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Pluripotent stem cells for uncovering the role of mitochondria in human brain function and dysfunction

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Abstract

Mitochondrial dysfunctions are a known pathogenetic mechanism of a number of neurological and psychiatric disorders. At the same time, mutations in genes encoding for components of the mitochondrial respiratory chain cause mitochondrial diseases, which commonly exhibit neurological symptoms. Mitochondria are therefore critical for the functionality of the human nervous system. The importance of mitochondria stems from their key roles in cellular metabolism, calcium handling, redox and protein homeostasis, and overall cellular homeostasis through their dynamic network. Here, we describe how the use of pluripotent stem cells (PSCs) may help addressing the physiological and pathological relevance of mitochondria for the human nervous system. PSCs allow the generation of patient-derived neurons and glia and the identification of gene-specific and mutation-specific cellular phenotypes via genome engineering approaches. We discuss the recent advances in PSC-based modeling of brain diseases and the current challenges of the field. We anticipate that the careful use of PSCs will improve our understanding of the impact of mitochondria in neurological and psychiatric disorders and the search for effective therapeutic avenues.

Mitochondria are intracellular organelles that are present in multiple copies in all nucleated cells. They contain their own genome and are maternally inherited (Dyall et al., 2004). The major function of mitochondria is to provide energy in the form of ATP through the process of oxidative phosphorylation (OxPhos). OxPhos occurs through the action of five protein complexes -known as respiratory chain (RC) complexes- localized in the mitochondrial inner membrane. Complexes I-IV transfer electrons and at the same time they expel protons into the space between the inner and outer mitochondrial membrane. This generates a proton gradient across the inner membrane that is known as mitochondrial membrane potential (MMP). The energy stored in this gradient is used by Complex V to produce ATP by allowing the entry of protons into the mitochondrial matrix (Vafai and Mootha, 2012).

In addition to bioenergetics, mitochondria are involved in the metabolism of fatty acids, amino acids, and steroids, as well as in numerous signaling pathways such as apoptosis, calcium homeostasis, and in the generation of reactive oxygen species (ROS) (Dyall et al., 2004). Hence, dysfunction of mitochondria can impact an array of cellular homeostatic processes. Mitochondrial impairment will cause more detrimental consequences on cells that are strictly dependent on their functionality.

In this review, we first describe the physiological and pathological importance of mitochondria for the human brain. The human nervous system is highly complex and significantly different from that of other species. In order to understand the mitochondrial contribution to human brain function and to the pathogenesis of brain diseases, it is important to investigate human brain cells, which can now be generated *in vitro* from human pluripotent stem cells (PSCs). In the second part of the review, we discuss the promises and challenges of PSCs for understanding the role of mitochondria in the function and dysfunction of the human nervous system.

Mitochondria in human brain physiology

Bioenergetics

Despite representing only 2–3% of total body weight, the human brain consumes around 25% of the daily intake of glucose (Clarke and Sokoloff, 1999). These requirements are even higher during development, as the brains of infants utilize more than 40% of the basal metabolic rate (Goyal et al., 2014). Mitochondria might therefore be particularly relevant for the function of the central nervous system (CNS) given its high energy demands. In fact, cell types with high bioenergetic needs -like skeletal muscle and heart muscle- commonly rely on OxPhos metabolism (Kunz, 2001; Padrão et al., 2011).

Nonetheless, bioenergetics alone may not be sufficient to explain the importance of mitochondria for CNS physiology. Other cell types with high energetic needs do not appear to rely on mitochondrial respiration. This is the case of proliferative cells like cancer cells and pluripotent stem cells (PSCs), which instead exhibit a glycolytic-dependent metabolism (Prigione et al., 2010; Vander Heiden et al., 2009). In these proliferative cells with high necessity for anabolic growth, there is an energy re-routing leading to enhanced rates of glycolysis and pentose phosphate pathway (PPP) as well as reduced entry of pyruvate into mitochondria (Prigione et al., 2015). This bears the crucial advantages of supporting the biosynthetic needs and maintaining low oxidative stress levels through the reduction of OxPhos-mediated ROS production and the increase of the level of the PPP-derived antioxidant glutathione (GSH) (Stincone et al., 2015). However, neuronal cells do not rely on glycolysis, since they favor a metabolic program that fully depends on mitochondrial respiration, despite its potential detrimental consequences on redox homeostasis **(Figure 1)**.

The metabolic profile of the cells can influence the epigenetic state, which refers to chromatin reorganization leading to a defined gene expression program in the absence of changes in the DNA sequence (Gut and Verdin, 2013). Metabolism-driven chromatin regulation is crucial for cellular plasticity, as it dictates the changes required to modulate the cell fate identity as it is in the case of reprogramming to iPSCs (Mathieu and Ruohola-Baker,

2017). Epigenetics is tightly regulated upon neural fate commitment (Imamura et al., 2014). However, the contribution of metabolism in the epigenetic regulation of neural fate remains to be investigated.

