Schizophrenia Research 210 (2019) 3–12

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Stem cell models of schizophrenia, what have we learned and what is the potential?

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ARTICLE INFO

Article history:
Received 5 July 2018
Received in revised form 14 December 2018
Accepted 16 December 2018
Available online 23 December 2018

Keywords:
Schizophrenia
Induced pluripotent stem cell
Neurogenesis
Risk factors
WNT signalling
Cellular models
Organdies
CRISPR

ABSTRACT

Schizophrenia is a complex disorder with clinical manifestations in early adulthood. However, it may start with disruption of brain development caused by genetic or environmental factors, or both. Early deteriorating effects of genetic/environmental factors on neural development might be key to described disease causing mechanisms. Establishing cellular models with cells from affected individual using the induced pluripotent stem cells (iPSC) technology could be used to mimic early neurodevelopment alterations caused by risk genes or environmental stressors. Indeed, cellular models have allowed identification and further study of risk factors and the biological pathways in which they are involved. New advancements in differentiation methods such as defined and robust monolayer protocols and cerebral 3D organoids have made it possible to faithfully mimic neural development and neuronal functionality while CRISPR-editing tools assist to engineer isogenic cell lines to precisely explore genetic variation in polygenic diseases such as schizophrenia. Here we review the current field of iPSC models of schizophrenia and how risk factors can be modelled as well as discussing the common biological pathways involved.

1. Introduction

Schizophrenia (SCZ) is a highly heritable, complex neurodevelopmental disorder (Sullivan et al., 2003; Weinberger, 1987) characterised by positive, negative and cognitive symptoms including delusions and hallucinations (positive symptoms; so-called psychosis); lack of motivation and emotion, and disorganised speech (negative symptoms); and attention deficits, problems in learning and memory, and slower processing speed (cognitive impairments). These symptoms lead to a severe economic burden on patient society, with SCZ costing £11.8 billion to the National Health Service in England (Commission, 2012). Cellular and molecular mechanisms contributing to this disease remain largely unknown, despite its high prevalence. This is mostly due to the lack of available tissue samples that can accurately model SCZ pathogenesis – in vivo imaging and studies of post-mortem tissue can be useful for showing disease neurobiology, and animal models can be used to certain extent, however due to the complexity of the human brain and the manifestations of SCZ symptoms, there are limitations to these methods. Advancements in induced pluripotent stem cell (iPSC) technology (Takahashi et al., 2007; Takahashi and Yamanaka, 2006) has allowed for reprogramming of cells from patients to iPSCs harbouring the patient genome, which can then be differentiated to disease-relevant cell types, allowing recapitulation of the disease in cells at various stages of development. Disease modelling with the iPSC technology is a promising tool for mimicking distresses during early and late neuro-development which could lead to increased risks of developing SCZ (for review see Millan et al., 2016). Moreover, engineering human pluripotent stem cells with CRISPR-Cas9 genome editing tools makes it possible to generate isogenic cell lines with site specific genome alterations (single nucleotide mutations and common/rare gene variations) at sites implicated in the development of SCZ (Forrest et al., 2017; Pak et al., 2015). Several of the SCZ risk factors have been functionally implicated during prenatal/perinatal stages (Jablensky et al., 2017) highlighting the possible usefulness of iPSC models for dissecting cellular mechanism behind such complex disorders and SCZ.

There are many hypotheses regarding the molecular mechanisms behind SCZ, however, there is no unifying mechanism underlying all cases (Lang et al., 2007) but rather evidence for a complex genetic disorder, with over 100 genetic variants identified as risk factors (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Furthermore, environmental factors, such as birth complications, stress or drug use, have also been found to play a key role in the development of SCZ (Brown, 2011). In many cases, environmental conditions interact

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https://doi.org/10.1016/j.schres.2018.12.023
0920-9964/

with genetic effects, meaning that individuals with a "vulnerable" genetic background may be more sensitive to environmental stressors. It could be difficult to distinguish between environmental and genetic contributions to SCZ development and progression (Brown, 2011) – a meta-analysis of twin studies of SCZ demonstrated that, although the heritability of SCZ is high (81%), there is a significant environmental contribution of 11% to the risk of SCZ (Sullivan et al., 2003).

