Title: "Neurodegeneration in a dish: advancing human stem-cell-based models of Alzheimer's disease"

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Abstract:

Induced pluripotent stem-cell-based models enable investigation of pathomechanisms in disease-relevant human brain cell types and therefore offer great potential for mechanistic and translational studies on neurodegenerative disorders, such as Alzheimer's disease (AD). While current AD models enable investigation of early disease phenotypes including Aβ accumulation and Tau hyperphosphorylation, they still fail to fully recapitulate later hallmarks such as protein aggregation and neurodegeneration. This impedes the identification of pathomechanisms and novel therapeutic targets. We discuss strategies to overcome these drawbacks and optimize physiological properties and translational potential of iPSC-based models by improving culture formats, increasing cellular diversity, applying genome editing, and implementing maturation and ageing paradigms.

Main text:

Introduction

Age-related brain disorders such as Alzheimer's disease (AD) are among the most common causes of dementia and death, posing increasing social and economic burden on our ageing society. Pathological hallmarks of AD are amyloid-β (Aβ)-containing extracellular plaques, Tau-containing intracellular tangles, neuroinflammation reflected by gliosis, and neurodegeneration. Despite extensive research, no effective treatments are available, and many clinical trials have failed. The lack of mechanism-based treatments is closely related to difficulties in modeling disease development and underlying human-specific molecular, cellular, and physiological processes.

As patient brain cells are inaccessible for experimental research, mechanistic studies largely rely on mouse models, which have significantly broadened our knowledge. However, mouse models lack central AD hallmarks such as combined formation of plaques, tangles, and neurodegeneration under physiological conditions (reviewed in [1,2]). Furthermore, murine neurons are less vulnerable to neurodegeneration than human neurons in an AD context [3]. Recent work further suggests fundamental differences in pathomechanisms between mice and men. For example, single-cell

transcriptome analysis revealed differences in disease-specific expression changes in human vs. mouse brains, especially regarding the AD-associated microglia signature [4–6]. While chimeric models may address some of these problems [7], the drawbacks of current mouse models still limit research on disease pathways and drug development. Hence, a central aim of dementia research is to overcome species barriers and develop human models recapitulating disease hallmarks that further our understanding of AD pathogenesis.

Induced pluripotent stem cell (iPSC) technology strongly advanced the generation of human neural models. Patient cells have been reprogrammed to model diseases, and gene-editing techniques such as CRISPR/Cas9 allow generation of isogenic controls (reviewed in [8]). Robust and versatile protocols enable differentiation of disease-relevant brain cell types including neurons, astrocytes, oligodendrocytes, and microglia (reviewed in [9]). Thus, iPSC-based approaches harbor great potential to complement existing disease models, as they allow studying relevant cell types, have the genetic configuration of patients, and display crucial cellular biological features found in the human brain (reviewed in [10]). These advances promote the investigation of pathomechanisms in human systems and allow to move beyond the neuron-centric research of past decades. This is particularly important since recent studies suggest central roles of other brain cell types, such as microglia, in AD pathogenesis. Furthermore, iPSC-based models are amenable to translational and high-throughput screening approaches, which is essential to foster drug development (reviewed in [11,12]).

Recent advances in modeling AD pathogenesis in human iPSC-based models were reviewed e.g. in [13]. Here, we discuss the major accomplishments of iPSC-based AD models in the past five years together with their prevailing drawbacks and suggest approaches to improve their applicability for disease research by addressing culture format, cellular diversity, gene editing, and maturation (**Figure**).

Culture format - Entering the third dimension

Initial iPSC-based AD models were 2-dimensional (2D) neuronal cultures, which are relatively simple to implement, but limited to cell-autonomous phenotypes recapitulated by neurons grown on plastic surfaces. Consequently, these models allowed insights into early disease phenotypes such as altered Aβ secretion [14], endoplasmic reticulum, and oxidative stress [15], enlarged endosomes [16] and impaired endocytosis [17], mitochondrial impairment [18], increased phospho-Tau [16] as well as intracellular Aβ accumulation [15]. These are likely early-stage pathological changes in neurons. In contrast, later phenotypes such as plaques and tangles were not described in 2D systems. This is possibly due to frequent wash-out of nucleating oligomer species during media changes or aberrant or missing cell-cell and cell-matrix interactions. Thus, 2D cultures recapitulate initial, cell-autonomous pathological changes but fail to model later, more complex stages of AD that are characterized by protein aggregation.