Redox and protein homeostasis

Oxygen is an important substrate for cellular energy production (Semenza, 2007). The use of oxygen for cellular energy generation is, however, not without risks. During the transfer of electrons in the mitochondrial respiratory chain, electrons may escape and prematurely react with oxygen to form ROS (Turrens, 2003). Oxidative stress, which can be described as an imbalance between the production of ROS and the capacity of the cell to counteract ROS, results in macromolecular damage (oxidizing of lipids, proteins, and nucleic acids) (Stadtman, 2006). This can in turn cause necrotic or apoptotic cell death (Balaban et al., 2005) **(Figure 1)**.

The adult human brain consumes about 20% of the oxygen that is inspired at rest (Erecińska and Silver, 2001). Hence, neuronal cells need to be prepared to balance ROS with antioxidant defenses. To this aim, the production of NADPH within the oxidative branch of the PPP is critical, as it is needed for the generation of GSH (Stincone et al., 2015) **(Figure 1)**. Any imbalance of this fine equilibrium may contribute to oxidative stress and neurodegeneration (Lin and Beal, 2006).

Among the detrimental consequences of oxidative stress, there is protein oxidation, which can be associated with the loss of protein function and the cytoplasmic accumulation of protein aggregates. Protein defects and protein aggregation may also be caused by an impairment of protein clearance pathways, including the ubiquitin-proteasome system and autophagy. Although it is still debated which kind of protein states (large aggregates or small oligomers) are the most toxic species, a disruption of protein homeostasis -in short proteostasis- is harmful to the cells (Díaz-Villanueva et al., 2015; Ruan et al., 2017). Aberrant

proteostasis could particularly affect neuronal cells, which are long-lived cells and may therefore sustain increased accumulation of damaged proteins over time. In accordance, the presence of intracellular protein aggregates is a common feature of neurodegenerative diseases (Lim and Yue, 2015).

Calcium homeostasis

Calcium homeostasis is essential for excitable cells like neurons that require cytoplasmic calcium for the regulation of neurotransmitter release (Neher and Sakaba, 2008). Calcium-activated potassium channels regulate plasma membrane polarization and cellular excitability as synaptic transmission requires the initial entrance of calcium into the cells (Sah and Louise Faber, 2002).

In order to avoid toxic consequences of calcium overload and to allow the cells to become excitable again, cytoplasmic calcium needs to be quickly buffered. This process is energetically demanding and relies on calcium-ATPases in the plasma membrane and in the endoplasmic reticulum (ER). The ER can efficiently clear low amounts of cytoplasmic calcium. When the amount of calcium increases in the cytoplasm, or within a microenvironment of the cytoplasm, mitochondria become responsible for the clearance. Mitochondria are in fact low-specificity high-capacity buffers, which means that they can take up the largest amount of cytoplasmic calcium once it reaches a certain level (Williams et al., 2013). The MMP is the driver for this mitochondrial calcium uptake (Rizzuto et al., 2012). Given their motility, mitochondria can travel to areas of high calcium concentration in order to reduce it to normal level (Wang and Schwarz, 2009). Mitochondrial calcium uptake is in turn beneficial for cellular energetics, since calcium within mitochondria induces ATP production via activation of calcium-dependent NADH dehydrogenases (McCormack and Denton, 1990; Wan et al., 1989).

The fine tuning of calcium homeostasis by mitochondria is critical, as mitochondrial calcium overload can trigger cell death through the opening of the permeability transition pore (PTP), which consists of dimers of ATP synthase (Giorgio et al., 2013) **(Figure 1)**. Defective mitochondrial calcium handling is therefore highly detrimental for neuronal cells, and may contribute to the neuronal cell death observed in neurodegenerative diseases (Abeti and Abramov, 2015) and in mitochondrial disorders (Abramov et al., 2010).

Mitochondrial dynamics

Mitochondria are highly dynamic organelles and continuously change their shape through the processes of fusion and fission (Chan, 2006). The plasticity and dynamics of the mitochondrial network enable mitochondria to reach all subcellular regions and to respond to local needs by distributing calcium, ATP, and ROS, thereby contributing to the maintenance of cellular homeostasis (van der Bliek et al., 2013). The energetic status of the cells and the MMP are important regulator of mitochondrial fusion, as loss of MMP results into mitochondrial fragmentation (Hoppins and Nunnari, 2009).

Mitochondrial dynamics is critical for highly polarized cells like neurons, where energy needs to be supplied to regions that are distant from the cell body (Hollenbeck, 2005). Accordingly, pathogenic mutations that disrupt proteins involved in fusion and fission cause neurological diseases (Burté et al., 2015). At the same time, disturbed mitochondrial dynamics has been implicated in the pathogenesis of many neurodegenerative disorders (Chen and Chan, 2009).

The degradation of dysfunctional mitochondria plays an important physiological role. Mitochondrial fission can generate a defective mitochondrial daughter unit that is eliminated by the autophagic machinery through a process called mitophagy (Twig and Shirihai, 2011). The degradation of impaired mitochondria is essential in maintaining the quality control for correct cellular function. This control may be particularly relevant for long-living cells like

neurons that have to maintain tight homeostatic control for a long time in order to avoid the persistence of dysfunctional mitochondria (de Castro et al., 2010).