The main challenge for iPSC-based modelling of SCZ is to understand how our findings of cellular phenotypes correlate to biological pathways involved in the development of SCZ and how the genetic and/or environmental risk factors would cause the phenotypes. Various altered cellular phenotypes in iPSC models of SCZ have been detected such as skewed neurodevelopment, skewed cell fate determination, altered electrophysiological activities and altered neuronal network connectivity [reviewed in details in (Falk et al., 2016)]. The first report on iPSC differentiation to neural progenitors and later to neural cells from patients with idiopathic SCZ, exhibited normal cellular morphology and normal electrophysiological activities however, decreased neural connectivity and less synaptic proteins in SCZ patient neural cells (Brennand et al., 2011). To decrease heterogeneity between patients, recent studies used more defined cohorts and explored the disease mechanisms at different stages of neural development [for review see (Hoffman et al., 2018)]. Patient versus control differences were observed at early phases of neural differentiation of iPSC at the neural progenitor cells (NPC) stage. For example, altered proliferation rate (Murai et al., 2016), de-regulated WNT signalling baseline and responsiveness (Srikanth et al., 2015; Topol et al., 2015) differences in migration capacity and cell polarity (Yoon et al., 2014) and increased susceptibility to oxidative stress (Brennand et al., 2015), which all were de-regulated mechanisms at the NPC stage. Further differentiation of the NPC to neuronal cells exhibited additional functional alterations in patient cells such as neuronal maturation (less synaptic connectivity and less neurotransmitter release) (Hook et al., 2014) and skewed polarization (Roussos et al., 2016).

Several cell types such as excitatory and inhibitory neurons (Wen et al., 2014), glial progenitors (Windrem et al., 2017) and microglial cells (Sekar et al., 2016) have been identified in the development of SCZ, additionally, the organisation, migration and polarity of these cells might be hard to recapitulate in homogenous monolayer iPSC models. Thus, some of these limitations could be addressed by using co-culturing and/or 3D culturing systems such as organoids and brain spheroids (Lancaster and Knoblich, 2014). Cellular phenotypes observed in SCZ patients' organoids includes, less proliferation, less migration potential and decreased intracortical connectivity (Stachowiak et al., 2017; Ye et al., 2017).

The literature describes several common de-regulated cellular phenotypes between iPSC models of idiopathic SCZ with different known genetic risk loci, making it difficult to pinpoint if observed phenotype is caused by the identified aberrant genetics or due to other unknown factors or the combination of the two. Genome editing approaches such as CRISPR-editing tools or TALENS could be utilized to correct or introduce genomic alteration and would then result in isogenic iPSC different to the parental cells only at the place of editing. Comparison studies between the parental and edited iPSC lines would uncover cellular phenotypes caused by the identified genetic alteration (Fig. 1).

2. Genetic risks in schizophrenia

Genetic studies, such as genome-wide association study (GWAS) and comparative genomic hybridization have been carried out on many SCZ cohorts (Kushima et al., 2018; Li et al., 2017; Rees et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics, 2014). These studies have identified vast genetic variation across patients, with risk alleles identified ranging from common alleles with low penetrance (weak effect) to rare alleles with high penetrance (Tansey et al., 2015). Genetic variation identified includes copy number variations (CNVs) and single nucleotide polymorphisms (SNPs). A large GWAS carried out by the Psychiatric Genomics Consortium in 2014 (Schizophrenia Working Group of the Psychiatric Genomics, 2014) identified 108 SCZ-associated genetic loci, including 83 novel loci, each with only a small effect on the risk of SCZ. Despite this fact, it is thought that common loci account for at least one-third of the genetic risk for SCZ (The International Schizophrenia, 2009).

The majority of risk genes found converge upon common biochemical pathways that are known to be disease-relevant. For example, a number of genes are involved in NMDA receptor signalling (Harrison, 2015). Previous studies have shown that NMDA receptor hypofunction (Gao and Snyder, 2013; Olney et al., 1999), along with abnormal function of glutamate synapses (Goff and Coyle, 2001), are core to the pathophysiology of SCZ. Genes including the NMDAR gene GRIN2A

