail, which significantly reduced Aβ level in patient-derived iN cultures (Kondo et al., 2017). In a similar manner, Brownjohn et al. performed a phenotypic small-molecule screen to identify modulators of APP processing in TS21 (Down syndrome) human neurons, and identified the avermectins, commonly used to treat parasitic worms, as compounds that increase the production of short Aβ38/40 peptides relative to longer, potentially more toxic peptides Aβ42. (Brownjohn et al., 2017). Notably, while assaying the efficacy of γ-secretase inhibitor and γ-secretase modulator to verify the use of iN cells of various disease genotypes (engineered overexpression or spontaneous mutations) for drug screening, the difference in the released Aβ profiles (relative levels of Aβ38, Aβ40, and Aβ42) drew attention to the artifacts introduced by overexpression of mutant proteins (Liu et al., 2014). In a recent study based on iN cells from SAD and FAD iPSCs, Young and colleagues modulated endosomal trafficking by stabilizing retromer with the small molecule chaperon R33, and suggested that this may have a potentially beneficial effect of regulating endogenous levels of Aβ and phosphorylated tau (Young et al., 2018).

Two recent breakthroughs of drug discoveries further corroborate the translational potential of induced brain cells. The small molecule PH002 was first identified to potentially function as a structural corrector for apoE4, chaperoning it back into apoE3 configuration (Brodbeck et al., 2011). A study using iPSCs from homozygous apoE3- or apoE4-expressing individuals found that PH002 effectively lowered tau pathology in apoE4 neurons and merits further therapeutic development (Wang et al., 2018a). The other breakthrough in screening inhibitors of aberrant tau phosphorylation using iN cells with APP duplications unexpectedly identified cholesterol metabolism as a drug target. Van der Kant et al. (2019) revealed a new mechanism of proteosomal degradation of phosphorylated tau that is negatively regulated by cholesteryl esters (the storage product of excess cholesterol). The lead compounds targeting the activation of enzyme CYP46A1 to lower cholesteryl esters were tested in both induced neurons and astrocytes with encouraging results (Van der Kant et al., 2019).

Aside from drug screens, iPSC-derived iN models have also proved useful in the search for new AD biomarkers for better and earlier diagnosis. To this end, Shirotani et al. recognized a reduction in alpha-1-acid glycoprotein levels in FAD iPSC-derived iN cultures and revealed a consistent change in CSF samples from early stage AD patients, suggesting the discovery of a biomarker to facilitate the early intervention (Shirotani et al., 2017).

8. Discussion

The latest conceptual breakthroughs in viewing AD from alternative angles to the standard amyloid cascade hypothesis have reshaped our interests in this research field and lead to important discoveries that were previously underappreciated (Fig. 1). Meanwhile, recent breakthroughs in cell reprogramming and induction of different brain cells have successfully developed humanized AD models that are capable of modeling features of human-specific, decades-long pathogenesis. This reductionist approach is particularly well-suited to addressing the experimental challenge posed by the inherent complexity of the brain, and can also help us examine the interactions between the different cell types of the brain. The recent rise of iPSC modeling has produced a solid body of evidence on the effects of AD-relevant mutations, particularly in neurons (Table 1). Other fundamental neuronal functions that are defective in AD pathogenesis, such as lipid metabolism, endocytosis, and mitochondria stress, have not yet been fully modeled with iN cells and their characterization will benefit from a combinatorial approach using the emerging non-neuronal cell models. From a translational angle, the aptitude of iN cells for high-throughput screening is particularly useful, and recent iN drug screens have identified several disease-modifying compounds that may be relevant for forthcoming clinical trials.

CRISPR-based technologies have significantly promoted the translational utility of human cellular models by making it possible to introduce or correct patient-specific mutations on a large scale (reviewed in Giau et al., 2018). As the list of AD risk genetic variants has grown exponentially after recent genome-wide investigations, modeling with induced human brain cells in conjunction with CRISPR gene editing is now an invaluable tool for deciphering cell autonomous and non-cell-autonomous disease mechanisms involving multiple cell types. While this joint methodology still faces various obstacles to its *in vivo* therapeutic application, the engraftment of stem cells and differentiated microglia, astrocytes, and oligodendrocytes demonstrates great promise for the development of regeneration and replacement therapies (reviewed in Bali et al., 2017; Canals et al., 2018; Duncan and Valenzuela, 2017; Lee and Park, 2017; Pocock and Piers, 2018).

As disease models are limited to known mutations and genetic variants, most of which are rare and affect only a small fraction of the total AD population, it is not yet known whether findings in induced brain cells will be generalizable and inform common pathogenetic mechanisms. A recent clinical study that scrutinized one of the largest cohorts for autosomal dominant FAD came to a similar conclusion. The longitudinal analysis of cognitive and biomarker changes in individuals enrolled in the ongoing Dominantly Inherited Alzheimer Network (DIAN) project revealed that, regardless of causes and onset age, FAD and SAD indeed share the same pathological features and disease course, once amyloidosis starts (McDade et al., 2018). This surprising result suggests a common pathway underlying AD caused by distinct genotypes with variable penetrance, and hence indicates that discoveries made by studying specific mutations may indeed translate to late-onset SAD.

An increasing refinement in the generation of human induced brain cells is highly anticipated over the next few years, and there is little doubt that upcoming AD-in-a-dish models will resemble the brain microenvironment closely enough to explicate fundamentals surrounding AD pathogenesis. With continued advances in our understanding and techniques for 3D iPSC modeling, a relevant brain-on-a-chip system will permit comprehensive examination of various hypotheses. Ultimately, induced brain cells will contribute to the development of effective intervention and prevention strategies for AD and other neurodegenerative and aging-related disorders.

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