Neuron-glia interactions

Glial cells are active components of synapses and contribute to neurotransmission (Auld and Robitaille, 2003; Kettenmann et al., 2013). Glial cells also provide metabolic support. Within the CNS, astrocytes regulate the flux of energy substrates to neurons, thereby generating a physiological metabolic compartmentalization (Pellerin and Magistretti 2012).

Astrocytes take up glucose and convert into lactate via glycolysis, and neurons take up the astrocyte-produced lactate and use it for ATP generation via OxPhos (Pellerin and Magistretti, 1994; Pellerin and Magistretti 2012). This leaves neurons free to use glucose in the PPP pathway for antioxidant defenses via GSH production (Herrero-Mendez et al., 2009) **(Figure 1)**. This dependency on PPP-based utilization of glucose in neuronal cells may make neurons more sensitive to mitochondrial dysfunction, as they are unable to increase glycolysis and glycolytic-based utilization of glucose. Neurons may even release their damaged mitochondria to take up healthy ones from astrocytes (Hayakawa et al., 2016).

Despite their apparent glycolytic metabolism, glia may also need active mitochondria. In fact, it has been suggested that the lactate that is released from glia may come not only from glycolysis-derived pyruvate but also from mitochondria-derived malate converted into pyruvate (Dienel and McKenna, 2014) **(Figure 1)**. Moreover, the communication between astrocytes and neurons occurs through elaborated “calcium waves”, whose homeostatic control requires functional mitochondria (Bazargani and Attwell, 2016; Jackson and Robinson, 2015; Skupin et al., 2010).

Mitochondria in human brain pathology

Neurodegenerative diseases

Neurodegenerative diseases are a group of disorders characterized by progressive degeneration of cells of the nervous system. Neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). All of these neurodegenerative diseases show a specific accumulation of dysfunctional mitochondria (Burté et al., 2015; de Castro et al., 2010; Johri and Beal, 2012; Lin and Beal, 2006) **(Figure 2)**.

In AD, mitochondrial dysfunction appears to precede Aβ deposition (Moreira et al., 2010; Swerdlow et al., 2010). The accumulation of Aβ-species leads to metabolic dysfunctions in the tricarboxylic acid (TCA) cycle and in the activity of mitochondrial complex IV (Lustbader et al., 2004; Morán et al., 2012). Defects in mitochondrial dynamics and mitochondrial biogenesis may also occur (Reddy et al., 2012; Sheng et al., 2012). Finally, reduced glucose metabolism in the brain is a recognized early feature of AD (Calsolaro and Edison, 2016), as also seen in a fly model of AD where reduced Aβ toxicity was achieved by counteracting glucose hypometabolism (Niccoli et al., 2016).

The involvement of mitochondrial dysfunction in PD has been assumed since the early 1980s, when drug addicts were observed to develop parkinsonism from the drug by-product 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes inhibition of the mitochondrial complex I (Winklhofer and Haass, 2010). Complex I inhibition via MTPT or rotenone is now commonly employed to generate animal models of PD. Deficiency in complex I activity was detected in the substantia nigra pars compacta (SNpc) of PD patients (Schapira et al., 1990) and oxidative stress has been observed in post-mortem studies of sporadic PD (Michel et al., 2014). Finally, most of the genes associated with familial PD encode for proteins that belong to mitochondria or that have a direct link to mitochondrial function, morphology, or dynamics (Bose and Beal, 2016).

In familial ALS, mutations in the gene superoxide dismutase 1 (*SOD1*) cause defective mitochondrial function, morphology, and distribution (Tafuri et al., 2015). Impaired

bioenergetics, mitochondrial calcium homeostasis, mitochondrial apoptosis, and axonal transport of mitochondria have all been reported in sporadic ALS (Shi et al., 2010). These defects also occur in peripheral cells, as ALS-derived fibroblasts display reduced MMP and decreased mitochondrial content (Kirk et al., 2014).

There is evidence for overall bioenergetic defects in HD, as indicated by weight loss and increased energy expenditure that HD patients exhibit in spite of sustained calorie intake (Lodi et al., 2000). Although largely cytosolic, mutant huntingtin (HTT) can associate with the outer mitochondrial membrane. There, it impairs mitochondrial protein import and proteostasis via cytoplasmic protein accumulation (Ruan et al., 2017; Yano et al., 2014) and affects mitochondrial dynamics by binding with the mitochondrial fission GTPase, dynamin-related protein-1 (DRP1), which induces mitochondrial fragmentation (Song et al., 2011). Finally, reduced activity of the respiratory complexes II, III and IV, impaired calcium homeostasis, and oxidative damage have all been implicated in HD pathogenesis (Panov et al., 2002; Sorolla et al., 2008).