To address these drawbacks, more physiological, 3-dimensional (3D) AD models were developed by embedding cells into hydrogels or using emerging organoid technology. The first model incorporated cells into a peptide matrix and showed phenotypes not seen in 2D due to specific effects of 3D culture on mechanosensitive proteins [19]. In 2016, Raja et al. pioneered the use of cerebral organoids (COs) differentiated from AD-patient-derived iPSCs, showing for the first time progressive extracellular accumulation of Aβ puncta, although no plaques were described [20]. Interestingly, Gonzalez et al. analyzed COs from patients carrying the same mutation but showed structures positive for a Thioflavin-derivative or Gallyas silver staining, resembling plaques and tangles, respectively [21]. This discrepancy may arise from differences in genetic background or organoid protocols, illustrating a major caveat of current technology. Despite recent improvements in reproducibility [22], organoids

develop by stochastic self-organization, especially when provided with only minimal developmental cues, yielding hardly controllable combinations of cell types and maturation stages [23]. This inherent variability limits the potential of organoids to study cell-type specific disease contributions and develop drug screening approaches. Scaffolded 3D cultures, where differentiated cells are embedded into hydrogels, circumvent these problems, but lack the intrinsic self-organization of organoids and have mostly been used with immortalized cell lines [24,25].

A problem of all 3D cultures is the necrotic core due to lack of vascularization and therefore nutrient supply. Air-liquid interface culture has been proposed to solve this problem but requires thin sections that may more closely resemble 2D format [26] with a possibly negative impact on protein aggregation and related phenotypes. Nutrient supply could be improved by *in-vivo*-like vascularization, which would also address the important roles of the vascular system in AD pathogenesis [27]. However, vascularization of COs has only been achieved after transplantation into mouse cortex [28]. This chimeric approach was also used to model AD with iPSC-derived neurons [3] or microglia [7,29] implanted into mouse brains, which resulted in specific degeneration of human neurons in AD mice, albeit without tangle pathology [3]. Nonetheless, the field still lacks fully human 3D models that combine vascularization with the presence of central cell types, including microglia, and that recapitulate late-stage AD phenotypes.

In summary, 3D models are superior to 2D models by forming additional AD phenotypes such as extracellular protein accumulation, but require further improvements regarding reproducibility, controllability, and cell survival before being used to faithfully study disease mechanisms and develop drug-screening approaches.

Increasing cellular diversity

While initial iPSC-derived AD models contained only neurons to study pathomechanisms, more recent work moved beyond this neuron-centric view and addressed the disease-relevance of astrocytes, microglia and oligodendrocytes. All these cell types are essential in the nervous system for network dynamics and maintenance [30]. Their addition therefore improves physiology and relevance of *in vitro* cultures modeling brain function and dysfunction. Supporting this, iPSC-based neuron-astrocyte co-cultures show increased spontaneous and synchronized activity [31] and synapse maturation [32], suggesting higher network activity and maturity. Such a dense and mature network may be necessary to recapitulate activity-dependent phenotypes, such as increased neuronal Aβ secretion [33] and Aβ-dependent neuronal hyperactivity [34]. Besides making the model more physiological, all glia types modulate pathogenesis [35], and possibly even promote it at later stages.

Increased astrocyte activation was found in AD patients and mouse models, concomitantly with increased Aβ uptake and gliosis around plaques [36]. Furthermore, increased Aβ levels impair general astrocytic function, leading to compromised support of neurons in mice [37]. This was confirmed in AD-patient-derived astrocytes that displayed increased Aβ production, altered cytokine release and a general increase in cellular stress, resulting in altered Ca^{2+}-transients in co-cultured, healthy neurons [38]. Other studies additionally found intra-cellular Aβ accumulation [15], abnormal morphology, and mislocalization of key cellular proteins in AD astrocytes [39].

Microglia likely play an ambivalent role in AD, as shown in mouse models [40]. Early in disease, microglia activation results in increased clearance of extracellular Aβ and initial plaque containment by formation of a microglial barrier. However, long-term activation of microglia leads to non-physiological, pro-inflammatory phenotypes. These are likely detrimental for neurons, directly by secretion of neurotoxic factors and overactivation of synaptic pruning, and indirectly by exacerbating tau pathology [40]. In mice, microglia also drive plaque assembly, spreading, and deposition [41–43].

Although iPSC-derived microglia were incorporated into organoids [44] and transplanted into mouse brains [7,29], microglial disease contributions have not been investigated in human, iPSC-based AD models.

