Received: 2 September 2020 | Revised: 28 October 2020 | Accepted: 17 November 2020

DOI: 10.1002/stem.3309

Check for updates

STEM CELLS®

EMBRYONIC STEM CELLS/INDUCED PLURIPOTENT STEM CELLS

# Metabolic and immune dysfunction of glia in neurodegenerative disorders: focus on iPSC models

Taisia Rõlova[1] | Šárka Lehtonen[1,2] | Gundars Goldsteins[2] | Pinja Kettunen[1] | Jari Koistinaho[1,2]

[1]Neuroscience Center, HiLIFE, University of Helsinki, Helsinki, Finland

[2]A. I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

**Correspondence**
Email: jari.koistinaho@uef.fi

## Abstract

The research on neurodegenerative disorders has long focused on neuronal pathology and used transgenic mice as disease models. However, our understanding of the chronic neurodegenerative process in the human brain is still very limited. It is increasingly recognized that neuronal loss is not caused solely by intrinsic degenerative processes but rather via impaired interactions with surrounding glia and other brain cells. Dysfunctional astrocytes do not provide sufficient nutrients and antioxidants to the neurons, while dysfunctional microglia cannot efficiently clear pathogens and cell debris from extracellular space, thus resulting in chronic inflammatory processes in the brain. Importantly, human glia, especially the astrocytes, differ significantly in morphology and function from their mouse counterparts, and therefore more human-based disease models are needed. Recent advances in stem cell technology make it possible to reprogram human patients' somatic cells to induced pluripotent stem cells (iPSC) and differentiate them further into patient-specific glia and neurons, thus providing a virtually unlimited source of human brain cells. This review summarizes the recent studies using iPSC-derived glial models of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis and discusses the applicability of these models to drug testing. This line of research has shown that targeting glial metabolism can improve the survival and function of co-cultured neurons and thus provide a basis for future neuroprotective treatments.

**KEYWORDS**

astrocytes, iPSC, metabolism, microglia, neurodegenerative disorders

## 1 | INTRODUCTION

Neurodegenerative diseases represent an increasing burden on society, but despite the decades of research, we still incompletely understand the disease mechanisms. Available treatments are symptomatic and, in most cases, do not have disease-modifying effects. We know that Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) are all hallmarked by cytoplasmic and extracellular insoluble protein deposits, metabolic abnormalities, gliosis, and synaptic/neuronal loss primarily affecting memory and cognition-controlling limbic areas in AD, motor function-controlling dopaminergic system in PD, and the primary motor cortex and motor neurons of the spinal cord in ALS. But what is the real cause and what is the mere effect of the disease is not clear in most cases. While initial research into neurodegenerative disorders concentrated on cell-

intrinsic neuronal dysfunction, it is more recognized now that glia can play a critical role in pathology development and progression. Notably, some of the most studied disease-causing mutations are located in genes exhibiting the highest expression in microglia in the human central nervous system, including hexanucleotide repeat GGGGCC in *C9orf72* associated with ALS and familial frontotemporal dementia, and *LRRK2* G2019S mutation associated with PD [1]. Also, many of the risk genes, identified by genome-wide association studies (GWAS) for AD [2], PD [3], and ALS [4], have a strong expression in microglia and astrocytes. For example, AD-associated GWAS genes belong to immune response, phagocytosis/endocytosis, lipid metabolism, and synaptic and axonal function pathways. The strongest genetic risk factor for AD is the ε4 allele of lipid carrier *APOE*, which increases the risk 4-fold on average. *APOE* has strong expression in mature human astrocytes and is upregulated in plaque-associated microglia in AD [5,6].

So far, the animal models have been the main tool for investigating the glial role in neurodegenerative disorders. Unfortunately, the majority of findings from the animal models have had poor translational value in clinical studies. One of the major reasons for this translational failure is thought to be the fact that human glia, and particularly astrocytes, are fundamentally distinct from rodent ones with their inherent differences in structure, calcium signaling, and metabolic processes [1].

Recent advances in stem cell technology make it possible to reprogram patients' somatic cells to induced pluripotent stem cells (iPSC) [7] and differentiate them further into disease-relevant cell types. Two types of iPSC-based disease models are widely used: patient-specific or gene-modified, where the causative mutation of interest is introduced into healthy donor iPSC. In this review, we will discuss the contribution of glial dysfunction to the pathogenesis of neurodegenerative disorders with the focus on iPSC-derived cell models and the use of these models in drug testing.

**Significance Statement**

This paper summarized recent advances in iPSC-based modelling of glial cell contribution to major neurodegenerative diseases. Glial cell have a crucial role in brain functions and are strongly affected in neurodegenerative diseases, Information on the role of human glial cells in neurodegenerative diseases is likely to accelerate development of novel therapies for these diseases.

## 2 | BASIC CHARACTERIZATIONS OF AD, PD, AND ALS

AD is the most common cause of dementia. Its main hallmarks include progressive cognitive and memory decline, deposition of amyloid-β (Aβ) in the hippocampal and cortical areas, the formation of intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein, selective synaptic and neuronal loss, as well as the proliferation of astrocytes and microglia. The accumulation of Aβ oligomers, especially Aβ42, is toxic for neurons and is believed to be the initial trigger of AD pathology. The strongest evidence for the critical role of Aβ is the familial form of AD caused by mutations in *APP* and *PSEN1* and *2* genes increasing the production of Aβ, especially Aβ42. Since Aβ plays physiological roles in synapse formation [8], and its secretion positively correlates with neuronal activity [9,10], it is not surprising that the formation of Aβ deposits occurs in the areas of life-long synaptic remodeling. However, there is a lot of person to person variation in how much Aβ is accumulated over the lifetime and in the rate of aging-associated cognitive decline, and the reasons for that are poorly understood. Aβ42 is also proposed to play a role in immune defenses [11] and be overproduced in the brain in response to an invading pathogen, such as herpes simplex virus type I or periodontal pathogens [12]. A deficient glial response might lead to a chronic neurodegenerative process. Regardless of the mechanism, the accumulation of aggregated Aβ in the brain is followed by a decrease in glucose consumption, especially in aerobic glycolysis [13] and fatty acid metabolism [14]. These metabolic changes are taking place prior to the obvious brain atrophy.

Pathologically, PD involves a loss of dopaminergic (DA) neurons in the substantia nigra (SN) and subsequent loss of dopamine in the striatum, leading to the development of motor symptoms. An aberrantly-folded protein α-synuclein has emerged as a hallmark of PD, being present in Lewy bodies and Lewy neurites. Moreover, α-synuclein aggregates are also prominent in astrocytes. The molecular mechanisms underlying DA neurons' selective vulnerability, especially in SN pars compacta, remain poorly understood. Earlier investigations identified several highly penetrant monogenic rare mutations, including *SNCA* and *LRRK2*, responsible for autosomal-dominant PD forms. G2019S mutation in the *LRRK2* gene, which enhances LRRK2 activity and impairs endolysosomal trafficking, is of particularly great interest due to its high incidence. Another common genetic risk factor for PD is a mutation in *GBA1*, increasing the risk by 20-30%. *GBA1* encodes glucocerebrosidase, a lysosomal enzyme involved in the metabolism of glycosphingolipids.