Psychiatric diseases

Mitochondrial defects have been observed in many psychiatric conditions, including schizophrenia (SCZ), bipolar disorder (BPD), autism spectrum disorder (ASD), and alcohol use disorder (AUD) (Marazziti et al., 2011).

In SCZ altered metabolic pathways have been found in brain tissue from schizophrenic patients, pointing towards a disturbance in brain energy metabolism and oxidative stress as contributing causes of SCZ (Michel et al., 2011; Prabakaran et al., 2004). The mitochondrial defects in SCZ vary within different brain regions (Roberts, 2017). The perturbation of mitochondrial network dynamics in SCZ may also contribute to the dysfunction of immuno-inflammatory pathways that are associated with the origin on SCZ (Rajasekaran et al., 2015).

BPD has been proposed to result from a phasic dysregulation of mitochondrial function (Morris et al., 2017), thereby leading to recurrent fluctuations in mood and energy. The reduced pH observed during manic phases may in fact be a consequence of lactate accumulation caused by defective mitochondrial metabolism in neurons (Weber et al., 2013). In accordance, complex I activity has been found decreased in the prefrontal cortex of BPD patients (Andreazza et al., 2010) and depressed BPD patients showed reduced glucose brain metabolism (Hosokawa et al., 2009).

The prevalence of mitochondrial dysfunction was reported to be higher in ASD compared to the general population (Rossignol and Frye, 2012; Hollis et al., 2017). Almost one third of ASD patients have increased lactate-to-pyruvate ratio (Correia et al., 2006). Post-mortem brain tissues studies identified decreased activity of RC complexes, elevated ROS, and mtDNA mutations in individuals with ASD (Chauhan et al., 2011; Tang et al., 2013). RC defects included complexes II and V in frontal lobe, temporal lobe, cortex, and cerebellum (Chauhan et al., 2011) and complexes I, III, IV and V in temporal lobe (Tang et al., 2013). Additionally, decreased pyruvate dehydrogenase activity has been associated with reduced activity in RC complexes I and II and increased copy number variations (CNVs) in the genes encoding for these complexes (Gu et al., 2013).

In the context of AUD, it is known that ethanol intoxication has detrimental effects on mitochondria of CNS cells (Hoek et al., 2002). Mitochondria might also play a role in the establishment of the alcohol addiction behavior. The abnormally increased release of dopamine (DA) in the ventral tegmental area, which is a key region regulating central reward, depends on calcium homeostasis, and therefore may be influenced by mitochondrial functionality. Reduced DA release, and subsequently reduced alcohol consumption, were observed in mice upon administration of the liver hormone FGF21 (Talukdar et al., 2016), suggesting the presence of metabolic-based regulatory mechanisms for glucose and alcohol-reward behaviors (Potthoff, 2017).

Mitochondrial disorders

Mitochondrial diseases are a group of inherited metabolic disorders caused by OxPhos defects due to mutations in the nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) (Koopman et al., 2012). Unlike mitochondrial dysfunctions in neurological and psychiatric diseases, which may be secondary within the pathogenic mechanism, in mitochondrial disorders the mitochondrial defects are primary and of clear genetic origin. Mutations in over 150 genes encoding for proteins of the mitochondrial respiratory chain have been associated with mitochondrial disorders (Vafai and Mootha, 2012). Although any organ or tissue can be affected, patients generally display neurological symptoms (Carelli and Chan, 2014; Koopman et al., 2013; McFarland et al., 2010).

Neurological conditions caused by mtDNA mutations include Leber's hereditary optic neuropathy (LOHN), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), and neurogenic weakness, ataxia and retinitis pigmentosa (NARP).

The most severe mitochondrial disease is Leigh syndrome (LS), a progressive encephalopathy with basal ganglia involvement. LS can be caused by several mutations of OxPhos components encoded by nDNA, such as *NDUFS4* (complex I) and *SURF1* (assembly factor of complex IV), or by mtDNA, like *MT-ATP6* (complex V) and *MT-ND2* (complex I) (Koopman et al., 2013).

Mitochondria in PSC models of neurological and psychiatric diseases

PSC-based disease modeling

Human pluripotent stem cells (PSCs) include human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs), which are obtained from somatic cells through the process of cellular reprogramming (Takahashi et al., 2007). iPSCs exhibit mitochondrial properties that are comparable to those of ESCs, regardless of the donor age of the parental

somatic cells (Bukowiecki et al., 2014; Xu et al., 2013). Upon conversion of iPSCs into neural cells, a mitochondrial maturation occurs, as mitochondria develop into an elongated network and the metabolism shifts from glycolysis towards OxPhos (Choi et al., 2015; Lorenz et al., 2017). A number of iPSC-based models have been generated to investigate neurodegenerative, psychiatric, and mitochondrial disorders. Several of these models identified mitochondrial dysfunctions in the patient-derived neural cells **(Figure 2)**.

In the context of AD, neurons generated from iPSCs derived from patients with sporadic AD showed defective expression of genes involved in mitochondrial function and respiratory chain, including nuclear-encoded OxPhos genes (Hossini et al., 2015). Oxidative stress has been detected also in neurons and astrocytes derived from familial AD patients (Kondo et al., 2013).