Oligodendrocytes (OLGs) are closely associated with AD pathogenesis as loss of myelin is among the earliest changes seen in patients [45]. OLG-differentiation protocols were established and co-culture with neurons or transplantation into mouse brains was achieved [46,47]. Brain organoids also generate OLG-like cells after prolonged culture [48,49], but compact myelination of neurons was observed only after transplantation into mice [46,47]. Likewise, no iPSC-based AD model containing OLGs has been described.

Importantly, glia are the main cells expressing many known AD risk genes such as ApoE and TREM2, and effects of risk variants in these genes have been studied in recent iPSC-based models. Astrocytes carrying two ApoE4 alleles, the strongest risk factor for AD, show reduced ApoE expression, increased cholesterol accumulation, and impaired Aβ clearance [50] as well as altered ApoE lipidation status and diminished support of neurons [51]. ApoE4 microglia display a pro-inflammatory transcriptome and impaired Aβ clearance [50]. TREM2-mutant microglia show specific impairments in the uptake of apoptotic bodies [52], impaired phagocytosis, and plaque clearance [53]. Importantly, microglia carrying the R47H variant show effects on TREM2 expression in mice but not in humans, highlighting the importance of studying human cells [54]. Likewise, many AD risk factors, including the different ApoE alleles, have no clear mouse orthologue or show low similarity between mouse and human [29], and human microglia behave differently in the AD brain compared to mouse microglia in disease models [4,5,7]. Also, it is unclear whether human microglia need human exogenous factors, cell-cell contacts, etc. to faithfully recapitulate disease-associated phenotypes, which may limit chimeric models.

In summary, future human AD models should incorporate a greater cellular diversity, ideally including all human glia types to better mimic human brain tissue and promote development of additional phenotypes. Furthermore, as mentioned above, the vascular system plays an important role in AD pathogenesis, thus vascular cell types should be incorporated into models to achieve formation of a neuro-glio-vascular unit and eventually *in vivo*-like vascularization.

Genetic background and genome editing

Initial iPSC-based AD models compared single stem cell lines from patients harboring familial AD mutations to unrelated healthy controls [13]. Hence, phenotypes detected in these lines may not only be attributed to AD mutations but also to differences in genetic background. Therefore, while these studies gave important insights, the results need to be interpreted carefully.

The caveat of genetic background variation in disease and control lines was subsequently addressed by applying gene editing tools to generate isogenic lines that only differ in the mutation of interest. This can be achieved by either correcting a mutation to wildtype in a patient line or inserting the mutation into a reference line from an unaffected donor. Initial approaches used zinc-finger nucleases (ZFNs) or transcription-activator-like effector nucleases (TALENs) for editing, but these were mostly replaced by CRISPR/Cas [8]. We and others developed CRISPR/Cas into a reliable tool to edit human iPSCs with high efficiency, and we demonstrated that knock-in of early-onset AD mutations in APP or PSEN1 elicited disease-relevant phenotypes [55,56].

Both patient lines with endogenous mutations and lines with edited disease-relevant mutations have advantages and disadvantages. Patient iPSCs not only harbor disease-causing mutations but represent the entire genetic background, including additional factors that may be required for formation of AD phenotypes. However, as patients with AD-causing mutations develop pathology only after decades,

patient-derived cells may not form late-stage phenotypes within experimentally trackable time frames. Mutation knock-in into healthy donor iPSCs facilitates studying and comparing strong APP and PSEN1 mutations in established lines that differentiate well into brain cells. Additionally, combinations of mutations, which do not occur together in patients, may accelerate pathology formation without generating overexpression artefacts [56]. AD overexpression mouse models are hampered by such artefacts not only due to unphysiological levels of mutant proteins [57], but also unintended consequences of transgene insertions [58]. In addition, knock-in lines allow to compare effects of different disease-associated mutations in an isogenic human background, thus facilitating the identification of fundamental pathomechanisms in a highly controlled experimental system. To illustrate this benefit, we contributed to the generation of a large resource of isogenic lines bearing diverse APP and PSEN1 mutations. In these lines increased β-CTF levels were associated with enlarged endosomes, confirming and extending previous findings on β-CTFs in mouse and cellular models ([56] and references therein).