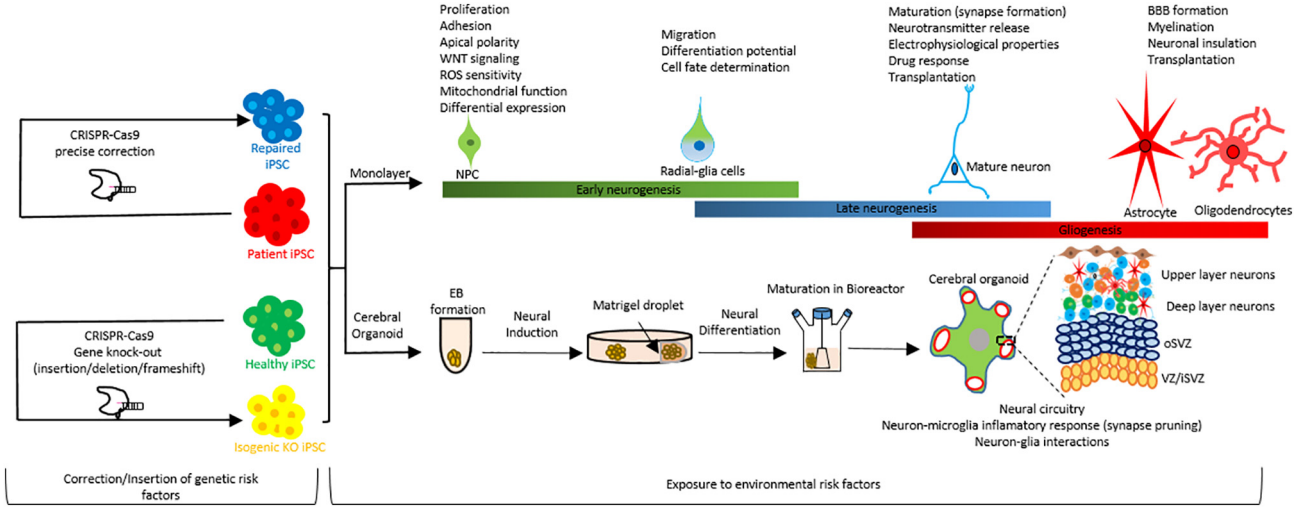

Fig. 1. Using iPSC technology to model schizophrenia in vitro. Schematic example of how human iPSC disease modelling could be performed in vitro using various technologies, cell stages, cell type, patient samples and assays. iPSC (red) with known genetic aberration could be a source to produce precisely corrected iPSC line (blue) using the CRISPR-Cas9 technology. Meanwhile healthy iPSC (green) can be targeted for specific gene(s) to generate an isogenic knock-out iPSC line to investigate roles of genetic variations during neurogenesis. All iPSC lines can be studied through differentiation processes in monolayer or 3D by the generation of cerebral organoids. Altered phenotypes during the differentiation of SCZ patient's iPSC or KO iPSC vs healthy control iPSC or corrected iPSC known so far have been listed on top of each cellular process. Environmental stressors involved in development of SCZ could be added and the effect investigated during the differentiation procedure. VZ: ventricular zone, iSVZ: inner sub-ventricular zone, oSVZ: outer sub-ventricular zone, BBB: blood-brain barrier, ROS: reactive oxygen species.

and other NMDAR signalling genes (GRIA1, GRM3 and SRR (Schizophrenia Working Group of the Psychiatric Genomics, 2014)) have been found to have genome-wide significance in the development of SCZ. It has also been found that a number of SCZ-associated genes are regulated by the microRNA mir-137. The gene encoding this microRNA has genome-wide significance, as do genes encoding several mRNA targets of mir-137, including TCF4, ZNF804A and CACNA1C. This suggests that dysregulation of mir-137 targets could also lead to SCZ.

Although rare SNPs have recently been identified to have a role in SCZ, with an overrepresentation of genes involved in calcium signalling and the ARC (activity-related cytoskeleton-associated scaffold protein) complex (Purcell et al., 2014), most rare genetic variation takes the form of larger regions of structural variation, usually encompassing multiple genes (Sebat et al., 2009). For example, deletions at 22q11.2 result in the loss of around 30 genes and are the strongest identified risk factor for SCZ (Bassett and Chow, 2008; Van et al., 2017). These rare CNVs tend to confer a large effect on risk, with each patient usually only possessing one pathogenic CNV. Genetic susceptibility to intellectual disability (ID), autism spectrum disorder (ASD), and SCZ could arise from mutations in the same genes, suggesting that they might share common mechanisms (Shohat et al., 2017). It has been suggested that the spatiotemporal activity of the genes contribute to specific disease phenotypes (Shohat et al., 2017). Thus, not only the specific gene (s) mutated but also the type of mutation (loss of function or missense) in addition to the expression time point during neurodevelopment would change the clinical manifestation of neuropsychiatric diseases (reviewed in details in Hoffman et al., 2018). For instance, de novo mutations in SCZ are enriched in adolescent cortex genes while they in autism are overrepresented in genes expressed in foetal cortex, cerebellum, and striatum (Shohat et al., 2017). Below we summarise the knowledge about known rare genetic risk factors involved in the development of SCZ.