For familial PD patients, defects in mitochondrial function and bioenergetics have been found in neural progenitor cells (NPCs) (Flierl et al., 2014), while mature neurons displayed dysfunctional mitochondrial dynamics and reduced response to oxidative stress (Cooper et al., 2012; Nguyen et al., 2011) and reduced mitochondrial volume fraction (Shaltouki et al., 2015). Decreased mitochondrial spare-respiration and increased production of basal ROS and increased mtDNA damage have been also described in familial PD-derived neurons (Ryan et al., 2013; Sanders et al., 2014). Mitochondrial morphology and mitochondrial motility appeared disrupted in human motor neurons obtained from ALS patients carrying mutant *SOD1* (Kiskinis et al., 2014).

NPCs derived from HD iPSCs displayed decreased intracellular ATP levels compared to control NPCs (HD iPSC Consortium, 2012). Aberrant bioenergetics in HD NPCs was also confirmed using genome-edited iPSCs (An et al., 2012; Xu et al., 2017). iPSC-derived neurons from HD patients showed store-operated channel (SOC)-mediated calcium dysregulation (Nekrasov et al., 2016) and impaired mitophagy (Guo et al., 2016). A small

molecule compound (P110-TAT) was found to inhibit mtHTT-induced mitochondrial fragmentation and to increase cell viability in HD patient-derived neurons (Guo et al., 2013).

In the context of psychiatric disorders, iPSC-derived NPCs from SCZ patients exhibited increased oxidative stress (Brennand et al., 2015), and dissipation of MMP together with perturbations of the mitochondrial network (Robicsek et al., 2013). Transfer of isolated normal mitochondria into iPSCs from SCZ patients rescued the defective neuronal differentiation, suggesting a direct effect of mitochondrial dysfunction in the pathogenesis of SCZ (Robicsek et al., 2017).

Hippocampal dentate gyrus-like neurons derived from iPSCs of BPD patients showed mitochondrial abnormalities, including upregulated mitochondrial gene expression, reduced organelle size, and higher MMP (Mertens et al., 2015a). Interestingly, these defects were rescued by lithium only if the cells were derived from BDP patients that responded clinically to lithium treatment.

For mitochondrial disorders, iPSCs lines have been generated and used to dissect the extent of neuronal dysfunction. In the case of MELAS, impaired mitochondrial dynamics and complex I degradation were observed during iPSC differentiation into neuronal-like cells (Hämäläinen et al., 2013). Defective bioenergetics was also found in NPCs carrying mtDNA mutations that cause MELAS and LS (Ma et al., 2015). NPCs and neurons carrying a mutation in the *MT-ATP6* gene showed aberrant bioenergetics, which was improved in response to mTOR inhibition (Zheng et al., 2016). NPCs and neurons carrying a different *MT-ATP6* mutation exhibited defective mitochondrial calcium homeostasis and abnormally increased MMP, which were rescued following treatment with the PDE5 inhibitor avanafil (Lorenz et al., 2017).

Genome engineering

Genome editing tools, including zinc finger nuclease (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the leading-edge clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system have become significantly important in PSC-based disease modeling (Kim and Kim, 2014). Their use allow the generation of so-called isogenic control PSC lines with respect to any disease-causing nDNA mutation (Hockemeyer and Jaenisch, 2016). This can be accomplished by either correcting the mutation in patient-derived iPSCs or by introducing the mutation into control PSCs (Grobarczyk et al., 2015). Given the heterogeneity of PSC lines, the use of isogenic iPSC controls can help uncovering the functional differences that are solely caused by the specific mutation (Ross and Akimov, 2014). This approach has been successfully employed in the context of several iPSC models of neurological diseases **(Figure 2)**.

TALEN technology has been used in iPSCs from familial AD patients to generate an allelic series of mutations in the gene Presenilin 1 (*PS1*) (Woodruff et al., 2013). In iPSCs from familial PD, ZFNs were used to correct mutations in the gene *LRRK2* (Reinhardt et al., 2013; Sanders et al., 2014) and in alpha-synuclein gene *SNCA* (Soldner et al., 2011) CRISPR/Cas9 system was employed to generate isogenic PSCs carrying specific disease-associated genetic risk variants associated with sporadic PD (Soldner et al., 2016). For familial ALS, SOD1 mutations were corrected in iPSCs using ZNF-based editing (Kiskinis et al., 2014) and CRISPR/Cas9 (Wang et al., 2017). CRISPR/Cas9 was also used to repair a mutation in the gene *C9ORF72* in familial ALS iPSCs (Mutihac et al., 2015). In the context of HD, the expanded CAG repeat in the *HTT* gene was corrected with homologous recombination (An et al., 2012) and, more recently, with CRISPR/Cas9 technology (Xu et al., 2017).