In addition, gene editing can be used to introduce risk alleles and study their contribution to AD pathology. For example, a recent study found increased Aβ accumulation and Tau hyperphosphorylation in iPSC-derived organoids upon addition of ApoE4 microglia compared to isogenic ApoE3 lines [50]. Furthermore, iPSC-derived TREM2-knockout microglia showed reduced plaque clearance when plated on murine APP/PS1 mouse brain cryosections [53].

We anticipate that future studies will integrate additional risk alleles into existing disease models to promote formation of more advanced phenotypes. In addition, iPSC-derived disease models will also be useful to study effects of newly identified risk loci, modifiers, or protective factors, which can be incorporated by CRISPR/Cas genome editing. Thus, human data could be generated to support findings of GWAS studies and elucidate underlying biological mechanisms.

Cell maturation and ageing

AD affects mature brain cells in adults and is not found in children. Therefore, the inherent rejuvenation that takes place during reprogramming of somatic cells into iPSCs, which causes iPSC-derived neurons to display a fetal rather than adult phenotype [59], could be a major challenge in generating iPSC-based AD models. Fetal neurons may lack certain pathways or factors involved in disease, which could impair phenotype formation. Alternatively, or in addition, formation of AD pathology may require build up over many years in affected cells. Several studies addressed these drawbacks by keeping differentiated cells in culture over extended periods of time. However, as maturation markers, such as four repeat (4R)-Tau, are upregulated only after months to years [60,61], this approach is limited. As an alternative Miller et al. applied accelerated aging by expressing Progerin, a mutant form of Lamin A, that causes a premature-ageing syndrome. This resulted in expression of age-related markers in iPSC-neurons and elicited late-stage parkinsonian phenotypes not seen without Progerin [62]. Another possibility is to transdifferentiate one cell type directly into another, e.g. fibroblasts into induced neurons, omitting intermediate iPSC generation [63]. In contrast to reprogramming into pluripotency, direct conversion preserves age-related transcriptomic signatures and cellular characteristics [64,65]. Constrains of transdifferentiation efficiencies, which initially performed below 10%, were overcome in recent years [66], but generation of sufficient quantities, e.g. for gene-editing, is still limited as omitting the iPSC state restricts proliferative potential of the cells. New single-step approaches that combine gene-editing and transdifferentiation into neurons could solve this problem [67]. However, ageing of fibroblasts or other primary cell types is not necessarily similar to or relevant for the converted cell type, as illustrated by differences between ageing

signatures of fibroblasts and neurons (reviewed in [68]). Hence, transdifferentiated brain cells do not necessarily model AD more faithfully than iPSC-derived ones.

A key feature of AD pathology that might require age-dependent factors is aggregation of Aβ and Tau. Thus far, it remains challenging to robustly generate protein aggregates in iPSC-derived AD models. While aggregates of Aβ have been described in some organoids [20,21], formation of tangle-like structures was so far only reported in one study [21]. Aggregation of Tau and Aβ in iPSC-derived neurons may be accelerated by addition of synthetic or brain-derived protein aggregates, which can yield important information on the propagation of pathology [69], but does not allow to study endogenous processes contributing to initial aggregation. To achieve endogenous aggregation, an aged (extra-)cellular environment may be necessary that promotes formation of ordered assemblies of Aβ and Tau, for example due to impairments in proteostasis [70], altered levels of co-factors, such as ASC-specks derived from microglia [41], or overactivation and exhaustion of phagocytic cells [40,71].

Another major maturation-dependent factor that plays a central role in AD pathology and potentially protein aggregation is Tau isoform expression. Two classes of Tau isoforms exist, 3R and 4R, which differ by inclusion of Exon 10 and thus an additional repeat region in 4R as opposed to 3R isoforms. Fetal neurons only express 3R Tau while the adult human brain expresses the same amount of 3R and 4R isoforms [72]. Since 4R Tau is more aggregation-prone [73] and Tau aggregates in AD consist of both 3R and 4R isoforms, 4R expression might be a prerequisite to recapitulate late-stage AD phenotypes such as Tau tangles. Recent Cryo-EM experiments have also shown involvement of the 4th repeat as a structural part of protofilaments in paired helical and straight filaments in AD brain [74]. Following differentiation, iPSC-derived neurons express mostly 3R Tau, while 4R Tau is only expressed after months to years. This maturation-dependent isoform expression likely contributes to difficulties in mimicking Tau-related pathologies. Although some Tau mutations increase expression of 4R Tau in iPSC-derived neurons and accelerate their maturation, adult-like 1:1 ratio of 3R and 4R is seen only after a year in culture [60,61]. To tackle this problem, 4R expression was elevated by modulating alternative splicing of Exon 10 [75]. However, this has not yet been tested in iPSCs. Another study demonstrated that 4R Tau levels increase to adult 1:1 ratio upon transplantation into mouse brain [3], indicating that some brain-derived soluble factor, cell-cell interaction, topology in 3D or other environmental effects are responsible for induction of adult Tau isoform expression. The field therefore needs to develop approaches to achieve adult expression of Tau isoforms *in vitro*.

