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Review

Human-induced pluripotent stem cell technology: toward the future of personalized psychiatry

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Abstract: The polygenic and multifactorial nature of many psychiatric disorders has hampered the personalized medicine approach implementation in clinical practice. However, induced pluripotent stem cell (iPSC) technology has emerged as an innovative tool for patient-specific disease modeling to expand the pathophysiology knowledge and treatment perspectives in the last decade. Current technologies enable adult human somatic cell reprogramming into induced pluripotent stem cells (iPSCs) to generate neural cells and direct neural cell conversion to model organisms that exhibit phenotypes close to human diseases, thereby effectively representing relevant aspects of neuropsychiatric disorders. iPSCs reflect patient pathophysiology and pharmacological responsiveness, particularly when cultured under conditions that recapitulate spatial tissue organization in brain organoids. Recently, the application of iPSCs has been frequently associated with gene editing that targets the disease-causing gene to deepen the illness pathophysiology and conduct drug screening. Moreover, gene editing has provided a unique opportunity to repair the putative causative genetic lesions in patient-derived cells. Here, we review the use of iPSC technology to model and potentially treat neuropsychiatric disorders by illustrating the key studies on a series of mental disorders, including schizophrenia, major depression disorder, bipolar disorder, and autism spectrum disorder. The future perspective will involve the development of organ-on-a-chip platforms that control the microenvironmental conditions to reflect individual pathophysiological by adjusting physiochemical parameters according to personal health data. This strategy could open new ways to build a disease model that considers individual variability and tailors personalized treatments.

Keywords: personalized psychiatry; psychiatric disorders; induced pluripotent stem cells; brain organoids

1. The limitations of personalized medicine in psychiatry

Personalized medicine aims to predict disease susceptibility, achieve an accurate diagnosis, and select the most effective therapeutic option with the least adverse effects for each patient [1,2]. Personalized predictive models of diagnosis and outcomes are based on the collection of a massive amount of information (big data) to analyze using advanced computational tools capable of making a prediction from the gathered data, such as machine learning methods [2].

Before its psychiatric application, personalized medicine has brought significant diagnostic and therapeutical advances in oncology where the identification of the molecular target of the drug, the mechanisms of resistance to targeted compounds, the best pharmacological combinations for a specific tumor, promoted the optimal individual treatment selection [3].

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The reliance on diagnostic manuals, due to the lack of practicable diagnostic tests, along with the inherent complexity of psychiatric disorders make personalized medicine in psychiatry implementation more challenging than in other medical specialties [4]. The currently available diagnostic tools, the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [5] and the International Statistical Classification of Diseases and Related Health Problems (ICD-11) [6], provide a classification that fails to capture the biological processes that are believed to underly the identified disorders and favor treatment personalization in psychiatry [7]. This drawback is partly related because even the more narrowly defined disorders with diagnostic tools generally represent heterogeneous endpoints of different underlying causal pathways, amply intertwined with social, cultural, and experiential factors [8].

A diagnostic system using a combination of several psychopathological dimensions defined along with a range of severity, from normality to the full pathological expression, has been proposed because the categorical approach fails to fully express mental illness complexity [9]. However, research evidence that demonstrated that patient evaluated using a dimensional approach improves with treatment is lacking. Replacing the categorical DSM-5/ICD-11 classification system with a dimensional one appears not feasible if clinical outcomes are not demonstrably better [10].

Animal models have widely contributed to understanding several brain pathological mechanisms and developing effective drugs; however, fully reproducing the complexity of human psychiatric diseases is substantially impossible [11]. Moreover, some biological limitations derive from species brain differences. Relative to the mouse, the dominant model organism in research, the human cortex has a >1000-fold number of neurons and contains an inner fiber layer and outer subventricular zone that are absent in the mouse brain [12]. Notably, genes associated with neuronal structure and function have different transcriptional regulations in mice, non-human primates, and humans [13]. Moreover, mouse models are adequate to demonstrate pathological consequence production by the altered single gene function, whereas studies of psychiatric disorders, generally characterized by different gene penetrance and complex interactions, are difficultly performed using mouse models.