Similarly to AD, energy metabolism is impaired in PD patients [15]. Transcriptomics analyses have shown that the most prominent pathways altered in PD include DA metabolism, oxidative stress, protein degradation, and neuroinflammation as well as mitochondrial function, vesicular transport, and synaptic transmission [16].

ALS is characterized by the degeneration of the upper and lower motor neurons in the motor cortex and spinal cord, resulting in paralysis of voluntary muscles. Although survival is variable, respiratory failure usually leads to death in 3-4 years after disease onset. Less than 10% of ALS cases have a family history, caused by mutations in at least 16 genes, among them in *SOD1*, *C9orf72*, *FUS*, *TARDBP* (TDP-43), and *OPTN* [17]. The expansion of a hexanucleotide repeat GGGGCC in *C9orf72* is the most common genetic cause of ALS. Over 95% of ALS cases exhibit cytoplasmic accumulation of TDP-43 protein aggregates with prion-like function [18]. A number of studies

using metabolomics, transcriptomics, and lipidomics in ALS models and human patients indicate significant metabolic dysregulation, including glucose, purine, pyrimidine, lysine, and glycerophospholipid metabolism pathways [19-22].

## 3 | ASTROCYTES

Astrocytes originating from neuroectoderm play a major role in maintaining tissue homeostasis and regulating brain metabolism. Astrocytes support neuronal activity by providing growth factors and nutrients, including lactate, recycling neurotransmitters, primarily glutamate, and regulating the ionic composition of the extracellular space. Astrocytes can also both secrete and uptake Aβ and α-synuclein. There is accumulating evidence that astrocyte metabolism is impaired in neurological conditions; hereby, the astrocytes cannot support efficiently neuronal synaptic activity and exert neurotoxic effects. This effect has been more extensively studied in the models of ALS.

Exposure of iPSC-derived motor neurons originating from healthy human individuals to sporadic ALS post-mortem tissue extracts containing aggregated TDP-43 induced intraneuronal TDP-43 aggregation, thus causing neurotoxicity [23] (Figure 1 A). Interestingly, a co-culture with healthy astrocytes had a neuroprotective effect by reducing neuronal TDP-43 aggregation. *TARDBP* (TDP-43) mutant astrocytes exhibited subcellular mislocalization of TDP-43 and increased cell death but did not have neurotoxic effects on co-cultured healthy control motor neurons [24] (Figure 1 A). On the other hand, iPSC-derived astrocytes carrying ALS-associated mutations in *SOD1* or *C9orf72* displayed both cell-intrinsic abnormalities as well as neurotoxic effects on healthy control motor neurons [25-28], which was confirmed by the generation of isogenic control astrocytes in one study [26]. The neurotoxic effects of ALS astrocytes have been attributed to impaired response to EphB1 released by injured neurons [25], dysregulation of neuronal voltage-activated Na + and K+ currents [26], premature acquisition of senescent phenotype and down-regulation of antioxidant molecules [27], and increased expression of connexin 43 gap junction protein [28]. Additionally, the astrocytes carrying *SOD1* A4V or *FUS* H517Q mutation were able to drive upregulation of multidrug resistance transporter *ABCB1* (P-Glycoprotein) in healthy control endothelial cells via the NF-κB pathway [29], thus potentially regulating molecular transport across the blood-brain barrier.

However, the most evident proof of concept for the deleterious phenotype of ALS astrocytes is the recent study using the transplantation of human sporadic ALS astrocytes into the mouse spinal cord [30] (Figure 1 B). In this study, transplanted ALS astrocytes displayed a neuroinflammatory phenotype, resulting in the degeneration of host motor neurons, followed by the appearance of motor deficits.

Similarly to ALS, it is becoming increasingly evident that the loss of DA neurons in PD does not arise from an intrinsic degenerative mechanism only but involves the interaction of neurons with neighboring glial cells (Figure 2). Healthy human astrocytes rescued the DA neurons exposed to a mitochondrial stressor, rotenone or potassium cyanide, by restoring the mitochondrial function and dynamics [31]. However, the studies on iPSC-derived astrocytes carrying PD-associated mutations *LRRK2* G2019S and *GBA1* N370S have suggested that PD astrocytes have impaired ability to degrade α-synuclein [32-34] and can induce α-synuclein accumulation, neurite dystrophy, and cell death in co-cultured neurons [35]. Our laboratory has demonstrated that PD astrocytes exhibit metabolic abnormalities, disturbed $Ca^{2+}$ homeostasis, impaired mitochondrial function, and an increased release of cytokines upon inflammatory stimulation [34]. Increased levels of polyamines and polyamine precursors and decreased levels of lysophosphatidylethanolamine reported in PD astrocytes [34] have also been observed in PD patients [36,37]. The activation of chaperone-mediated autophagy (CMA) reduced the toxic effects of PD astrocytes on co-cultured neurons in one study (Table 1) [35].

IPSC-derived astrocytes have also been generated to study the toxic effects of mutant glia in AD. As mentioned above, the strongest genetic risk factor for AD is the *APOE* ε4 allele. The primary function of APOE protein in the brain is to mediate lipid traffic between astrocytes and neurons. *APOE* ε4/ ε4 iPSC-derived astrocytes secrete less APOE, exhibit intracellular accumulation of cholesterol, display impaired clearance of extracellular Aβ42, and provide less metabolic support to neurons in co-culture [38,39] (Figure 3). These results are in accordance with recent single-cell transcriptomics data from human patients showing reduced expression of *APOE* gene in AD astrocytes [5,6]. Even more evidence for the deleterious effects of *APOE* ε4 expression in astrocytes comes from the study on brain organoids that allow generating a more in vivo-like 3D environment for the cells. At two months, the organoids contain mostly neurons and neural precursors, while at six months, there is a significant proportion of mature astrocytes. The six-month-old *APOE* ε4/ ε4 organoids, but not the two-month-old, exhibited a significantly higher level of Aβ42 and phospho-tau accumulation than ε3/ ε3 organoids, thus indicating that astrocyte dysfunction may have contributed to a higher level of AD-like pathology [38].