2.1. 15q11.2 deletion

The 15q11.2 (BP1-BP2) deletion encompasses four genes (NIPA1, NIPA2, CYFIP1 and TUBGCP5) and has been found to be a risk factor many neuropsychiatric diseases including SCZ, autistic spectrum disorder and intellectual disability (Malhotra and Sebat, 2012). As shown in Table 3, the 15q11.2 deletion is one of the most common CNVs associated with SCZ, yet many individuals who possess the 15q11.2 deletion have mild symptoms or no visible phenotype, showing incomplete penetrance and variable expressivity. Penetrance of the 15q11.2 deletion is estimated to be 10.4% (Rosenfeld et al., 2012), which is much lower than that other pathogenic CNVs, such as the distal 16p11.2 (also implicated in SCZ (Guha et al., 2013)) which has an estimated penetrance of 62.4%. Of the deleted genes, CYFIP1 is the most likely to be responsible for the SCZ and autism phenotypes seen in carries. The CYFIP1 protein interacts with FMRP (fragile X mental retardation protein); behavioural abnormalities found in patients with fragile X syndrome are similar to those found in SCZ patients. A recent study (Nebel et al., 2016) focusing on the effect of CYFIP1 deletion using NPC generated from three CYFIP1 knockdown iPSC lines found that reduced levels of CYFIP1 resulted in the differential expression of many genes involved in the cell cycle and cytoskeletal remodelling. It was also found that the knockdown of CYFIP1 and the subsequent reduction in CYFIP1 protein levels resulted in a significant reduction in WAVE1/2 protein levels. This is consistent with previous studies on neural rosettes and NPC derived from 15q11.2 deletion carrier iPSC (Yoon et al., 2014). The WAVE1/2 proteins, along with CYFIP1, are members of the Wave Regulatory Complex (WRC), a protein complex involved in the regulation of actin polymerisation and cytoskeletal dynamics. The down-regulation of these cytoskeletal genes resulting from CYFIP1 deletion indicates an important role for CYFIP1 in cytoskeletal regulation in NPC, however, the link between this and disease mechanism is currently unknown.

Another CNV within chromosome 15 associated with SCZ is a larger duplication encompassing the Prader-Wills Syndrome/Angelman Syndrome (PSW/AS) region (15q11–13) (Rees et al., 2014). Within this region is a cluster of GABA receptor genes (GABRA5, GABRB3 and GABRG3) (Simon et al., 2004). These genes encode for $GABA_A$ receptor subunits and are widely expressed throughout development. There is evidence to suggest that dysfunction of GABAergic neurons and the subsequent inhibition leads to SCZ (Schmidt and Mirnics, 2015), therefore it follows that imbalance of different receptor subunits could lead to problems with receptor assembly and function.

2.2. 15q13.3 deletion

Deletions at 15q13.3 leads to neuropsychiatric disorders in approximately 80% of cases (Lowther et al., 2015) while duplications having much lower penetrance (Szafranski et al., 2010). Genes involved in this region are: FAN1, MTMR10, TRPM1, KLF13, OTUD7A, CHRNA7 and miR-211. Among them, CHRNA7 is indicated as playing a significant role in the neuropsychiatric phenotypes observed in patients. CHRNA7 encodes the α7 subunit of the nicotinic acetylcholine receptor (α7-nAChR), which has been associated with SCZ. The α7 homomeric nAChRs are found in both the pre- and post-synaptic membranes and allow flux of cations, with the highest permeability to Ca^{2+} (Albuquerque et al., 2009) CHRNA7 is the strongest candidate gene for SCZ patients with 15q13.3 CNVs and is thought to be subject to dosage sensitivity (Gillentine and Schaaf, 2015); deletion of CHRNA7 leads to a reduction in α7-subunit number and therefore impacts on calcium signalling (Gillentine et al., 2017) and acetylcholine transmission. There is evidence in the literature suggesting duplications of CHRNA7 might be pathogenic, however with decreased penetrance (Schaaf, 2014). iPSC-derived neurons with 15q duplications have been shown to have disrupted common neuronal pathways which might influence chromatin structures (Germain et al., 2014).