Finally, research has been hindered by the inaccessibility to human live brain tissue and the difficulty of isolating and culturing primary human neurons. Postmortem brain studies are burdened by confounding variables, such as treatment history, drug or alcohol abuse, and cause of death, and can not provide information on temporal psychiatric disease changes in which molecular and cellular alterations may occur years before the clinical symptom presentation [14].

The discovery of induced pluripotent stem cells (iPSC) technology has opened unprecedented opportunities as the described limitations underscore the need for psychiatric disease models based on human cells. In the next sections we summarize main concepts concerning iPSCs and provide an overview of their preliminary applications in the field of personalized psychiatry (PP).

2. The Induced pluripotent stem cells

2.1. Definition and development

IPSCs consist of artificial stem cells that are induced (or “reprogrammed”) in culture from somatic cells using different transcription factors. Stem cells are pluripotent cells derived from the embryo (embryonic stem cells [ESCs]) and various postnatal tissue sources, characterized by the ability of self-renewal (proliferation) and differentiation into various adult cell types, and thus called pluripotent stem cells.

In 2006, Takahashi and Yamanaka [15] demonstrated for the first time that terminally-differentiated somatic cells could be reverted into undifferentiated pluripotent cells and produced a paradigm shift in developmental biology, thereby breaking the previous view of cellular differentiation as a unidirectional and irreversible process.

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Somatic cells were reprogrammed to iPSCs through genomic integration by virus of the transcription factors (TFs), Oct4, Sox2, Klf4, and c-Myc. TFs remodel chromatin to activate gene expression in the pluripotency network and suppress lineage-specific gene expression that promotes differentiation [16]. iPSCs can form neural progenitor cells (NPCs) that can be further stimulated to differentiate into various central nervous system (CNS) cell types, including astrocytes and functionally active neurons. Human iPSCs were first established from skin fibroblasts through a skin biopsy. More recently, peripheral blood mononuclear cells [17], which can be readily attained via phlebotomy, and even cells in the urine can be reproducibly converted into iPSCs [18].

Retroviruses are extensively used for integrating exogenous genes into the genome to reprogram somatic cells and produce iPSCs. However, some risks could limit their use in iPSC-based therapies, such as those proposed to regenerate cardiac cells after myocardial infarction [19] or insulin-producing β cells to treat type 1 diabetes mellitus [20]. Particularly, the over-expression of exogenous reprogramming factors, such as the oncogene c-Myc, could stimulate cancer growth and cause tumor development after iPSC-derived cell transplantation [21]. Strategies to produce iPSCs with minimal or absent genomic integration use lentiviruses, which show a safer integration profile [22], and Sendai virus, an RNA virus that exclusively replicates its genome in the cytoplasm [23]. A non-viral system, such as a DNA plasmid that carries the reprogramming factors, which usually do not integrate into the host genome, was also available. DNA plasmid limitation includes low transfection efficiency compared to traditional systems based on retroviral or lentiviral vectors due to the transient gene expression.

Over the recent years, iPSC application has been frequently associated with gene editing. This technique targets the disease-causing gene to deepen illness pathophysiology and conduct drug screening [28]. Gene editing is a group of technologies that, differently from traditional transgenic techniques, allow to add, remove or alter genetic material at specific genome locations [29]. An accurate and efficient approach to gene editing is CRISPR-Cas9, the acronym for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. CRISPR/Cas9 system, in which Cas9 endonuclease can target specific DNA sequences with the help of guide RNA, can be used to target and correct any specific gene sequences, including genetic risk factors for psychiatric disorders [30]. CRISPR-Cas9 is generally used in iPSCs to generate cell models to investigate CNS disorders with a defined genetic alteration. Moreover, the identified pathogenic mutation in these models can be corrected by inserting, deleting, or replacing nucleotides, or modulating gene expression. Another option is to introduce pathogenic mutations into iPSC lines from healthy individuals to replicate human disease and generate isogenic pairs of cell lines that identify the true impact of the engineered cellular phenotype changes [31].