Our laboratory has reported the generation of astrocytes from three familial AD patients (*PSEN1* ΔE9) and two isogenic controls [40], and found an increased secretion of Aβ42, and a number of abnormalities in calcium signaling, glycolysis, fatty acid oxidation, production of reactive oxygen species, and glutathione secretion in these familial AD astrocytes [40-42]. The findings suggest that these astrocytes do not efficiently utilize energy resources and cannot provide adequate metabolic support to neurons. Indeed, a co-culture of healthy human neurons with diseased astrocytes impaired neuronal calcium responses to glutamate and γ-aminobutyric acid (GABA) as compared to the co-culture with healthy human astrocytes [40]. The treatment with sulforaphane, a compound isolated from cruciferous vegetables, induced Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, normalized basal level glycolysis, decreased basal level Aβ42 secretion as well as ameliorated inflammatory response to pro-inflammatory cytokines TNFα and IL1β in *PSEN1* mutant iPSC astrocytes [41]. Another compound GW0742, a synthetic ligand agonist of PPARβ/δ,

normalized fatty acid oxidation and increased *CPT1A* gene expression in these cells without affecting glycolysis [42]. Interestingly, a very recent study has shown that glycolysis-derived L-serine production is impaired in astrocytes in an AD mouse model [43]. Deficient L-serine production by astrocytes contributed to cognitive defects that could be rescued by supplementation with L-serine in the diet. Immunoreactivity of PHGDH, a rate-limiting enzyme of the L-serine biosynthesis pathway, was significantly decreased in human AD brains in that study. It has also been shown that enhancing glycolysis can have a neuroprotective effect in PD models and clinical databases [44]. On the whole, these studies suggest that metabolic abnormalities in diseased human astrocytes can be corrected by pharmacological treatment.

There is also an indication that FTD- and AD-associated tau mutation can cause astroglial dysfunction, adversely affecting the co-cultured neurons [45]. The co-cultures of healthy neurons with FTD astrocytes were more sensitive to rotenone exposure than the co-cultures with control isogenic astrocytes. Mutant tau astrocytes upregulated a number of genes, especially the ones mediating endocytosis and cell-matrix interactions.

Interestingly, a very recent study using 3D co-culture of control unmodified iPSC-derived neurons and astrocytes embedded in silk sponge scaffold [46] has reported that low-grade human herpes simplex virus type 1 (HSV-1) infection can induce AD-like pathology, including the formation of amyloid plaques and gliosis, and antiviral medication with valaciclovir decreased pathological signs. This finding provides evidence for the theory of pathogen-induced AD pathology.

## 4 | OLIGODENDROCYTES

Similarly to astrocytes, oligodendrocytes are the cells of neuroectodermal origin. The primary function of oligodendrocytes was long considered to be the production of myelin, which ensheathes neuronal axons and enables fast signal transmission. However, it is increasingly recognized now that oligodendrocytes are indispensable in providing nutrients, especially lactate, to long neuronal axons [47]. Studies indicate significant demyelination in both white and gray matter in ALS [48] and AD patients [49]. Also, some microstructural myelin changes have been observed in PD patients [50]. It was believed that demyelination is mostly occurring due to intrinsic neuronal pathology. However, transgenic mouse studies have indicated that oligodendrocyte dysfunction exacerbates neuronal degeneration [47].

Several protocols for the generation of iPSC-derived oligodendrocytes have been established, with some producing up to 70% of O4 positive cells [51]. However, only a handful of studies have been published so far investigating the role of human oligodendrocytes in neurodegenerative conditions (apart from multiple sclerosis, which is not discussed in this review). Two studies have indicated that oligodendrocytes derived from human sporadic and familial ALS patients differentiate and maturate normally [52,53]. However, ALS oligodendrocytes of various genetic backgrounds exerted toxic effects on motor neurons through conditioned medium and in co-culture, killing about 50% of motor neurons on average [52]. All ALS oligodendrocytes, except the ones carrying *C9orf72* expansion, produced significantly lower levels of lactate as compared to healthy controls. The addition of lactate and the knock-down of human SOD1 in oligodendrocyte progenitors protected motor neurons treated with ALS oligodendrocyte-conditioned medium, with the exception of *C9orf72* expansion, and also slightly protected the neurons in co-culture with ALS astrocytes. Thus, the toxic effects of *C9orf72* expansion in oligodendrocytes may have a different mechanism than other common ALS-associated mutations.

Similarly to the ALS study, iPSCs carrying an FTD-associated tau mutation generated normal-looking oligodendrocytes [54]. However, these oligodendrocytes were more sensitive to rotenone-induced oxidative stress. It is known that astrocytes can affect myelination, but the mechanisms are not very clear. A recent study has shown that human iPSC-derived astrocytes carrying a mutation in *GFAP* inhibit the proliferation of iPSC-derived oligodendrocyte progenitors in co-culture and reduce their ability to myelinate nanofibers [55].

Overall these studies provide evidence that in neurodegenerative disorders oligodendrocytes get dysfunctional and provide inadequate metabolic and structural support to neuronal axons, although more research is clearly needed.

## 5 | MICROGLIA

Microglia are endogenous brain cells of myeloid origin constituting approximately 5-15% of the adult CNS population with predominance in the white matter [56]. Microglia help to maintain and restore tissue homeostasis and regulate synaptic remodeling and neurogenesis. However, in neurological conditions, microglia get overwhelmed and do not respond appropriately to stimuli anymore, thus impairing neuronal function. Many known AD-associated risk genes are expressed in microglia, including *TREM2*, *APOE*, *PLCG2*, *CR1*, *CD33*, *CHI3L1*, *CLU*, and *ABI3* [2,57].

Recently, several relevant protocols for the differentiation of iPSC-derived microglia-like cells have been published and extensively reviewed by Speicher and coworkers [58]. So far, there are no studies of parallel comparison of various microglia differentiation protocols regarding the achieved human microglia phenotype. However, the protocols by Dr. Blurton-Jones' group [59,60] and Haenseler and coworkers [61] have reported the highest yield and purity.

Despite some differences in protocols used, several studies have shown that the *APOE* ε4 allele impaired the function of iPSC-derived microglia/macrophage-like cells as compared to the *APOE* ε3 allele. *APOE* ε4/ε4 microglia-like cells upregulated genes involved in immune response, response to oxygen-containing component and response to an external stimulus, and downregulated genes involved in the movement of cellular component and cell development [38] (Figure 3). Also, *APOE* ε4/ ε4 cells displayed impaired uptake of extracellular Aβ42, impaired chemokinesis, impaired phagocytosis of zymosan-coated beads, and deficits in both mitochondrial respiratory capacity and glycolytic capacity [38,62] (and our unpublished data) when compared to

*APOE* ε3/ε3 cells. Similar metabolic deficits were observed in iPSC-derived microglia-like cells carrying the missense *TREM2* mutations associated with different forms of dementia, including AD, FTD, and Nasu-Hakola disease [63,64]. These deficits could be partially rescued by treatment with pioglitazone, a potent agonist of PPARγ [64], and are consistent with Nanostring transcriptomics analysis of parietal cortex of human AD patients showing that the brains carrying *TREM2* R47H mutation displayed elevated expression of genes involved in oxidative stress and lipid metabolism, along with a decreased expression of the genes involved in autophagy, growth-factor signaling, and neural connectivity [65]. Also, another immunohistochemical study showed the accumulation of autophagosomes inside microglia in AD brains with *TREM2* mutations [66]. The effect is likely mediated through impaired mTOR signaling [67,68] and PLCγ2 [69]. In a very recent study, iPSC-derived microglia carrying protective P522R mutation in PLCγ2 [57] exhibited enhanced cholesterol metabolism [69].