2.3. 22q11.2 deletion

The 22q11.2 deletion was one of the first CNVs linked to SCZ and is arguably the most common risk factor for SCZ (Bassett and Chow, 2008), with the homozygous 22q11.2 deletion found in 0.5–1% of SCZ patients (Horowitz et al., 2005; Van et al., 2017). This deletion has a heterogeneous phenotype, with clinical manifestations including multi-organ dysfunction, immune defects, gastrointestinal and endocrine problems and brain dysfunctions, including developmental delays, cognitive defects and neuropsychiatric illnesses. It is not entirely known why there is such variability in phenotype, however proposed mechanisms include variation in the size of the deletion (and therefore genes deleted), SNPs and other variants on the intact 22q11.2 region and additional variants in regions outside of the 22q11.2 region. These theories can also be applied to many other CNVs with variable expression. The 22q11.2 CNV usually encompasses at least 30 genes, with several disease-relevant genes being identified within this, such as TBX1, COMT, GNB1L, PRODH and DGCR8. However, due to the vast number of genes involved in the CNV, it is difficult to ascertain whether any individual gene deletion causes SCZ. DGCR8, which encodes a key regulator of microRNA (miRNA) synthesis (Toyoshima et al., 2016) has been identified as a gene within the 1.5 Mb deletion which may confer a risk of SCZ. Analysis of miRNA expression in 22q11.2 patient iPSC-derived neurospheres identified down-regulation of eight miRNAs previously found to be abnormally expressed in the brains of SCZ patients. A transcriptome network analysis of SCZ patient iPSC-derived neurons carrying a 22q11.2 deletion found that almost all genes within the 22q11.2 region were down-regulated 2-folds, confirming the deletion (Lin et al., 2016). Many genes outside of the 22q11.2 region, were also found to be down-regulated, including genes involved in cell cycle regulation and cytoskeletal organisation (as found in cells with the 15q11.2 deletion), meanwhile genes involved in apoptosis and immune

responses were found to be enriched. The up-regulation of apoptosis genes may explain synaptic loss found in SCZ and other neurodegenerative diseases, as well as reduced neuronal and glial viability. Other synaptic proteins associated with SCZ in this region include COMT, which is involved in dopamine regulation and neurotransmitter inactivation and PRODH, which regulates glutamine activity. Furthermore, iPSC studies on SCZ patient-derived cells carrying the 22q11.2 microdeletion show dysregulation of microRNAs targeting genes involved in neurotransmitter function, synapse formation and synaptic transmission (Zhao et al., 2015).

2.4. DISC1

DISC1 is a strong candidate for the link between dysregulated WNT-signalling and SCZ. Several mouse models have shown the DISC1-mutant mice have SCZ-like symptoms, including depressive-like phenotypes and reduction in social interactions (Clapcote et al., 2007), as well as deficits in neuroanatomy. It has been found that DISC1 plays a role in normal neuronal development, including regulating neurite outgrowth, neuronal migration and integration of new neurons into the existing neural circuitry. DISC1 also regulates proliferation of neural progenitor cells – silencing of DISC1 expression in adult hippocampal progenitor cells resulted in decreased cell proliferation in comparison to controls (H. Lee et al., 2015); furthermore, overexpression of the DISC1 protein gave a marked increase in proliferation (Srikanth et al., 2015). This was also confirmed in vivo through GFP labelling in mice (Mao et al., 2009). DISC1 has been found to regulate β-catenin stability through interactions with GSK3β, and DISC1 knockdown has been shown to abolish WNT-induced proliferation (Srikanth et al., 2015).

2.5. NRXN1

Several SCZ risk genes are involved in cell-cell adhesion and synapse formation. NRXN1 encodes for Neurexin-1 and is required to form synapses. Neurexins are pre-synaptic membrane proteins and interact with neuroligins (post-synaptic membrane proteins) to form synapses (Sudhof, 2017). Copy number variations in NRXN1 have been linked to both SCZ and autism. Studies in mice have shown that heterozygous Nrxn1α-knockout mice exhibit altered social behaviours similar to those found in SCZ (Dachtler et al., 2015). The effect of NRXN1 knockdown in iPSC has been studied in vitro and significant dysregulation of expression of genes involved in cell adhesion, as well as neuron differentiation and transcription factor pathways, was observed. Genes including those coding for synaptotagmins, neurexophilins and vesicular trafficking proteins were found to be significantly down-regulated; these genes directly interact with NRXN1 (Zeng et al., 2013).