In summary, future models could be optimized by accelerated ageing paradigms to more accurately mimic the molecular conditions present in aged brain and promote formation of late-stage AD phenotypes.

Conclusions

iPSC-based AD models harbor great potential to study disease in a human system using patient-derived and gene-edited cells. Currently available models carrying AD mutations recapitulate early disease phenotypes such as cell stress, Aβ accumulation, and tau hyperphosphorylation, confirming findings from other cellular systems and mice. They also allow insights into disease processes difficult to study before, for example the effect of AD risk factors such as ApoE4 in disease-relevant, human brain cells with physiological expression of disease-associated genes.

Concomitant advances in differentiation protocols, gene editing, and tissue engineering recently enabled the development of more complex, mature and physiological disease models that include additional, genetically manipulated cell types grown in 3D, for example in brain organoids. These models show more advanced phenotypes such as progressive, extracellular protein accumulation. Nevertheless, no human iPSC-based model exists that faithfully and reproducibly recapitulates a

complete AD phenotype, including protein aggregation in plaques and tangles, gliosis and neurodegeneration. This would be prerequisite to study pathomechanisms underlying the Aβ-Tau axis, neuroinflammation, and cell death in a human context. Additionally, translational research to identify novel therapeutic targets depends on physiological models that display these central pathological hallmarks.

To enhance current AD models, we suggest combined implementation of the following improvements (see Box): 1) apply 3D cultures with controllable and reproducible culture parameters (cell density, composition, maturity), to enable physiological cell-cell and cell-matrix interactions, stimulate maturation of cells, and facilitate protein aggregation, 2) incorporate glial and vascular cells, to eventually include all relevant brain cell types and address their disease-modulating roles, for example in neuroinflammation, 3) use multiple and isogenic lines to increase relevance and reproducibility of disease phenotypes and minimize the impact of non-relevant genetic background effects, 4) mature and age cells by prolonged culture or genetic manipulations, in particular to increase 4R tau expression and elicit tangle formation. Together, these enhancements will promote the development of next generation iPSC-models with advanced phenotypes, that will provide novel insights into pathomechanisms and a new basis for drug development.

Figure Legend:

Strategies to generate current and future iPSC-based models of Alzheimer's disease.
Current iPSC models do not reproduce all AD hallmarks observed in patient brains. Most models (first column) consist of neurons cultured in 2D format or 3D organoids and vary considerably regarding cell type composition and recapitulation of disease phenotypes. Furthermore, patient iPSC models with single, often weak, mutations may not generate AD pathology within an experimentally trackable time frame. Epigenetic rejuvenation of cells during iPSC reprogramming may further impede phenotype formation. We therefore propose several improvements (second column): To achieve more physiological conditions, other disease-relevant, non-neuronal cell types should be incorporated, including astrocytes, microglia, oligodendrocytes and vascular cell types. Growing the cells in reproducible and controllable 3D cultures or organoids may further increase robustness of the disease models. Additionally, gene editing can be used to combine strong mutations and genetic risk factors to promote phenotype formation. Lastly, artificial ageing paradigms could enhance cellular maturation to more closely recapitulate the state and environment of affected cells in aged patient brains. These models might reproducibly generate late-stage AD phenotypes such as Aβ plaques, neuroinflammation, Tau tangles, and neurodegeneration, and thus help uncover novel pathomechanisms and therapeutic targets.

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Special:

[5] Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Menon M, He L, Abdurrob F, Jiang X, et al.: **Single-cell transcriptomic analysis of Alzheimer's disease**. *Nature* 2019, **570**:332–337.

[6] Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, et al.: **A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease**. *Cell* 2017, **169**:1276-1290.e17.