Finally, it should be noted that iPSC-derived neurons express embryonic features that directly show the genetic mutation effect on the cell phenotype but erase epigenetic changes due to age or disease progress [18]. If this feature may be useful to identify the specific genetic mutations, it may hinder the understanding of changes related to time or disease progression. To overcome this potential limitation, in recent years several methods have been implemented for directly reprogramming one somatic cell type into neurons (induced Neurons [iN]) without the transition through iPSCs (transdifferentiation). Transdifferentiated cells have the ability to be identical to their native counterparts; however, *in vitro* and *in vivo* assays are necessary to fully characterize them and compare them to native cells [24]. The first method of direct cell reprogramming consists of cell exogenous transgene introduction to overexpress key transcription factors that start the transdifferentiation process [25]. Other methods target endogenous genes that are vital to the transdifferentiation by directly manipulating the DNA or the epigenetic environment [26]. Finally, cells can be treated with pharmacological agents that can modify the genetic and epigenetic environment to promote transdifferentiation [27].

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2.2. Three-dimensional (3D) brain iPSC culture models (organoids)

Brain organoid (BO) is a stem cell-derived 3D tissue that recapitulates early cerebral developmental events and simulates the human brain's architecture and functionality *in vitro*. The possibility of generating organoids relies on stem cells' intrinsic self-organization properties into 3D architectures that contain multiple cell types and maintain some specific function and spatial human brain organization [32,33].

3D context, more than 2D culture systems, allows the study of alterations in neuronal migration, cortical layering, and axon guidance that characterize neurodevelopmental and neuropsychiatric disorders, which often do not display observable neuroanatomical phenotypes [34]. Technical advances in 3D culture systems have reproduced different human brain regions [35], and the combination of these independent regional BO explores complex neuropathological conditions.

BO generation began with the development from iPSCs or ESCs of embryoid bodies (Ebs) containing three layers, namely, endoderm, mesoderm, and ectoderm. Neural tissue in humans develops from the ectoderm; thus, Ebs were placed in neural induction media that promote neuronal differentiation after 1 month of culture and then embedded in scaffold support to grow complex organoids. The embedded organoids were transferred into a spinning bioreactor for further maturation and preservation through enhanced nutrient absorption. Over the next 1–2 months, the cerebral tissue gradually expanded to form different brain regions surrounded by cortical tissue [36].

3. The potential of induced pluripotent stem cells in personalized psychiatry

The best medical application of iPSCs is generally thought to be in cell transplantation therapy; however, disease modeling and drug screening could be as relevant as cell therapy, making iPSCs attractive candidates for future PP development. iPSC-disease modeling reproduces a pathologic condition *in vitro* by reprogramming the patient's somatic cells into iPSCs, followed by redifferentiating the patient-specific iPSCs into disease-specific cells. Additionally, drug screening procedures can use the derived cells from humans for which the test compounds are therapeutically intended.

iPSCs may be derived from individuals with various monogenic and polygenic disorders that provide a precious resource for understanding the underlying molecular and cellular mechanisms through personalized models of psychiatric diseases. Moreover, cells are reprogrammed to a very early stage of development; thus, they can provide information on developmental or differentiation defects, as well as the temporal sequence of events, in the early stages of disease progression.The timeline for iPSC differentiation into CNS cells follows the same trajectory as in the developing embryo; thus, these cells are handy tools to study psychiatric disorders, including schizophrenia (SCF) and neurodevelopmental disorders, such as autism spectrum disorder (ASD), where neurodevelopmental alterations are believed. Moreover, BO, which contains multiple organ-specific cell types with function and spatial organization similar to a human brain, opened up a new avenue for investigating psychiatric disorders.

Finally, iPSCs could offer the possibility of overcoming the ethical concern of destroying a potential human life and the technical limitations related to ESC use. Producing a large number of ESCs or deriving them from patients with diseases is impossible; thus, their use is limited to studies of normal cellular function or the introduction of known engineered genetic changes [31].