2.6. CNTNAP2

Similarly, deletions in the CNTNAP2 (contactin-associated protein-like 2) have been also associated with SCZ. CNTNAP2 encodes the CASPR2 protein, which is required for localisation of potassium channels to the juxtaparanodal region of axons. CASPR2 is involved in axonal organisation and neuronal organisation; although it is not known how CNTNAP2 deletions cause SCZ, myelin-related neuronal dysfunction and aberrant neuronal migration have been found in brains of SCZ dysfunction, leading to a potential explanation for the association of CNTNAP2 deletions and SCZ (Friedman et al., 2007).

2.7. Other CNV loci

Certain SCZ-associated CNVs have implications in WNT-signalling. For example, BCL-9, which maps to 1q21.1, promotes transcriptional activity of β-catenin, resulting in increased WNT-signalling (Elkouby et al., 2010). Both deletions and duplications of 1q21.1 have been linked to SCZ (Chang et al., 2016; Marshall et al., 2016), although other genes within this region have also been studied in association with SCZ (Ni et al., 2007) and it is not yet possible to identify a single causal gene. However, the link between BCL-9 and WNT-signalling, along with the evidence of aberrant WNT-signalling in SCZ, suggests the BCL-9 is a good candidate gene and should be further studied to elucidate a disease mechanism.

Besides these single-gene CNVs, identified risk genes within other CNVs associated with SCZ are also involved in cell adhesion including GJA5 (1q21.1), DLG1 (3q29) and CLDN5, SCARF2 and ARVCF (all 22q11.2) (Kushima et al., 2017).

3. Environmental risks in schizophrenia

Environmental factors (Table 1) could explain abnormal brain pathology found in post-mortem brain studies of SCZ patient brains (van Os et al., 2010). For example, ventricular enlargement is one of the most stable morphological abnormalities found in SCZ patients and can have both genetic and environmental causes (Wright et al., 2000). Therefore, loss of cortical volume in SCZ patients as a result of decreased levels of synaptic pruning can be caused by either early exposure to neural hazards such as hypoxia (Brennand et al., 2015) or genetic variation of the HLA locus such as C4 complement (Sekar et al., 2016). Several studies show higher scores on the obstetric complication scales for patients with SCZ than for healthy individuals and the main complication being hypoxia (Ballon et al., 2008). Altered levels of oxygen and production of reactive oxygen species (ROS) during development have been associated with impaired neuronal differentiation (Do et al., 2009) and also regarded as a risk factor for SCZ (Bitanihirwe and Woo, 2011). Indeed, alterations in levels of oxygen consumption and ROS production have been investigated in iPSC-derived cellular models in several studies (Brennand et al., 2015; Paulsen Bda et al., 2012; Robicsek et al., 2013). The main findings at the NPC stage suggested that the elevated levels of ROS in SCZ cells could be rescued by antipsychotic drugs such as valproic acid (Paulsen Bda et al., 2012). Moreover, the amount of ROS was higher (Brennand et al., 2015) in NPC of the SCZ patient and mitochondrial dysfunction was observed in iPSC, NPC and dopaminergic neurons of patients (Robicsek et al., 2013). Taken together, abnormal fluctuations in oxygen during prolonged periods will result in ischemic response and might influence critical process such as angiogenesis and neurogenesis in developing brain. The crosstalk between NPC and endothelial cells is crucial in formation of blood brain barrier (BBB), which depends on secretion of angiogenic factors from NPC. Recently, it has been shown that, the secretome of iPSC-derived NPC from SCZ patients lack fundamental factors such as VEGF and result in neurovascular dysfunction (Casas et al., 2018). Other environmental factors such as exposure to glucocorticoids can adversely affect the ROS production in iPSC-derived neuroepithelial stem cells leading to de-regulated neurogenesis, further supporting the role of proper NPC regulation in psychiatric disorders (Raciti et al., 2016). However, in complex diseases such as SCZ, it is important to note that variations in oxygen levels and angiogenic potential can impact on gene expression during development and gene expression variants can also affect the reaction to hypoxic-ischemic response (Schmidt-Kastner et al., 2012). Other environmental causes such as exposure to ethanol and methylmercury and maternal seizures have been investigated during mice embryo development in comparison to human iPSC-derived NPC (Hashimoto-Torii et al., 2014). Exposure of embryos to these environmental factors activated heat shock factor 1 (HSF1) in cerebral cortical cells and iPSC-derived NPC from SCZ patients showed higher variability in the levels of HSF1 activation (Hashimoto-Torii et al., 2014). It could be possible that early neural cells in SCZ patients might be more vulnerable to environmental stressors due to genetic reasons.