When *APOE* ε4/ε4 microglia-like cells were studied in parallel with the microglia carrying familial AD mutations in *APP* and *PSEN1* genes, familial AD microglia exhibited only a mild phenotype, comprising lower cytokine responses to inflammatory stimuli, higher uptake of fluorescent Aβ42, and normal mitochondrial metabolism [62]. This indicates that there is no primary microglial pathology in familial AD.

Since it is widely acknowledged that microglial behavior differs significantly between *in vivo* and *in vitro* conditions, the transplantation of microglial precursors into the mouse brain was proposed to offer a more relevant study model. There have been four studies so far [70-73] reporting the transplantation of human embryonic stem cell or iPSC-derived microglial progenitor cells into the brains of newborn immunodeficient mice. The mice used express the human form of CSF1, which is necessary for human microglial survival [74]. By two months post-transplantation, numerous ramified human iPSC-derived microglia (xenomicroglia) were observed in the striatum, cortex, and hippocampus [70-73], and exhibited gene expression pattern similar to *ex vivo* human microglia from previous studies but distinct from *in vitro* differentiated microglia. These promising results suggest that xenomicroglia could provide valuable insights into the role of human microglia in neurodegenerative disorders. Notably, the response of xenomicroglia to oligomeric Aβ42 and fibrillar Aβ42 deposits differed from that of endogenous mouse microglia [70,73]. In the AD transgenic mouse model, *TREM2* R47H mutant xenomicroglia displayed a lower association with amyloid plaques as compared to isogenic control microglia, thus confirming a dysfunction of *TREM2* mutant cells [70].

Overall, iPSC studies show that AD-associated microglia exhibit metabolic and immune dysfunction rather than bona fide inflammatory phenotype.

Studies on microglial function have relied mainly on rodent models and post-mortem studies of PD and ALS patient brains in which microglial activation has been shown. Similarly to astrocytes, microglia express genes associated with PD pathology (*LRRK2*, *SNCA*, *GBA1*). No studies reporting significant defects in microglia-like cells carrying PD-related mutations have been published so far. The studies using iPSC-derived monocytes/macrophages have not reported strong phenotypes either [75,76], except in macrophages carrying *SNCA* triplication mutations [77] and *GBA1* mutation [78], *both causing* impairment in phagocytosis and elevated production of inflammatory cytokines. A very recent study using iPSC-derived macrophages and microglia-like cells has shown that LRRK2 is upregulated by IFN-γ stimulation and is recruited to maturing phagosomes [75]. However, *LRRK2* G2019S macrophages did not show a significant abnormality in this study.

ALS-associated *C9orf72* is highly expressed in microglia in the mouse brain, and its deficiency leads to the accumulation of lysosomes, increased ROS production triggered by phagocytosis of zymosan-coated particles, and altered immune responses in macrophages and microglia, with aging-related neuroinflammation [79]. However, the protocols for iPSC-derived microglia were generated only in the past few years, and so far, no abnormalities in ALS iPSC-derived microglia or macrophages have been reported.

## 6 | CONCLUSION

Recent advances in stem cell technology have enabled the generation of patient-specific brain cells, including neurons, astrocytes, oligodendrocytes, and microglia, thus providing novel platforms for modeling human brain diseases and testing therapeutic compounds. The iPSC models of AD, PD, and ALS have been useful to confirm metabolic dysfunction in glial cells. However, in order to take full advantage of iPSC-based models, several significant challenges need to be overcome, including high line-to-line and lab-to-lab variation and immaturity of the differentiated brain cells exhibiting a phenotype closer to embryonic or neonatal rather than mature adult cells. It is essential to make sure that what we see in the dish is the real patient-specific phenotype and not an artifact. Engle and coworkers [80] have suggested that the key to successful iPSC-based disease modeling is the high quality of starting iPSCs and a sufficient number of lines (3-4 pairs of isogenic clones or 4-6 individuals per group). Also, the phenotype of the glial cells or neurons derived from the iPSC lines from patients with a particular genetic brain disease needs to resemble and have at least some of the features expected based on the studies of brain biopsy, autopsy, or animal models. Since the generation of new iPSC lines and their differentiation is still a laborious and costly process, most studies reviewed above have failed to include the minimum recommended number of iPSC lines per group. However, when studies using different lines and different protocols point to the same direction and largely correlate with what we already know about the diseases, this can give more confidence in the truthfulness of the results. Thus, a way forward is the establishment of multi-center collaborations, allowing to test the same hypothesis in a substantially higher number of different patient-derived lines simultaneously.

A critical part of disease modeling using iPSCs is to show non-cell-autonomous effects of patient-derived glia on other types of brain cells in 2D and 3D co-cultures. As discussed above, there are relatively numerous studies reporting the toxic effects of diseased astrocytes on neurons in 2D co-cultures. There are also some reports

of incorporating human microglia-like cells into brain organoids, which increased microglial maturity [59,62,63]. However, no clear disease-related phenotypes have been published so far using this model. Thus, more work is needed to develop co-cultures of neurons and different glial types that would show disease-relevant phenotypes and be adaptable for high-throughput screening of drugs.

A more advanced way to circumvent the immaturity of glia in culture and to show non-cell-autonomous effects is to transplant human cells into immunodeficient mice. Both astrocyte [30] and microglial precursors [70-73] have already been successfully transplanted. The caveat here is that the surrounding cells are of mouse, and not human, origin and some important interactions may be lacking. Further, the transplantation of human brain organoids containing microglia into mice could provide a novel tool for drug screening *in vivo*.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

J.K. declared Consultant/Advisory role with a US based start-up on Alzheimer's disease, stocks in Aranda Pharma Ltd. The other authors declared no potential conflicts of interests.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## ORCID