In the following paragraphs we provide an overview of preliminary studies that used IPSCs in severe psychiatric disorders.

3.1. Schizophrenia

Schizophrenia (SCF) is a severe mental illness that is associated with subtle brain cortical structure changes and is characterized by the following symptoms: delusions,

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hallucinations, disorganized thinking, abnormal motor behavior, and negative symptoms [5].

IPCS studies in patients with SCF are mainly devoted to investigating the pathogenesis of the illness. The first human study by Brennand et al. found diminished neuronal connectivity, neurite number, PSD95 synaptic protein levels, as well as altered gene expression profiles of the cAMP, wingless-related integration site (Wnt) signaling pathways, and glutamate receptors in iPSC-derived neurons of patients with SCF, compared to controls. Wnts are secreted factors that regulate cell proliferation and differentiation during embryonic development and act by activating diverse signaling cascades inside the target cells. These alterations are consistent with those described in the postmortem brain of patients with SCF and animal models of SCF and could be ameliorated with the antipsychotic loxapine [37].

Narla et al. identify the nFGFR1 signaling, which integrates signals from diverse pathways that are characterized by schizophrenia-linked mutations, as a common altered mechanism and a potential therapeutic target in investigated patients [38].

Hippocampal neurogenesis aberrations have been implicated in SCF pathogenesis [39]. Thus, iPSC-based case/control studies investigated the early development in iPSC-derived hippocampal neurons of patients with SCF. The dentate gyrus (DG) of the hippocampus is one of the two areas of the brain where neurogenesis continues throughout life and its generated neurons play a key role in learning and memory. Yu [40] described reduced neuronal activity and reduced spontaneous neurotransmitter levels released in the dentate granule neurons from SCF-iPSC-derived hippocampal NPCs. Moreover, hippocampal CA3 neurons that are derived from these patients had altered network connectivity when co-cultured with human dentate granule neurons [41].

The mitochondrial tricarboxylic acid (TCA) cycle (also referred to as the Krebs cycle) is known to largely satisfy the energetic neuron demand, thereby generating approximately 90% of cellular reactive oxygen species (ROS). Mitochondrial dysfunction enhances ROS formation, which negatively acts on mitochondrial function, leading to oxidative damage that affects several cellular components, such as lipids, DNA, and proteins. The human fetal brain is particularly vulnerable to oxidative damage due to its high oxygen consumption, high ROS basal level production, and still less developed antioxidant defense mechanisms relative to the adult brain. Collectively, the studies in iPSC-NPCs and neurons of patients with SCF showed perturbed mitochondrial respiration and morphology along with signs of increased oxidative damage compared to controls [42].

Globally, SCF iPSC-derived NPCs demonstrated altered migration and Wnt signaling [49], increased oxidative stress [43], and perturbed environmental stressor responses [44].

BO studies in patients with SCF have revealed several phenotypes that may be associated with early neurodevelopmental defects, such as nFGFR1 signaling [45] and immune response alterations [46]. The BOs produced with iPSCs of eight patients with SCZ and eight healthy controls were compared with a transcriptomic approach to identify disease-specific differences [47]. RNA sequencing demonstrated aberrant gene expression in pathways involved in synaptic biology, nervous system development, immune response, mitochondrial function, and excitatory and inhibitory neurotransmission modulation.

Notaras et al. observed that SCF BO principally differed from healthy controls in the total quantity of molecular factors (rather than their diversity) and the altered expression of an ensemble of neuronal factors using iPSC-derived BO to analyze molecular factors regulating CNS development [48]. Moreover, the differential regulation of two novel specific disease candidates identified through genome-wide association study (GWAS) arrays (namely, Pleiotrophin and Podocalyxin) was observed [48].