These two studies investigate gene expression changes in microglia in AD. Studying 5xFAD mice, Keren-Shaul et al. identified a microglia subtype that is associated with neurodegenerative diseases (disease-associated microglia - DAM). DAMs are found close to Aβ plaques and have a unique transcriptional signature (DAM signature). Mathys et al. provided the first single-cell transcriptomic analysis of human AD brain, analyzing six major cell types and two different stages of pathogenesis. Mouse and human microglia disease signatures differed, but potential key modulators such as ApoE showed the same effects.

[26] Giandomenico SL, Mierau SB, Gibbons GM, Wenger LMD, Masullo L, Sit T, Sutcliffe M, Boulanger J, Tripodi M, Derivery E, et al.: **Cerebral organoids at the air–liquid interface generate diverse nerve tracts with functional output**. *Nat Neurosci* 2019, **22**:669–679.

Addressing insufficient nutrient and oxygen supply of current organoids this study established air-liquid interface culture of sliced mature cerebral organoids, which improved long-term cell survival, especially in the center of the organoids, as well as axon outgrowth, including long-range projections that can innervate adjacent mouse tissue.

[28] Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, Johnston S, Parylak SL, Jin X, Gage FH: **An in vivo model of functional and vascularized human brain organoids**. *Nat Biotechnol* 2018, **36**:432–441.

The authors established a method for transplanting human brain organoids into the adult mouse brain, leading to vascularization by mouse vessels which was essential for graft survival. Grafts showed progressive maturation, gliogenesis, integration of microglia, and growth of functional connections to the host brain.

[41] Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, Vieira-Saecker A, Schwartz S, Santarelli F, Kummer MP, et al.: **Microglia-derived ASC specks crossseed amyloid-β in Alzheimer's disease**. *Nature* 2017, **552**:355–361.

[42] Sosna J, Philipp S, Albay RI, Reyes-Ruiz JM, Baglietto-Vargas D, LaFerla FM, Glabe CG: **Early long-term administration of the CSF1R inhibitor PLX3397 ablates microglia and reduces accumulation of intraneuronal amyloid, neuritic plaque deposition and pre-fibrillar oligomers in 5XFAD mouse model of Alzheimer's disease**. *Mol Neurodegener* 2018, **13**:1–11.

[43] Spangenberg E, Severson PL, Hohsfield LA, Crapser J, Zhang J, Burton EA, Zhang Y, Spevak W, Lin J, Phan NY, et al.: **Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model**. *Nat Commun* 2019, **10**:3758.

These 3 studies highlight the importance of microglia in plaque assembly, spreading and deposition in AD mouse models. Venegas et al. showed that ASC specks, protein complexes secreted by activated microglia, enhance Aβ aggregation and plaque deposition. Sosna et al. and Spangenberg et al. showed that long-term depletion of microglia during the plaque-forming period prevents intraneuronal amyloid accumulation and neuritic or parenchymal plaque development, respectively.

[50] Lin YT, Seo J, Gao F, Feldman HM, Wen HL, Penney J, Cam HP, Gjoneska E, Raja WK, Cheng J, et al.: **APOE4 Causes Widespread Molecular and Cellular Alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived Brain Cell Types**. *Neuron* 2018, **98**:1141-1154.

This study examined the effects of ApoE4 in iPSC-derived neurons, astrocytes and microglia compared to isogenic ApoE3 cells. The authors found detrimental effects in all three cell types, including slightly increased Aβ generation and elevated early endosomes in neurons, cholesterol accumulation and impaired Aβ clearance in astrocytes and a pro-inflammatory signature as well as impaired Aβ clearance in microglia.

[55] Paquet D, Kwart D, Chen A, Sproul A, Jacob S, Teo S, Olsen KM, Gregg A, Noggle S, Tessier-Lavigne M: **Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9**. *Nature* 2016, **533**:125–129.

This study describes a CRISPR genome editing platform that allows efficient editing of mutations in iPSCs. Using this platform, the authors demonstrated that knock-in of APP_{Swe} and $PSEN_{M146V}$ mutations elicit mutation- and zygosity-dependent phenotypes in iPSC-derived neurons.

[56] Kwart D, Gregg A, Scheckel C, Murphy E, Paquet D, Duffield M, Fak J, Olsen O, Darnell R, Tessier-Lavigne M: **A Large Panel of Isogenic APP and PSEN1 Mutant Human iPSC Neurons Reveals Shared Endosomal Abnormalities Mediated by APP β-CTFs, Not Aβ**. *Neuron* 2019, **104**:256–270.