4. Molecular pathways associated with schizophrenia

Two linked hypotheses surrounding the molecular mechanism of SCZ are the glutamate hypothesis and the GABA (gamma-aminobutyric

Table 1
Environmental risk factors for schizophrenia.

Early life	Childhood	Later life	Other
Complications during pregnancy	Living in an urban area	Drug abuse	Left-handedness
Complications at birth	Childhood trauma	Migration	Older father (>40 years)
Winter birth	Head injury	Low socioeconomic status	Young parents (<20 years)
Premature birth		Social isolation	Autoimmune diseases
Viral infection			Epilepsy
Poor prenatal nutrition			
Micronutrient deficiency			

acid) hypothesis. Glutamate is an excitatory neurotransmitter that can act on a wide range of receptors, with a key receptor being the NMDA (*N*-methyl-D-aspartic acid) ionotropic receptor. The NMDA receptor is involved in the regulation of many neuronal pathways, including synaptic plasticity, cortical development and long-term potentiation, and NMDA receptor hypofunction has been associated with cognitive defects and negative symptoms found in SCZ patients (Lau and Zukin, 2007). Glutamatergic neurons directly interact with GABA interneurons within the cortico-limbic system; GABA interneurons suppress the excitatory activity from glutamate-expressing pyramidal neurons, resulting in an inhibitory feedback loop.

WNT-signalling has been implicated in SCZ during neurodevelopment, the WNT-signalling pathway is crucial for regulating patterning of neural stem cells and for establishing the anterior-posterior axis in early vertebrate development (Elkouby et al., 2010) and forebrain specification (Inestrosa and Varela-Nallar, 2015) in late development (Harrison-Uy and Pleasure, 2012). WNT proteins and their signalling pathways are involved in a number of processes central to brain development, including cell proliferation (Pei et al., 2012), cell adhesion (Amin and Vincan, 2012) and cell fate determination; the WNT pathway and its role in cell fate determination is highly conserved across eukaryotic organisms (Holstein, 2012). The best-characterised WNT pathway is the canonical WNT/β-catenin pathway. In the absence of WNT ligands, β-catenin is degraded by a destruction complex consisting of a number of proteins that facilitates the phosphorylation of β-catenin by GSK3β, leading to the degradation of β-catenin. Due to its central and important role, disruption of WNT-signalling will affect normal brain development. Accordingly, WNT-signalling has been implicated in neurodevelopmental and neurotransmitter theories of SCZ, suggestive of a central role in SCZ pathogenesis.

Also crucial to neurodevelopment is cell adhesion and the cell adhesion molecules (CAMs) are involved in axon growth and migration, synapse formation and synaptic plasticity, and do also have a role in neurotransmission at synapses (Corvin, 2010). Genes such as NRXN1, CNTNAP2 and CASK known as synaptic adhesion molecules are known to be disrupted in many psychiatric disorders such as autism spectrum disorders, SCZ and bipolar disorder. However, there are recent evidences that these adhesion molecules are not exclusively involved in late neuronal development, but their expression pattern implicate their extended role in early neurogenesis (van de Leemput et al., 2014).