In the context of mutations in the mtDNA, however, genome editing is still not practicable. The generation of isogenic lines remains a major challenge for iPSC-based modeling of mitochondrial DNA disorders (Inak et al., 2017). There are only two solutions

available for mtDNA mutations. First, to replace the whole mitochondria via somatic cell nuclear transfer (SCNT) (Ma et al., 2015). This approach, however, does not correct a specific mutation but creates a novel cell carrying a mismatch between the original nDNA and mtDNA, which in itself may cause altered cellular phenotypes (Sterneckert et al., 2014). In fact, the introduction of a distinct mtDNA into a recipient cell can cause extensive transcriptional reprogramming (Picard et al., 2015). Second, to decrease the level of mutation by selective elimination of the mutated mtDNA molecules (Gammage et al., 2014; Moraes, 2014). This technology however is only applicable for heteroplasmic mtDNA mutations, where a mixture of wild type and mutated mtDNA is present in the cells.

It is interesting to notice that a change in the level of heteroplasmic mtDNA mutations can also occur naturally during the derivation of iPSCs from somatic cells (Prigione et al., 2011). This effect can lead to the spontaneous derivation of iPSCs with reduced mtDNA mutation levels that may act as isogenic control lines (Folmes et al., 2013; Fujikura et al., 2012; Hämäläinen et al., 2013; Kodaira et al., 2015; Ma et al., 2015; Perales-Clemente et al., 2016).

Challenges and future directions

PSC-based disease modeling is now widely used and can be coupled with conventional disease modeling approaches. In the context of neurological, psychiatric, and mitochondrial diseases, human PSCs are becoming particularly relevant given that animal models may not be available or may not fully recapitulate the disease phenotypes (Sandoe and Eggan, 2013). In particular, the use of PSCs is shedding light on the importance of mitochondrial function for brain pathologies, as highlighted above. Nonetheless, critical challenges still remain.

One of the major issues is the degree of maturation of PSC-derived neurons and glia (Tao and Zhang, 2016). A long time of *in vitro* culture is needed in order for these cells to

reach a certain degree of maturity. The use of PSC-derived neurons and glia is therefore highly costly and time-consuming. Overexpression of neural specific transcription factors can be used to speed up the process (Zhang et al., 2013). However, the generated neurons and glia may still be more resembling cells of the fetal rather than adult brain. This is also the case for PSC-derived three dimensional brain organoids, which are starting to be used given their more faithful mirroring of brain development (Lancaster et al., 2013; Paşca et al., 2015; Yang and Ng, 2017). Furthermore, due to the epigenetic reprogramming occurring during the generation of iPSCs, aging-related features may be erased. The use of neurons and glia directly derived from fibroblasts may thus represent a promising strategy to allow capturing aging-related phenomena (Mertens et al., 2015b). However, this approach may not yet be ideal for disease modeling applications due to limited number of neurons and glia generated. Alternatively, aging may be accelerated *in vitro* using progerin overexpression (Miller et al., 2013) or by inducing cellular stresses (Studer et al., 2015).

Among the most interesting applications of PSC-derived cells of the nervous system is their use in drug discovery platforms. In order to reach this goal, however, several aspects need to be met including cost-effective cell generation and high reproducibility (Avior et al., 2016). The differentiation of iPSCs into post-mitotic neurons is in fact costly and time-consuming and thus not amenable yet for high-throughput compound screening studies. On the other hand, iPSC-derived neural progenitor cells (NPCs) are homogenous and easy to expand and their transcriptional profile resemble that of NPCs residing in the human adult brain (Lorenz et al., 2017). iPSC-derived NPCs may thus potentially be suitable for large-scale screenings, once disease-specific and relevant phenotypes have been identified and confirmed both in NPCs and post-mitotic neurons (Inak et al., 2017). The gene editing of human PSCs still presents numerous challenges. Most importantly, there is the risk to introduce off-target effects that may mask or alter the actual disease phenotypes (Hockemeyer and Jaenisch, 2016). Secondarily, this approach may be highly valuable only for monogenic

diseases, while complex sporadic forms may be hard to tackle. Finally, editing of mtDNA still remains to be demonstrated.

Future studies should apply genome-editing technologies in iPSCs to target nuclear genes regulating mitochondrial function. By investigating the consequences on PSC-derived neurons and glia, it may be possible to gain important new knowledge regarding the role of mitochondria in human brain function **(Figure 2)**.

Overall, the use of PSCs may help unveiling the contribution of mitochondria in the healthy and diseased human brain. This information may ultimately lead to improved therapies for debilitating neurological and psychiatric diseases.

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Figure legends

Figure 1. Mitochondria in human brain cells. Mitochondria contribute to the physiological functionality of human neurons and glia. Their roles include the control of cellular bioenergetics, redox balance, apoptosis, and calcium homeostasis (see text for details). Abbreviations: PPP: pentose phosphate pathway; GSH: glutathione; Glucose-6P: glucose-6-phosphate; ROS: reactive oxygen species; TCA cycle: tricarboxylic acid cycle; Acetyl-CoA: acetyl coenzyme A; ATP: adenosine triphosphate.