The authors generated a large panel of fully isogenic iPSC lines carrying a variety of both homo- and heterozygous APP and PSEN1 mutations. Differentiated mutant neurons showed early

endosome enlargement, mediated by APP β-CTFs, confirming the important roles of APP β-CTFs and endosomal abnormalities in early AD pathogenesis.

Outstanding:

[3] Espuny-Camacho I, Arranz AM, Fiers M, Snellinx A, Ando K, Munck S, Bonnefont J, Lambot L, Corthout N, Omodho L, et al.: **Hallmarks of Alzheimer's Disease in Stem-Cell-Derived Human Neurons Transplanted into Mouse Brain**. *Neuron* 2017, **93**:987-989

This study pioneered the use of human-mouse chimeric models for AD research by transplantation of wildtype iPSC-derived neurons into AD (APP/PS1) mouse brain, where they integrated and e.g. upregulated 4R-Tau to the adult isoform ration of 3R:4R = 1:1. The authors observed human-specific neurodegeneration in the absence of Tau tangle formation, but in the presence of hyperphosphorylated and misfolded tau.

[7] Hasselmann J, Coburn MA, England W, Figueroa Velez DX, Kiani Shabestari S, Tu CH, McQuade A, Kolahdouzan M, Echeverria K, Claes C, et al.: **Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo**. *Neuron* 2019, **103**:1016–1033.

To investigate human microglia in an experimental AD model, the authors transplanted human iPSC-derived hematopoietic precursor cells (HPCs) into mouse brain ventricles, where they engrafted and differentiated into functional microglia. The microglia signature of transplanted human microglia differed from the mouse DAM signature as well as other human AD RNAseq data (4, 5). Again, changes in potential key genes such as ApoE and TREM2 were conserved.

[20] Raja WK, Mungenast AE, Lin Y-TT, Ko T, Abdurrob F, Seo J, Tsai L-HH: **Self-organizing 3D human neural tissue derived from induced pluripotent stem cells recapitulate Alzheimer's disease phenotypes**. *PLoS One* 2016, **11**:1–18.

This study pioneered the use of cerebral organoids differentiated from AD-patient-derived iPSCs using cells carrying APP Duplication, $PSEN_{M146I}$ or $PSEN1_{A246E}$ mutations, and showed progressive, extracellular amyloid deposition in Aβ puncta. However, no Aβ plaques or Tau tangles were observed.

[21] Gonzalez C, Armijo E, Bravo-Alegria J, Becerra-Calixto A, Mays CE, Soto C: **Modeling amyloid beta and tau pathology in human cerebral organoids**. *Mol Psychiatry* 2018, **23**:2363–2374.