*Jari Koistinaho* https://orcid.org/0000-0001-6559-1153

## REFERENCES

1. Zhang Y, Sloan SA, Clarke LE, et al. Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. *Neuron*. 2016;89(1):37-53.
2. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat Genet*. 2019;51(3):404-413.
3. Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet Neurol*. 2019;18(12):1091-1102.
4. Huisman MH et al. Population based epidemiology of amyotrophic lateral sclerosis using capture-recapture methodology. *J Neurol Neurosurg Psychiatry*. 2011;82(10):1165-1170.
5. Grubman A, Chew G, Ouyang JF, et al. A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nat Neurosci*. 2019;22(12):2087-2097.
6. Mathys H, Davila-Velderrain J, Peng Z, et al. Single-cell transcriptomic analysis of Alzheimer's disease. *Nature*. 2019;570(7761):332-337.
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663-676.
8. Nicolas M, Hassan BA. Amyloid precursor protein and neural development. *Development*. 2014;141(13):2543-2548.
9. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron*. 2005;48(6):913-922.
10. Satir TM, Nazir FH, Vizlin-Hodzic D, et al. Accelerated neuronal and synaptic maturation by BrainPhys medium increases Abeta secretion and alters Abeta peptide ratios from iPSC-derived cortical neurons. *Sci Rep*. 2020;10(1):601.
11. Brothers HM, Gosztyla ML, Robinson SR. The Physiological Roles of Amyloid-beta Peptide Hint at New Ways to Treat Alzheimer's Disease. *Front Aging Neurosci*. 2018;10:118.
12. Harris SA, Harris EA. Herpes Simplex Virus Type 1 and Other Pathogens are Key Causative Factors in Sporadic Alzheimer's Disease. *J Alzheimers Dis*. 2015;48(2):319-353.
13. Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nat Rev Neurosci*. 2019;20(3):148-160.
14. Chew H, Solomon VA, Fonteh AN. Involvement of Lipids in Alzheimer's Disease Pathology and Potential Therapies. *Front Physiol*. 2020;11:598.
15. Anandhan A, Jacome MS, Lei S, et al. Metabolic Dysfunction in Parkinson's Disease: Bioenergetics, Redox Homeostasis and Central Carbon Metabolism. *Brain Res Bull*. 2017;133:12-30.
16. Borrageiro G, Haylett W, Seedat S, Kuivaniemi H, Bardien S. A review of genome-wide transcriptomics studies in Parkinson's disease. *Eur J Neurosci*. 2018;47(1):1-16.
17. Taylor JP, Brown RH Jr, Cleveland DW. Decoding ALS: from genes to mechanism. *Nature*. 2016;539(7628):197-206.
18. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science*. 2006;314(5796):130-133.
19. Blasco H, Corcia P, Pradat PF, et al. Metabolomics in cerebrospinal fluid of patients with amyotrophic lateral sclerosis: an untargeted approach via high-resolution mass spectrometry. *J Proteome Res*. 2013;12(8):3746-3754.
20. Blasco H, Nadal-Desbarats L, Pradat PF, et al. Untargeted 1H-NMR metabolomics in CSF: toward a diagnostic biomarker for motor neuron disease. *Neurology*. 2014;82(13):1167-1174.
21. Patin F, Corcia P, Vourc'h P, et al. Omics to Explore Amyotrophic Lateral Sclerosis Evolution: the Central Role of Arginine and Proline Metabolism. *Mol Neurobiol*. 2017;54(7):5361-5374.
22. Cistaro A, Valentini MC, Chiò A, et al. Brain hypermetabolism in amyotrophic lateral sclerosis: a FDG PET study in ALS of spinal and bulbar onset. *Eur J Nucl Med Mol Imaging*. 2012;39(2):251-259.
23. Smethurst P, Risse E, Tyzack GE, et al. Distinct responses of neurons and astrocytes to TDP-43 proteinopathy in amyotrophic lateral sclerosis. *Brain*. 2020;143(2):430-440.
24. Serio A, Bilican B, Barmada SJ, et al. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. *Proc Natl Acad Sci U S A*. 2013;110(12):4697-4702.
25. Tyzack GE, Hall CE, Sibley CR, et al. A neuroprotective astrocyte state is induced by neuronal signal EphB1 but fails in ALS models. *Nat Commun*. 2017;8(1):1164.
26. Zhao C, Devlin AC, Chouhan AK, et al. Mutant C9orf72 human iPSC-derived astrocytes cause non-cell autonomous motor neuron pathophysiology. *Glia*. 2020;68(5):1046-1064.
27. Birger A, Ben-Dor I, Ottolenghi M, et al. Human iPSC-derived astrocytes from ALS patients with mutated C9ORF72 show increased oxidative stress and neurotoxicity. *EBioMedicine*. 2019;50:274-289.
28. Almad AA, Doreswamy A, Gross SK, et al. Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. *Glia*. 2016;64(7):1154-1169.
29. Qosa H, Lichter J, Sarlo M, et al. Astrocytes drive upregulation of the multidrug resistance transporter ABCB1 (P-Glycoprotein) in endothelial cells of the blood-brain barrier in mutant superoxide dismutase 1-linked amyotrophic lateral sclerosis. *Glia*. 2016;64(8):1298-1313.
30. Qian K, Huang H, Peterson A, et al. Sporadic ALS Astrocytes Induce Neuronal Degeneration In Vivo. *Stem Cell Reports*. 2017;8(4):843-855.