Figure 2. Probing mitochondrial brain function with PSCs and genome editing. The use of human PSCs, combining patient-derived material with precise genome engineering, can allow dissecting the contribution of mitochondria to human brain function. Numbers in brackets refer to the following references: (1) Hossini et al., 2015; (2) Kondo et al., 2013; (3) Woodruff et al., 2013; (4) Fierli et al., 2014; (5) Shaltouki et al., 2015; (6) Cooper et al., 2012; (7) Nguyen et al., 2011; (8) Ryan et al., 2013; (9) Sanders et al., 2014; (10) Reinhardt et al., 2013; (11) Soldner et al., 2011; (12) Soldner et al., 2016; (13) Kiskinis et al., 2014; (14) Wang et al., 2017; (15) Mutihac et al., 2015; (16) The HD Consortium, 2012; (17) An et al., 2012; (18) Xu et al., 2017; (19) Nekrasov et al., 2016; (20) Gou et al., 2013; (21) Brennand et al., 2015; (22) Robicsek et al., 2013; (23) Mertens et al., 2015a; (24) Zheng et al., 2016; (25) Ma et al., 2015; (26) Lorenz et al., 2017; (27) Johri et al., 2012; (28) Reddy et al.,2012; (29) Sheng et al., 2012; (30) Lustbader et al., 2004; (31) Schapira et al., 1990; (32) Michel et al., 2013; (33) Bose et al., 2016; (34) Tafuri et al., 2015; (35) Shi et al., 2010; (36) Yano et al., 2014; (37) Song et al., 2011; (38) Panov et al., 2002; (39) Sorolla et al., 2008; (40) Chauhan et al., 2011; (41) Tang et al., 2013.