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It should be noted that some of the biological alterations that emerged from iPSC-based studies appeared not to be specific to SCF but shared by several disorders. Particularly, studies have identified in disrupted in schizophrenia 1 (DISC1), a gene associated with diverse mental disorders, upon the finding that its coding sequence is interrupted by a balanced translocation in a Scottish family, in which the translocation co-segregates with SCF, bipolar disorder, and major depressive disorder [49]. The variety of disorders in subjects that harbor the translocation supports the hypothesis that the translocation leads to a subtle disruption in neural development that predisposes to mental disorders by increasing vulnerability to other environmental and genetic risk factors. An isogenic iPSC model demonstrated that DISC1 gene disruption at the site of the balanced translocation causes loss of expression of longer DISC1 transcripts, which increases baseline Wnt signaling and alters the transcriptional profile of neural progenitor cells and neurons, resulting in neurodevelopmental disorders [49].

Generated cerebral organoids by DISC1-disrupted iPSCs showed disorganized structural morphology and impaired proliferation, which is phenocopied by Wnt agonism and rescued by Wnt antagonism [50]. The shared changes in BO morphology and gene expression with DISC1 interruption and Wnt agonism highlight the link between DISC1 mutation, Wnt signaling abnormalities, and neuropsychiatric diseases.

Overall, the iPSC-based studies in patients with SCF suggest the presence of an early genic dysregulation leading to altered neuronal and brain development.

3.2. Major Depressive Disorder

Major Depressive Disorder (MDD) is a common and severe mental disorder that is characterized by one or more major depressive episodes, defined as discrete periods of at least 2-week duration, but generally longer, in which at least five of the following nine symptoms are present: depressed mood, loss of pleasure or interest, significant appetite disturbance/body weight change, sleep disturbance, loss of energy, psychomotor changes, excessive guilt and/or worthlessness, decreased concentration, and recurring death and/or suicide thoughts [5]. Unsatisfactory response rates to currently approved antidepressant drugs, which are effective in approximately half of the treated patients, contribute to the heavy medical and economic burden of MDD [51].

iPSC-based studies on MDD focused on treatment-resistant depression (TRD), which is defined as the failure to achieve a reduced baseline depressive symptomatology of at least 50%, after at least two antidepressant treatment trials of adequate dosage and duration. A study on patients with MDD showed that iPSCs derived from patients who are non-remitted with selective serotonin reuptake inhibitors (SSRIs) antidepressant therapy displayed serotonin-induced hyperactivity downstream of upregulated excitatory serotonergic receptors (5-HT2A and 5-HT7) in contrast to what was seen in healthy and remitted patient-derived iPSCs. Lurasidone, which is a high-affinity 5-HT2A and 5-HT7 antagonist, partially rescued 5-HT-induced hyperactivity in non-remitted patient-derived iPSCs [52].

Another study of the same group revealed no significant differences in serotonin release/reuptake or genes related to serotonin pathways in iPSCs and serotonergic neurons of SSRIs treated patients with MDD [53]. However, non-remitted patient-derived serotonergic neurons exhibited altered neurite growth and morphology downstream of lowered key Protocadherin alpha gene expression compared to healthy controls and remitted patients [53]. The protocadherin-α family includes cell adhesion molecules that are required for serotonergic projections to appropriately innervate target brain areas so that their loss from serotonergic neurons, leads to unbalanced distributions of serotonergic axons [54].

Ketamine, a glutamate N-methyl-d-aspartate (NMDA) receptor antagonist, is a promising treatment for patients with TRD, providing significant depressive symptom improvement within hours of intravenous administration. An iPSC-based study of

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ketamine's antidepressant mechanisms of action supported the leading hypothesis that ketamine can enhance structural plasticity through the AMPA glutamate receptor-driven raise of Brain-Derived Neurotrophic Factor levels, leading to an increased synaptic number and function in the prefrontal cortex [55].