31. Du F et al. Astrocytes Attenuate Mitochondrial Dysfunctions in Human Dopaminergic Neurons Derived from iPSC. *Stem Cell Reports*. 2018;10(2):366-374.
32. Booth HDE, Wessely F, Connor-Robson N, et al. RNA sequencing reveals MMP2 and TGFB1 downregulation in LRRK2 G2019S Parkinson's iPSC-derived astrocytes. *Neurobiol Dis*. 2019;129:56-66.
33. Aflaki E, Stubblefield BK, McGlinchey RP, McMahon B, Ory DS, Sidransky E. A characterization of Gaucher iPS-derived astrocytes: Potential implications for Parkinson's disease. *Neurobiol Dis*. 2020;134:104647.
34. Sonninen TM, Hämäläinen RH, Koskuvi M, et al. Metabolic alterations in Parkinson's disease astrocytes. *Sci Rep*. 2020;10:14474.
35. di Domenico A, Carola G, Calatayud C, et al. Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease. *Stem Cell Reports*. 2019;12(2):213-229.
36. Lewandowski NM, Ju S, Verbitsky M, et al. Polyamine pathway contributes to the pathogenesis of Parkinson disease. *Proc Natl Acad Sci U S A*. 2010;107(39):16970-16975.
37. Cheng D, Jenner AM, Shui G, et al. Lipid pathway alterations in Parkinson's disease primary visual cortex. *PLoS One*. 2011;6(2):e17299.
38. Lin YT, Seo J, Gao F, 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.e7.
39. Zhao J, Davis MD, Martens YA, et al. APOE epsilon4/epsilon4 diminishes neurotrophic function of human iPSC-derived astrocytes. *Hum Mol Genet*. 2017;26(14):2690-2700.
40. Oksanen M, Petersen AJ, Naumenko N, et al. PSEN1 Mutant iPSC-Derived Model Reveals Severe Astrocyte Pathology in Alzheimer's Disease. *Stem Cell Reports*. 2017;9(6):1885-1897.
41. Oksanen M, Hyötyläinen I, Trontti K, et al. NF-E2-related factor 2 activation boosts antioxidant defenses and ameliorates inflammatory and amyloid properties in human Presenilin-1 mutated Alzheimer's disease astrocytes. *Glia*. 2020;68(3):589-599.
42. Konttinen H, Gureviciene I, Oksanen M, et al. PPARbeta/delta-agonist GW0742 ameliorates dysfunction in fatty acid oxidation in PSEN1DeltaE9 astrocytes. *Glia*. 2019;67(1):146-159.
43. Le Douce J et al. Impairment of Glycolysis-Derived l-Serine Production in Astrocytes Contributes to Cognitive Deficits in Alzheimer's Disease. *Cell Metab*. 2020;31(3):503-517 e8.
44. Cai R, Zhang Y, Simmering JE, et al. Enhancing glycolysis attenuates Parkinson's disease progression in models and clinical databases. *J Clin Invest*. 2019;129(10):4539-4549.
45. Hallmann AL, Araúzo-Bravo MJ, Mavrommatis L, et al. Astrocyte pathology in a human neural stem cell model of frontotemporal dementia caused by mutant TAU protein. *Sci Rep*. 2017;7:42991.
46. Cairns DM et al. A 3D human brain-like tissue model of herpes-induced Alzheimer's disease. *Sci Adv*. 2020;6(19):eaay8828.
47. Philips T, Rothstein JD. Oligodendroglia: metabolic supporters of neurons. *J Clin Invest*. 2017;127(9):3271-3280.
48. Kang SH, Li Y, Fukaya M, et al. Degeneration and impaired regeneration of gray matter oligodendrocytes in amyotrophic lateral sclerosis. *Nat Neurosci*. 2013;16(5):571-579.
49. Nasrabady SE, Rizvi B, Goldman JE, Brickman AM. White matter changes in Alzheimer's disease: a focus on myelin and oligodendrocytes. *Acta Neuropathol Commun*. 2018;6(1):22.
50. Pozorski V, Oh JM, Adluru N, et al. Longitudinal white matter microstructural change in Parkinson's disease. *Hum Brain Mapp*. 2018;39(10):4150-4161.
51. Chanoumidou K, Mozafari S, Baron-van Evercooren A, Kuhlmann T. Stem cell derived oligodendrocytes to study myelin diseases. *Glia*. 2020;68(4):705-720.
52. Ferraiuolo L, Meyer K, Sherwood TW, et al. Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism. *Proc Natl Acad Sci U S A*. 2016;113(42):E6496-E6505.
53. Livesey MR, Magnani D, Cleary EM, et al. Maturation and electrophysiological properties of human pluripotent stem cell-derived oligodendrocytes. *Stem Cells*. 2016;34(4):1040-1053.
54. Ehrlich M, Mozafari S, Glatza M, et al. Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors. *Proc Natl Acad Sci U S A*. 2017;114(11):E2243-E2252.
55. Li L et al. GFAP Mutations in Astrocytes Impair Oligodendrocyte Progenitor Proliferation and Myelination in an hiPSC Model of Alexander Disease. *Cell Stem Cell*. 2018;23(2):239-251 e6.
56. Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol*. 2001;101(3):249-255.
57. Sims R et al. Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease. *Nat Genet*. 2017;49(9):1373-1384.
58. Speicher AM, Wiendl H, Meuth SG, Pawlowski M. Generating microglia from human pluripotent stem cells: novel in vitro models for the study of neurodegeneration. *Mol Neurodegener*. 2019;14(1):46.
59. Abud EM et al. iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases. *Neuron*. 2017;94(2):278-293 e9.
60. McQuade A, Coburn M, Tu CH, Hasselmann J, Davtyan H, Blurton-Jones M. Development and validation of a simplified method to generate human microglia from pluripotent stem cells. *Mol Neurodegener*. 2018;13(1):67.
61. Haenseler W, Sansom SN, Buchrieser J, et al. A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response. *Stem Cell Reports*. 2017;8(6):1727-1742.
62. Konttinen H, Cabral-da-Silva MC, Ohtonen S, et al. PSEN1DeltaE9, APPswe, and APOE4 Confer Disparate Phenotypes in Human iPSC-Derived Microglia. *Stem Cell Reports*. 2019;13(4):669-683.
63. Brownjohn PW, Smith J, Solanki R, et al. Functional Studies of Missense TREM2 Mutations in Human Stem Cell-Derived Microglia. *Stem Cell Reports*. 2018;10(4):1294-1307.
64. Piers TM, Cosker K, Mallach A, et al. A locked immunometabolic switch underlies TREM2 R47H loss of function in human iPSC-derived microglia. *FASEB J*. 2020;34(2):2436-2450.
65. Zhou Y, Song WM, Andhey PS, et al. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med*. 2020;26(1):131-142.
66. Ulland TK et al. TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. *Cell*. 2017;170(4):649-663 e13.
67. Baik SH et al. A Breakdown in Metabolic Reprogramming Causes Microglia Dysfunction in Alzheimer's Disease. *Cell Metab*. 2019;30(3):493-507 e6.
68. Oddo S. The role of mTOR signaling in Alzheimer disease. *Front Biosci (Schol Ed)*. 2012;4:941-952.
69. Andreone BJ, Przybyla L, Llapashtica C, et al. Alzheimer's-associated PLCgamma2 is a signaling node required for both TREM2 function and the inflammatory response in human microglia. *Nat Neurosci*. 2020;23(8):927-938.
70. Hasselmann J et al. Development of a Chimeric Model to Study and Manipulate Human Microglia In Vivo. *Neuron*. 2019;103(6):1016-1033 e10.
71. Xu R, Li X, Boreland AJ, et al. Human iPSC-derived mature microglia retain their identity and functionally integrate in the chimeric mouse brain. *Nat Commun*. 2020;11(1):1577.
72. Svoboda DS, Barrasa MI, Shu J, et al. Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the

neonatal mouse brain. *Proc Natl Acad Sci U S A*. 2019;116(50):25293-25303.
73. Mancuso R, van den Daele J, Fattorelli N, et al. Stem-cell-derived human microglia transplanted in mouse brain to study human disease. *Nat Neurosci*. 2019;22(12):2111-2116.
74. Elmore MR et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*. 2014;82(2):380-397.
75. Lee H, Flynn R, Sharma I, et al. LRRK2 Is Recruited to Phagosomes and Co-recruits RAB8 and RAB10 in Human Pluripotent Stem Cell-Derived Macrophages. *Stem Cell Reports*. 2020;14(5):940-955.
76. Speidel A et al. Leucine-Rich Repeat Kinase 2 Influences Fate Decision of Human Monocytes Differentiated from Induced Pluripotent Stem Cells. *PLoS One*. 2016;11(11):e0165949.
77. Haenseler W, Zambon F, Lee H, et al. Excess alpha-synuclein compromises phagocytosis in iPSC-derived macrophages. *Sci Rep*. 2017;7(1):9003.
78. Panicker LM, Miller D, Awad O, et al. Gaucher iPSC-derived macrophages produce elevated levels of inflammatory mediators and serve as a new platform for therapeutic development. *Stem Cells*. 2014;32(9):2338-2349.
79. O'Rourke JG et al. C9orf72 is required for proper macrophage and microglial function in mice. *Science*. 2016;351(6279):1324-1329.
80. Engle SJ, Blaha L, Kleiman RJ. Best Practices for Translational Disease Modeling Using Human iPSC-Derived Neurons. *Neuron*. 2018;100(4):783-797.