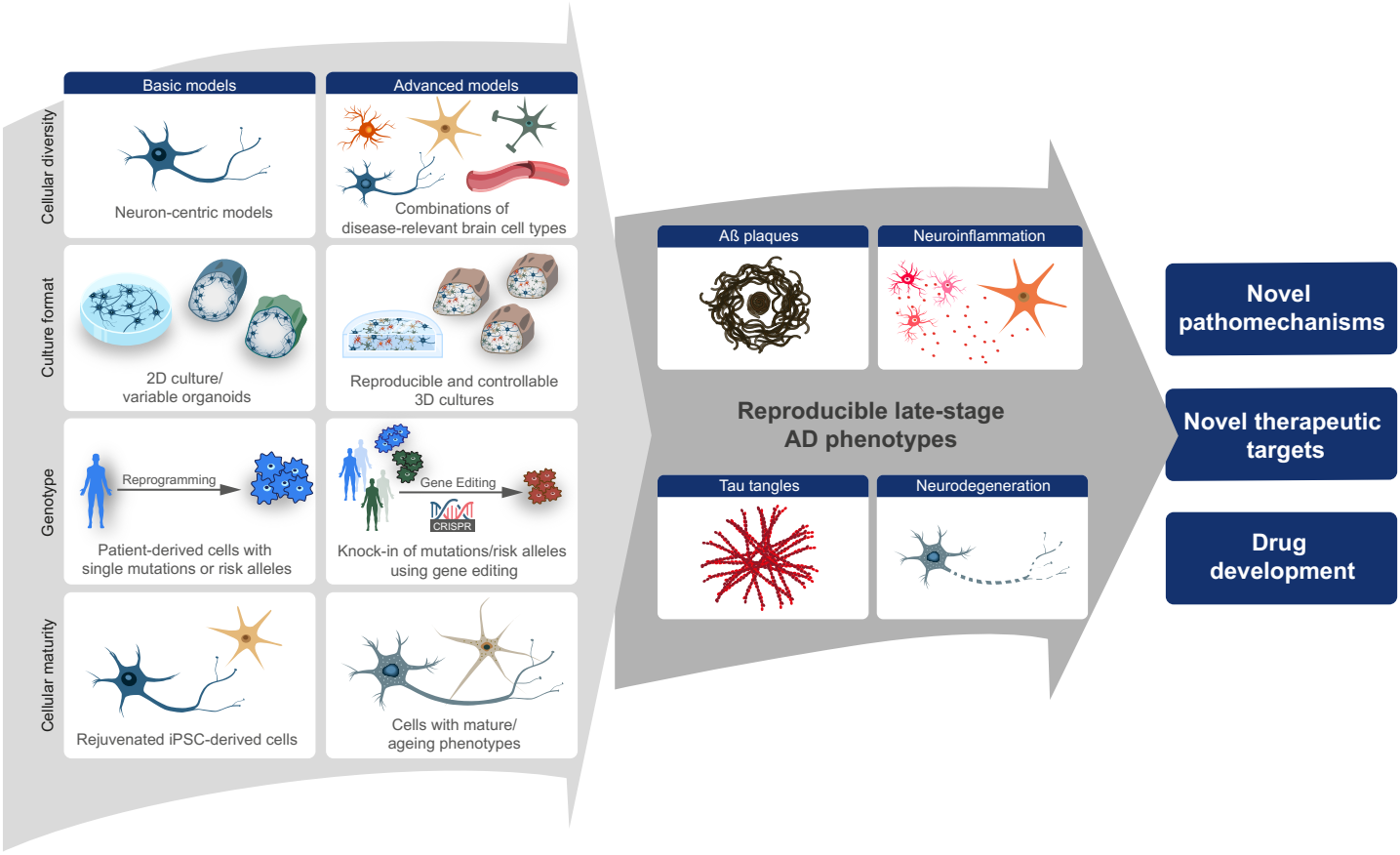
Gonzalez et al. used cerebral organoids differentiated from a down-syndrome patient or, as in Raja et al., an AD patient carrying the $PSEN1_{A246E}$ mutation. The authors described structures positive for a Thioflavin-derivative and Gallyas silver, resembling Aβ plaques and Tau tangles, respectively.

[22] Velasco S, Kedaigle AJ, Simmons SK, Nash A, Rocha M, Quadrato G, Paulsen B, Nguyen L, Adiconis X, Regev A, et al.: **Individual brain organoids reproducibly form cell diversity of the human cerebral cortex**. *Nature* 2019, **570**:523–527.

Addressing the high variability of current cortical organoids this study described a protocol to generate dorsally patterned forebrain organoids with high consistency, reproducibility and similarity to fetal human brain, as shown by single-cell RNA sequencing.

Highlights:

- iPSCs allow modeling of neurodegenerative disorders in disease-relevant human systems
- recapitulation of late-stage disease phenotypes remains challenging
- new strategies have emerged to advance iPSC models and tackle these challenges
- increased cellular diversity and 3D culture allow more physiological disease models
- editing mutations/risk factors and improving maturation may promote pathology


Basic models
Advanced models
Cellular diversity
Neuron-centric models
Combinations of disease-relevant brain cell types
Culture format
2D culture/ variable organoids
Reproducible and controllable 3D cultures
Genotype
Reprogramming
Patient-derived cells with single mutations or risk alleles
Gene Editing
CRISPR
Knock-in of mutations/risk alleles using gene editing
Cellular maturity
Rejuvenated iPSC-derived cells
Cells with mature/ ageing phenotypes
Aß plaques
Neuroinflammation
Reproducible late-stage AD phenotypes
Tau tangles
Neurodegeneration
Novel pathomechanisms
Novel therapeutic targets
Drug development

Milestones for the optimization of human iPSC-based AD models

- Reproducibility of cultures regarding cellular composition and phenotype formation
- Representation of all disease-relevant brain cell types and ability to modulate proportions and genotypes
- Generation of complex tissues with near-physiological cell interactions and homogeneous nutrient supply
- Maturity of utilized cell types and representation of age-related pathological changes
- Exclusion of genetic background variation by using isogenic controls
- Recapitulation of central AD hallmarks, including plaques, tangles, neurodegeneration, and -inflammation
- Formation of phenotypes within experimentally trackable time frames
- Scalability of cultures with reproducible AD phenotypes to enable drug screening