The mechanisms underlying the prolonged antidepressant effects (1 week) after a single ketamine infusion are poorly understood. The triggering of synaptic function and plasticity was hypothesized because a ketamine half-life of approximately 2 hours cannot explain the long-lasting antidepressant effects. A study that supported this view demonstrated that dopaminergic neurons that are differentiated from iPSCs obtained from healthy donors who are exposed for 6 hours to ketamine metabolite (2R, 6R)-hydroxynorketamine (half-life 6–12 h) produced dendrite outgrowth when measured 3 days after exposure [56].

3.3. Bipolar disorders

Bipolar disorder (BD) is characterized by the recurring swing between the opposite mood states of major depressive episodes and mania (BD type I) or hypomania (BD type II). Mania is more severe than hypomania and causes markedly impaired social or occupational functioning or necessitates hospitalization. Both manic and hypomanic episodes are characterized by expansive or irritable mood, increased activity and self-confidence, talkativeness, and distractibility, as well as decreased need for sleep, racing thoughts, and poor judgment (5). First-line BD treatment includes mood stabilizers, mainly lithium. IPSC-based studies in BD are focused on elucidating the pathogenetic mechanism also by examining the detailed effects of lithium and/or other BD drug exposure on the different signaling pathways.

The first study that investigated iPSC-derived neuronal cells from patients with BD revealed that the gene expression encoding membrane-bound receptors and ion channels, particularly transcripts involved in calcium signaling, was significantly increased in the neurons generated from 3 patients with BD compared to those obtained from 3 healthy controls [57]. Additionally, *in vitro* lithium pretreatment significantly altered the calcium signaling and electrophysiological properties in BD neurons but not in controls [57]. Notably, calcium signaling has a central role in controlling inappropriate neuronal responses and tonic excitability. A proteomic study in iPSC-derived dorsal anterior forebrain cortical neurons suggested that the molecular lithium-response pathway in patients with BD may function via collapsin response mediator protein-2, which acts to modify neuronal dendrite and dendritic spinal formation [58].

The expression of multiple BD-linked genes that are involved in neuronal development, differentiation, and neuroplasticity are regulated by microRNAs (miRNAs), which are small, non-coding RNAs. miR-34a is predicted to target genes implicated as risk factors for BD and is reduced by lithium and valproic acid, two mood stabilizers that are widely prescribed to prevent depressive and manic recurrences [59].

A miR-34a increased expression has been detected compared to healthy controls in postmortem brain tissue, directly induced neuronal cells, and iPSC-derived neuronal cells from patients with BD [60]. The overexpression of miR-34a *in vitro* was reported to decrease CACNB3 and ANK3 gene expression, previously identified as bipolar risk genes, leading to impaired neuronal differentiation, synaptic protein expression, and neuronal morphology [60]. Afterward, several studies [61, 62] investigated the specific risk gene variants in iPSC-derived neuronal cells from patients with BD.

CXCR4(CXC chemokine receptor-4) expressing NPCs were analyzed from two BD-affected brothers and their two unaffected parents in a family-based paradigm that has the advantage of controlling for the genetic background [63]. The study revealed that patients with BD, compared with their unaffected parents, displayed multiple

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phenotypic differences at the neurogenesis level and gene expression critical for neuroplasticity, including Wnt pathway components and ion channel subunits.

Kim et al. studied iPSC-derived neurons that are obtained from patients with BD type I and their unaffected siblings from an Old Order Amish pedigree with a high BD incidence [64]. The observed significant disease-associated differences in gene expression suggested that RNA biosynthesis and metabolism, protein trafficking, and receptor signaling pathway alterations may play a role in BD pathophysiology.

Studies on iPSC-derived neuronal cells' electrophysiological activity may help predict lithium response and develop novel drugs for BD treatment. Differential hyperexcitability responses to *in vitro* lithium treatment in iPSC-derived hippocampal dentate gyrus-like neuronal cells from six patients with manic BD type I and four unaffected individuals were detected. Particularly, the hyperexcitability phenotype in BD was selectively reversed by lithium treatment only in neurons that are derived from patients who responded to lithium treatment, suggesting that this model of iPSCs might help develop new drugs [65]. Notably, the electrophysiological data obtained from iPSC-derived neuronal cells treated with lithium was used to train an algorithm that can predict the lithium responsiveness of a new patient with a success rate of over 92% [66].