**How to cite this article:** Rõlova T, Lehtonen Šárka, Goldsteins G, Kettunen P, Koistinaho J. Metabolic and immune dysfunction of glia in neurodegenerative disorders: focus on iPSC models. *Stem Cells*. 2020;1–10. https://doi.org/10.1002/stem.3309

**Table 1** In vitro drug testing using human iPSC-derived glia

| Compound | Pathway | Model | Effect observed | Reference |
|---|---|---|---|---|
| CMA activator | Chaperone-mediated autophagy | LRRK2 G2019S astrocytes | Intracellular α-synuclein ↓<br>Neurodegeneration ↓ | [35] |
| Sulforaphane | NRF2 | PSEN1Δ*E*9 astrocytes | Glycolysis ↑<br>Aβ42 secretion ↓<br>Inflammatory cytokine production ↓ | [41] |
| GW0742 | PPARβ/δ | PSEN1Δ*E*9 astrocytes | Fatty acid oxidation ↑ | [42] |
| Valaciclovir | Anti-viral | HSV-1-infected neuron/astrocyte co-culture | Aβ deposition ↓<br>Astrocyte activation ↓ | [46] |
| Pioglitazone | PPARγ/p38MAPK | TREM2 R47H microglia | Maximal respiration ↑<br>Glycolysis ↑<br>Aβ42 uptake ↑ | [64] |

**Figure 1** **A summary of recent iPSC based studies on ALS.** A, iPSC-based co-culture studies support the involvement of astrocyte and oligodendrocyte dysfunction in ALS. No study has reported so far disease-related phenotype in iPSC-derived microglia, although patient data and mouse model data suggest that these cells play an important role in ALS pathogenesis. B, transplanted human ALS astrocytes induce ALS-like symptoms in mice. TDP-43, transactive response DNA binding protein 43 kDa; EphB1, ephrin type-B receptor 1; Cx43, connexin 43; NFκB, nuclear factor kappa B; SCID, severe combined immunodeficiency

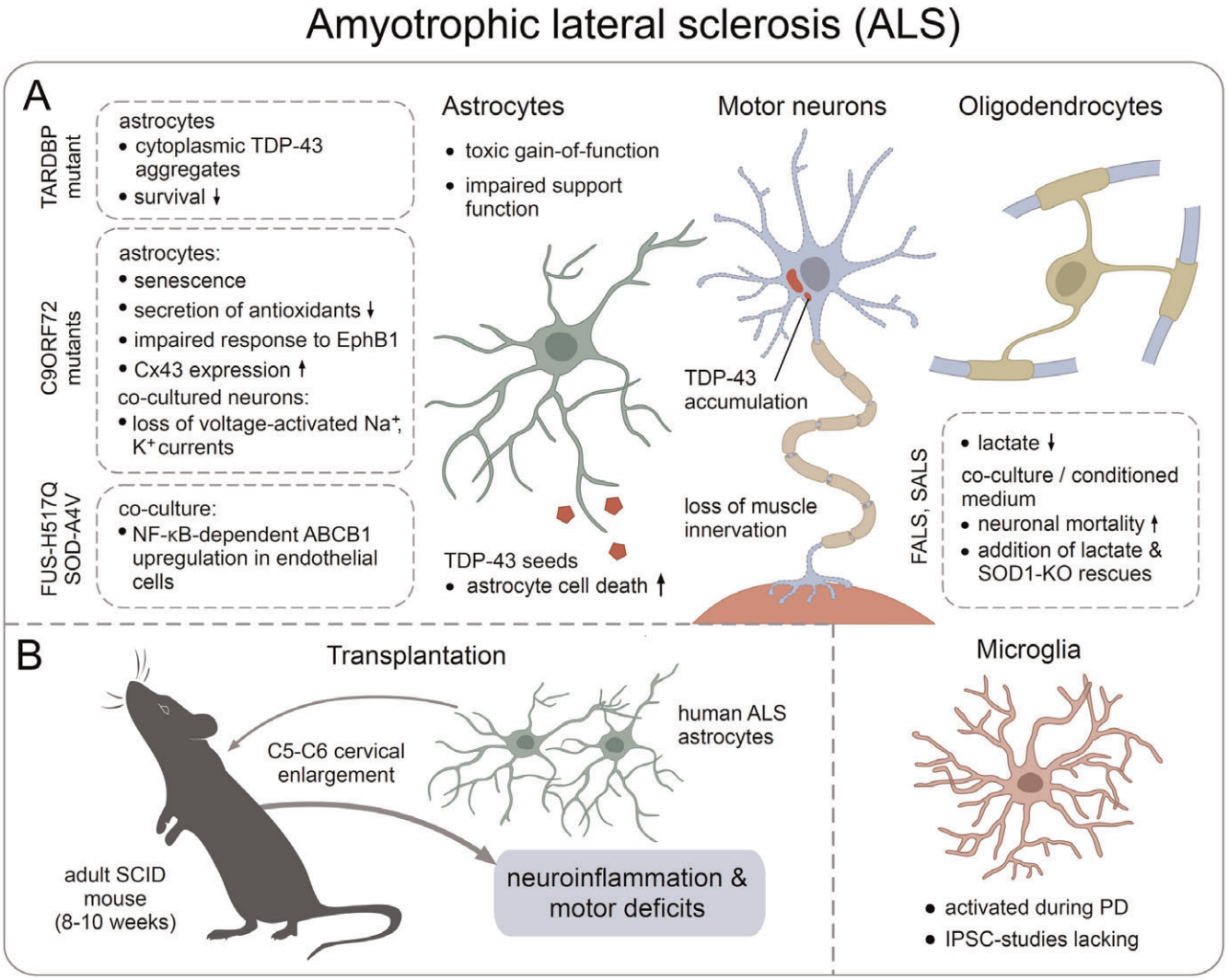


**Figure 2** **A summary of recent iPSC-based studies on PD.** IPSC-based co-culture studies support the involvement of astrocyte dysfunction in PD pathology. No study has reported so far disease-related phenotype in iPSC-derived microglia and oligodendrocytes, although patient data suggest that these cells play an important role in PD pathogenesis. GBA1, glucocerebrosidase; LRRK2, leucine-rich repeat kinase 2; LPE, lysophosphatidylethanolamine