Figure 1

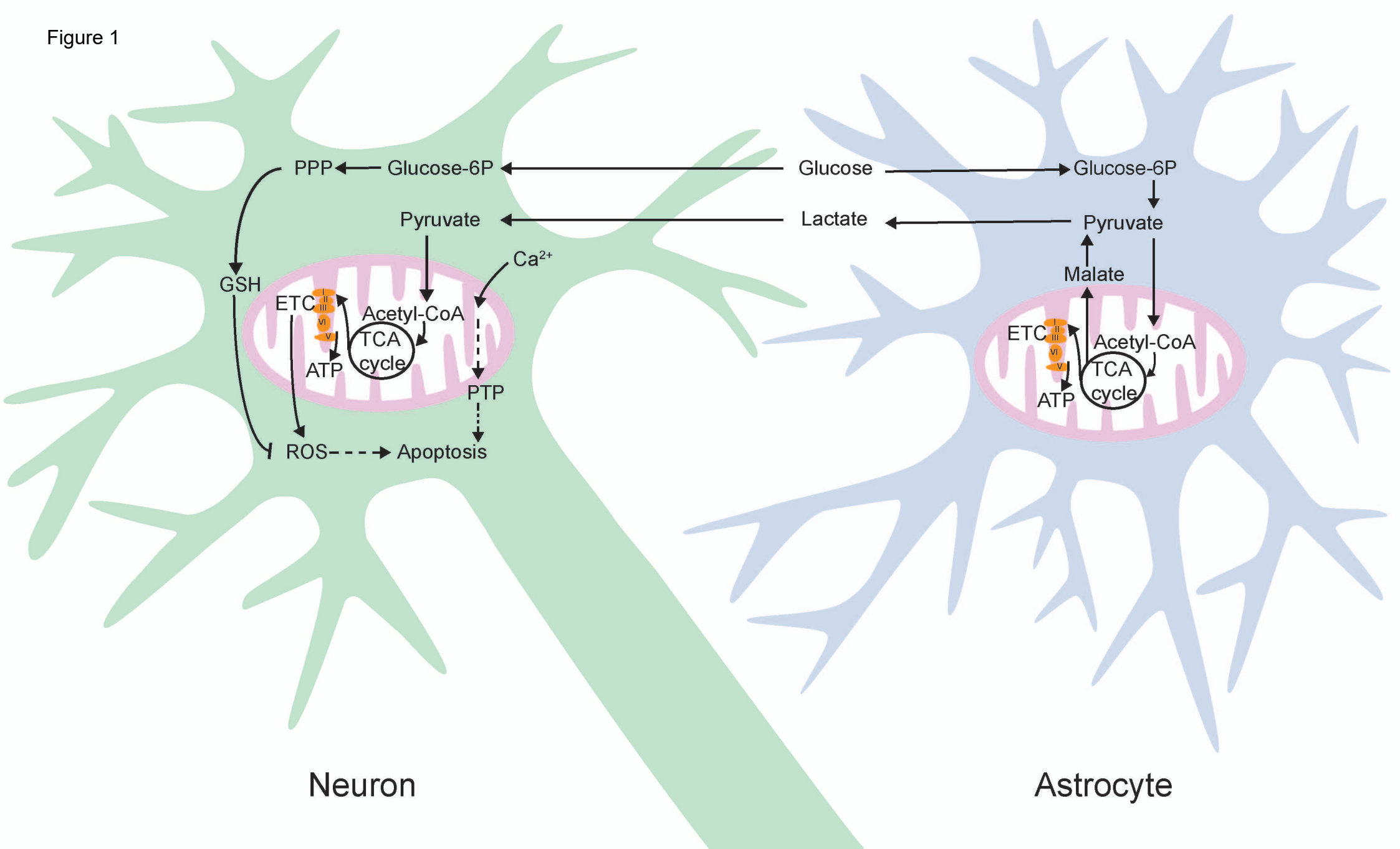
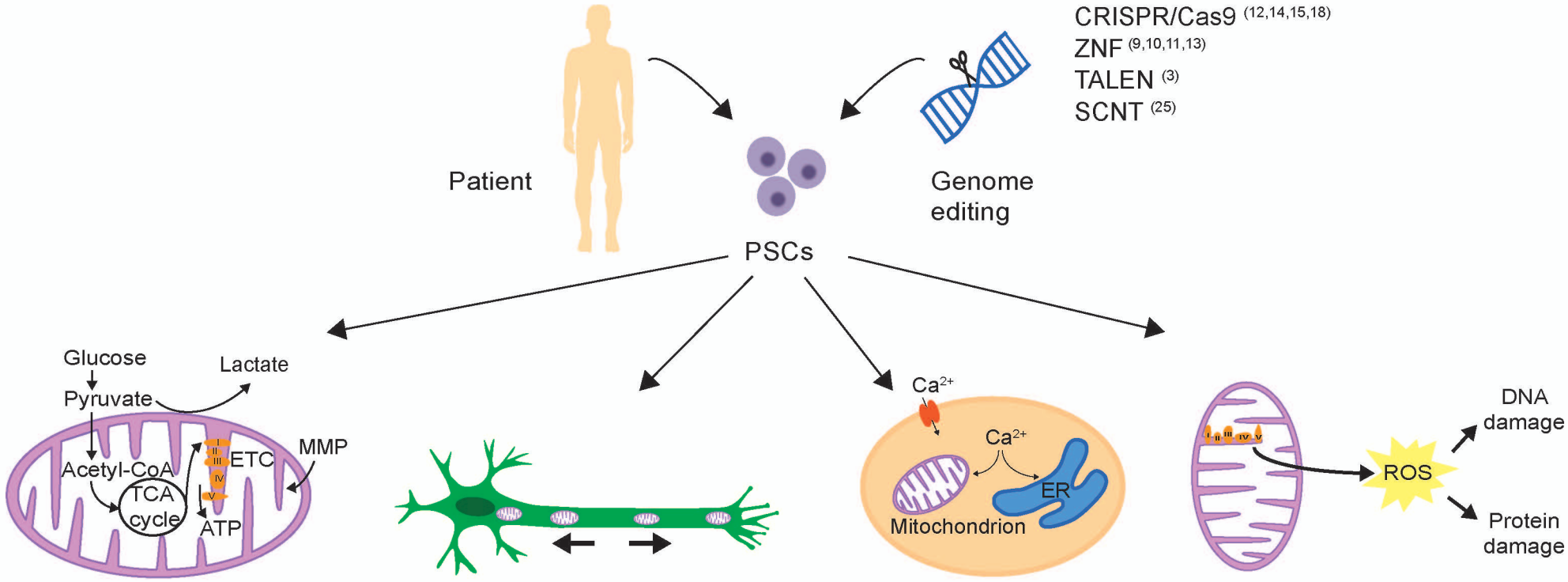

PPP
Glucose-6P
Glucose
Glucose-6P
Pyruvate
Lactate
Pyruvate
Ca2+
GSH
Malate
ETC
I
II
III
VI
V
Acetyl-CoA
TCA cycle
ATP
PTP
ROS
Apoptosis
Neuron
Astrocyte

Figure 2



	Bioenergetics	Mitochondrial dynamics	Calcium homeostasis	Oxidative stress
Mitochondrial defects in PSC-derived neural cells	Alzheimer's disease (1) Parkinson's disease (4,5) Huntington's disease (16-18) Leigh syndrome (24-26) Schizophrenia (22) Bipolar disorder (23)	Parkinson's disease (6) Amyotrophic lateral sclerosis (13) Huntington's disease (20) Schizophrenia (22) Bipolar disorder (23)	Huntington's disease (19) Leigh syndrome (26)	Alzheimer's disease (2) Parkinson's disease (7, 8) Schizophrenia (21)
Mitochondrial dysfunction	Alzheimer's disease (27) Parkinson's disease (27, 31) Amyotrophic lateral sclerosis (27, 35) Huntington's disease (27) Autism spectrum disorder (40, 41)	Alzheimer's disease (28, 29) Parkinson's disease (33) Amyotrophic lateral sclerosis (34, 35) Huntington's disease (36, 37)	Parkinson's disease (33) Amyotrophic lateral sclerosis (35) Huntington's disease (38)	Alzheimer's disease (30) Parkinson's disease (32) Huntington's disease (39) Autism spectrum disorder (40, 41)