The first comprehensive study that compared BO generated from patients with BP type I and healthy individuals demonstrated transcriptomic differences with gene downregulation involved in cell adhesion, neurodevelopment, and synaptic biology, along with the gene upregulation involved in immune signaling in patients with BP type I [50].

3.4. Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) refers to a broad range of lifelong neurodevelopmental conditions characterized by impaired social abilities and cognitive functions. ASD can be of unknown polygenic etiology (idiopathic) or a specific syndromic disorder, such as Fragile X, caused by a single gene mutation [67].

iPSCs recapitulate aspects of the neuronal development process while preserving the patient's genetic background and are frequently used to model idiopathic ASD, for clarifying pathogenetic mechanisms. The increased proliferation and differentiation abnormalities of iPSC-derived NPCs from patients with idiopathic ASD with macrencephaly compared to non-ASD controls with normal brain size were suggested to result in early brain overgrowth [68]. Schafer et al. followed iPSCs from patients with idiopathic ASD with early brain overgrowth during their neuronal development to examine when and how the earliest ASD-specific abnormalities arise [69]. ASD-associated changes, involving temporal dysregulation of specific gene networks and morphological growth acceleration, tracked back even before the neuronal stage in neural stem cells (NSCs). Bypassing NSC-like stages by direct ASD iPSC conversion into neurons prevents the manifestation of the observed neuronal ASD-associated [69].

Mariani et al. showed the overexpression of the gene FOXG1, which generates an overproduction of GABAergic neurons, and, in turn, an increased brain volume and imbalanced excitation and inhibition systems in the developing cortex, using gene expression analyses on patients with ASD iPSC-derived BO [70]. Unfortunately, studies on idiopathic ASD NPCs show conflicting results relating to the proportion of GABAergic inhibitory precursors compared to glutamatergic precursors [71].

Fragile X syndrome (FXS) is the most prevalent single-gene form of ASDs and is characterized by cognitive impairment, defective communication, hyperactivity, and impulsivity. FXS is caused by transcriptional FMR1 gene silencing on the X-chromosome during embryonic development with the consequent loss of Fragile X Mental Retardation Protein (FMRP) expression. FMRP is a selective RNA-binding protein that regulates the translation of many genes at the synaptic sites. The lack of FMRP leads to aberrant differentiation in human iPSC-derived neural progenitor cells [72].

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Conventional bidimensional and 3D FXS models based on isogenic FMR1 knock-out mutant iPSCs display altered cortical neuron gene expression and impaired differentiation compared with the wild-type human iPSCs. Cortical BO models show an increased number of glial cells, such as astrocytes, and bigger organoid size, which suggests that FMRP is required to correctly support neuronal and glial cell proliferation and the correct excitation/inhibition ratio in human brain development [73].

Nonsense-mediated RNA decay (NMD) is a cellular surveillance pathway that safeguards the quality and stability of mRNA transcripts by targeting them for degradation if altered. FMRP deficiency results in hyperactivated NMD in FXS fibroblast-derived iPSCs, with a negative consequence on iPSC maturation to neurons [74].

4. Limitations of iPSC technology and future perspectives

Although iPSC models provide an innovative tool to understand the pathophysiology of neuropsychiatric disorders and perform drug screening in disease-relevant cells, several limitations remain. iPSC-based studies are impacted by variation in reprogramming and neuronal differentiation efficiencies between iPSC lines derived from both the same and different donors. Two main strategies were developed to face this variation. The first tries to decrease the heterogeneity by selecting patients with shared clinical and biological characteristics or drug responses, with the expectation of inter-individual variation reduction *in vitro*. The second strategy uses a large cohort of disease-relevant iPSC cell lines to minimize sample variability. This effort is costly and time-consuming, thus iPSC banks were created to provide repositories from which many iPSC lines that are available for a particular disease are stringently checked for quality [75].