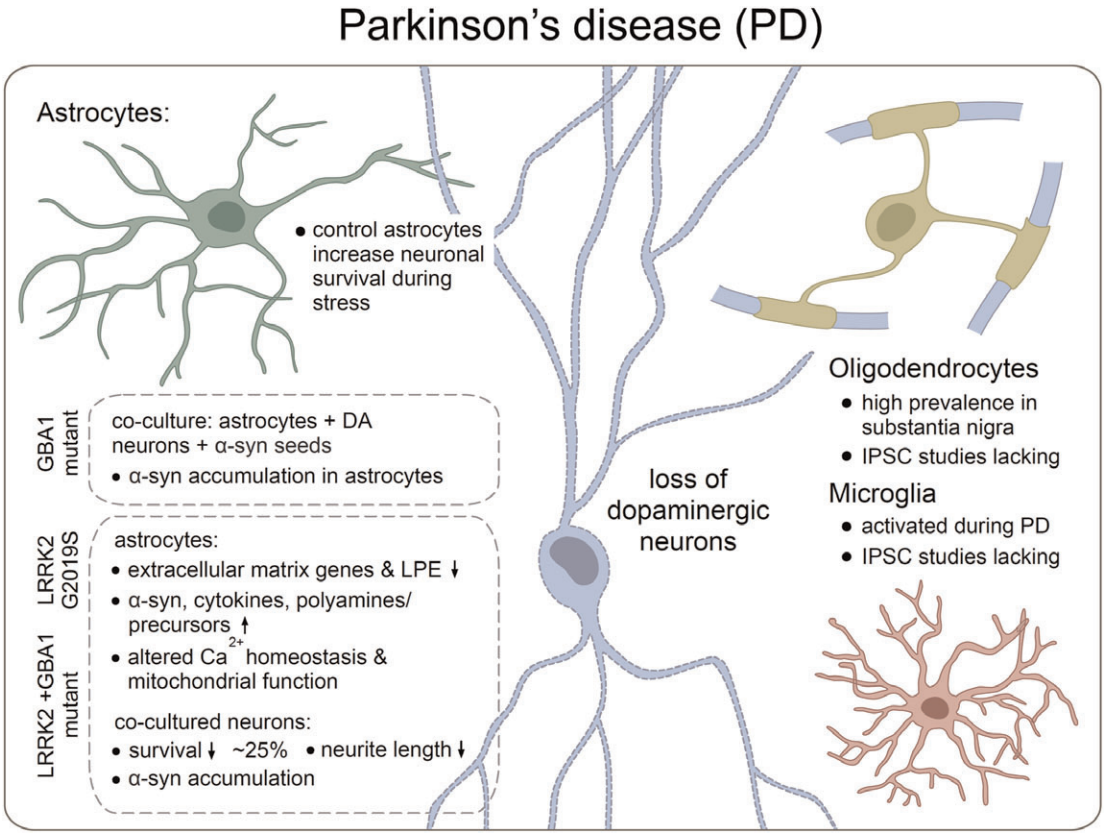
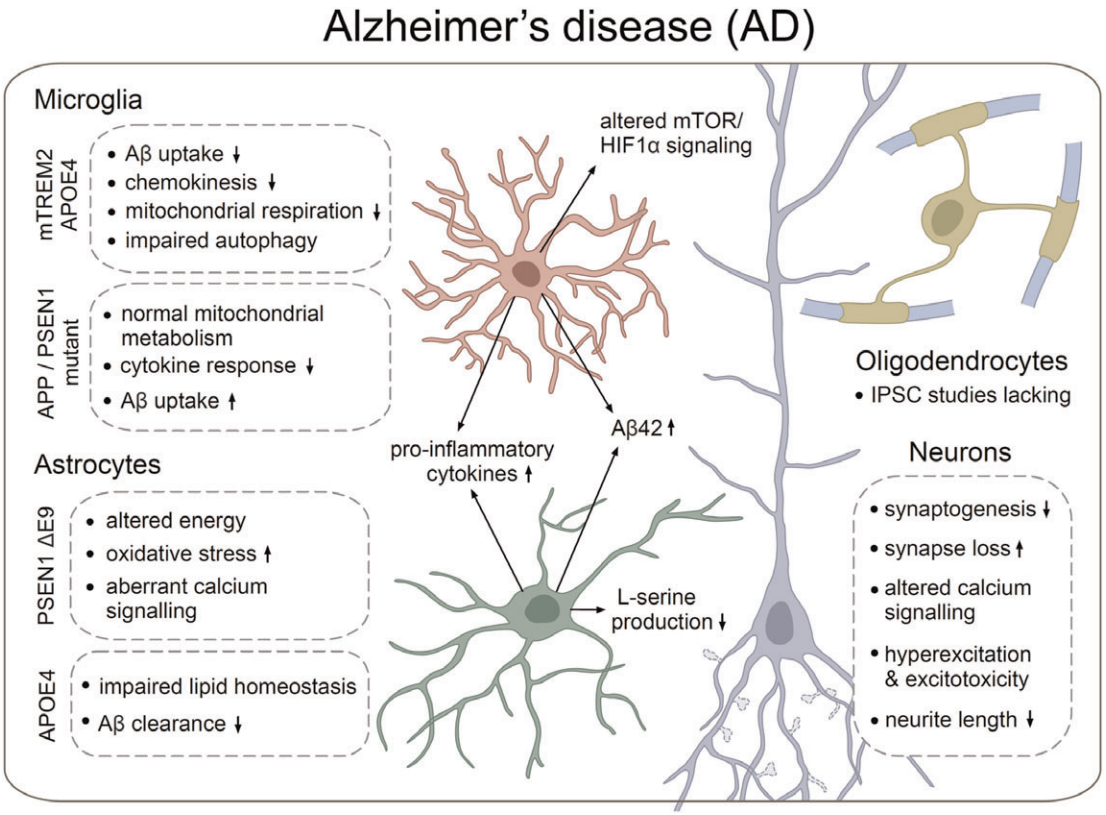
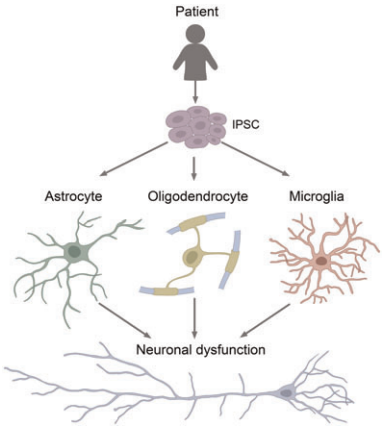


**Figure 3** **Recent iPSC-based studies suggest the involvement of both microglial and astrocyte dysfunction in AD-related neuronal dysfunction and neuronal loss**. TREM2, triggering receptor expressed on myeloid cells 2; APOE, apolipoprotein E; APP, amyloid precursor protein; PSEN1, presenilin 1; Aβ, beta-amyloid; mTOR, mammalian target of rapamycin; HIF1α, hypoxia-inducible factor 1 alpha



# Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only.
It will not be published as part of main.




Induced pluripotent stem cells (iPSC) created from fibroblasts or other easily accessible human cells can be differentiated into patient-specific glia such as oligodendrocytes, astrocytes and microglia, and grown together with neurons in culture or in vivo for investigating the role and molecular mechanisms of the glial cell in neurodegenerative diseases.