One critical issue with iPSC-based disease modeling is the generation of appropriate control iPSCs. Initially, control iPSCs were obtained from healthy, gender-matched family members, but these iPSCs exhibited substantial heterogeneity due to genetic differences. More recently, genetically identical (isogenic) iPSC lines that are created by gene editing approaches from well-characterized healthy control iPSC lines have been employed.

A further drawback of iPSC technology refers to the reprogramming process itself, which erases the epigenetic memory of cells. iPSC-derived neurons are immature and maintain the fetal neuron properties, independently of the age of the initial somatic cell donor [76]. This aspect represents a limit for both the adult-onset psychiatric disorders and those which are influenced by environmental factors that are known to modify epigenetics. Therefore, iPSC-derived models are an opportunity for studying susceptibility rather than normal disease or disease progression [77]. Techniques to artificially induce age in iPSC-derived lineages for modeling late-onset disorders, such as the exposure to compounds that trigger mitochondrial stress or reactive oxygen species, have been developed [76].

The discovery of direct reprogramming technology has enabled iNs production, bypassing the iPSC stage. This approach produces a reliable model for late-onset psychiatric disorders, aging-related neurodegenerative diseases, and drug discovery as it does not reset aging information [78]. Unfortunately, iN, unlike iPSC, does not maintain self-renewal, which is required for maintenance and stock; hence, it needs to acquire a larger quantity of original cells from a patient to obtain enough iNs.

A central limitation of BO is the absent development past the stage of a prenatal brain, probably because the organoids do not elaborate a vascular system. This inadequacy additionally leads to necrotic core formations during tissue development. Therefore, engineering approaches that allow the proper exchange of oxygen, nutrients, and waste products, such as porous scaffolds, are recently produced using computer-aided design, and 3D printing was implemented to address this issue [79].

Another constraint, especially for 3D cultures, is the extended period. Achieving later stages of cell maturation, including astrocyte maturation takes approximately 9

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months. This challenges the feasibility of using these 3D cultures on a very large scale and considerably slows the experimental processes [80].

From a therapeutic perspective, gene-editing techniques could be used to repair the genetic mutations that contribute to disease in an *in vivo* approach, which involves direct cell modification in the individual, and to repair known causative lesions in patient-derived iPSCs (*ex vivo* gene therapy). This last intervention requires the culture and modification of patient-derived iPSCs *in vitro* and the transplantation of the modified cells back into the recipient [81].

iPSC replacement therapies for neurodegenerative disorders, such as Parkinson's disease, are challenged by the transplantation into an environment where pathogenic mechanisms occur, thereby causing neuronal degeneration and apoptosis. In theory, inserting healthy cells in this type of environment can either have a positive effect due to the neuroprotective factor secretion or result in graft failure. In a recent study, a personalized cell-therapy strategy that used iPSC-derived dopaminergic cells in a patient with Parkinson's disease leads to a clinical improvement 24 months post-transplantation, a time frame consistent with gradual putamen reinnervation by projections from dopaminergic neurons [82].

A more recent and innovative advancement in iPSC technology is the development of the organ-on-a-chip platforms (OOC) that introduces microfluidic techniques in cell cultures to reproduce the microenvironment through the control of biochemical factors, mechanical forces, and fluid flow. OOC aims to reflect individual pathophysiological conditions by including blood samples and patient-derived iPSCs and by adjusting biochemical parameters according to personal health data [83]. This strategy could open new ways to build a disease model that considers individual variability and should lead to a "personalized" representation of the single patient and therefore be directly applied in the clinic to inform targeted prevention and treatment strategies. The personalized OOC has not yet been applied in clinical practice. The key steps for future successful implementation include the demonstration that OOC has added value in directing personalized treatment and prevention strategies.

It is essential to highlight that even the most innovative PP advances should be understood not as a replacement but as an integration of patient assessment. Every therapeutic choice must be inserted into the individual patient's reality in light of his/her personal and clinical history, complying, to the extent possible, with the preferences regarding the type of intervention.

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