5. Stem cell models of schizophrenia

The discovery of reprogramming and the iPSC technology have given us the opportunity to derive, expand and study close to unlimited numbers of neural cells from living people carrying a diagnose or not. Recent years have proved that iPSC models of psychiatric disorder in general and also specifically SCZ are useful complementary tools for identifying molecular mechanisms causing disorder. Patient-derived stem cells can be used to model SCZ caused by rare copy number variations or idiopathic causes. Using donors with specific genetic mutations as opposed to those with no known genetic cause can reduce the variability of samples and help establish mechanisms behind the pathophysiology of the disease (Table 2). In most cases, fibroblasts are harvested from patients and reprogrammed using a range of protocols to obtain pluripotent stem cells that can be cultured and differentiated to the desired cell types. Functional assays, gene expression analysis and many other experiments are carried out on iPSC, neural progenitor cells (NPC) and differentiated neurons; such studies have uncovered disease mechanisms and pathways involved. Proliferation decline is usually the first obvious phenotype to recognise in SCZ NPC, which for example has been shown to be linked to up-regulation of miR-219 (a brain-specific miRNA) (Murai et al., 2016). Decreased proliferation in SCZ iPSC-derived NPC or prolonged cell cycle in iPSC-derived forebrain organoids (Ye et al., 2017) might cause a delay in or might be the cause of subsequent biological events including aberrant migration (Brennand et al., 2015; Brennand et al., 2011; Ye et al., 2017), de-regulated adherent junctions and apical polarity (Yoon et al., 2014). Ye et al. found in an organoid model of cells from patients with a DISC1 mutation that the interaction of DISC1-Ndel1 regulated mitosis and cell-cycle progression (Ye et al., 2017). Further investigation of patient derived NPC usually continue to the neurogenesis process and the maturation of neurons by measuring electrophysiological activities via patch clamping recording or Multi-Electrode Array (MEA) recording. Using the former approach, it has been shown that iPSC-derived neurons form SCZ patients have deficits in spontaneous and evoked activity (Sarkar et al., 2018). Another functional assay is imaging calcium dye fluctuations using calcium sensitive fluorescent dyes. Single-cell measurement of activity may not reflect the exact connectivity loss and network activity deficits in SCZ, in addition, by doing this in homogenous neuronal cultures could oversight phenotypes. Increasing the complexity of the cellular models to include several types of brain cells and/or to mimic in 3D, like organoids (Lancaster et al., 2017; Lancaster and Knoblich, 2014) and brain spheroids (Pasca et al., 2015) has the potential to reconstruct in vivo-like neural circuits. iPSC-derived region-specific cerebral organoids (Qian et al., 2018) generate a four-layered cortex-like structure containing electrophysiologically mature neurons that form functional synapses, however, the full six-layer structure of human cortex is yet to be achieved and robust methods for quantification of the pathophysiology of SCZ. One limitation for increasing the complexity of the brain organoids is the lack of blood supply and thus the necrosis of cells in the central parts of the organoid.

Faithful models of human brain development in health and disease would open up for identifying drug targets and possibilities for screening setups using disease relevant human cells. Development of new treatments for SCZ would be a large step forward considering the limited options there are today. iPSC models of SCZ have successfully been used to evaluate the effect of previously known treatments like valproate that bring potassium and zinc content back to normal in patient NPC (Paulsen Bda et al., 2014), but since one third of the SCZ patients do not respond to available antipsychotic treatments (Hasan et al., 2012), justifies adding resources into screening for novel drug candidates. Recently, an expression-based study investigated SCZ-linked transcriptional responses to 135 small molecules when introduced to iPSC-derived NPCs from several individuals with SCZ and healthy controls (Readhead et al., 2018). They showed that 52 drugs improved the SCZ-related transcriptomic signature in the iPSC-derived NPC lines, 61% of these in a diagnosis-dependent manner. They also showed that some of those drugs differentially regulate neuropsychiatric disease-associated genes. Moreover, many susceptibility genes of

Table 2
iPSC models of schizophrenia cases with a known genetic cause. CNV, copy number variation; DISC1, disrupted-in-schizophrenia 1; NRXN1, neurexin-1; SAD, schizoaffective disorder; SCZ, schizophrenia, NPC, neural progenitor cells; CYFIP1, cytoplasmic FMR1 interacting protein 1; VCFS, velocardiofacial syndrome; MD, major depression; TALEN, transcription activator-like effector nucleases; iPSC, induced pluripotent stem cells; KO, knockout; LTR, long terminal repeats; NSCs, neural stem cells, ESC, embryonic stem cell.

CNV loci	Genetic mutation	Sample details	Reprograming method	Cell type	Phenotype	Reference
15q11.2	382 kb deletion at 15q11.2	SAD proband and unaffected mother carrying 15q11.2 deletion; 1 unrelated control	Sendai viral	NPC and neurons	Altered dendritic morphology; reduction in CYFIP1 and PSD-95 protein levels	(Das et al., 2015)
	15q11.2 microdeletion	3 SCZ patients carrying 15q11.2 deletion; 3 unrelated controls and 2 controls carrying DISC1 mutation	Episomal vectors or sendai viral	iPSC-derived NPC	NPC exhibit defects in maintenance of adherens junctions, apical polarity and WAVE complex stability	(Yoon et al., 2014)
		3 hiPSC-derived NPC lines with lentiviral shRNA targeting *CYFIP1*		*CYFIP1*^{KD} NPC	Differential expression of FMRP targets and postsynaptic density genes in *CYFIP1*^{KD} lines	(Nebel et al., 2016)
22q11.2	22q11 deletion	3 SCZ patients (1 carrying 22q11 deletion, 2 sporadic cases); 2 unrelated controls	Retroviral LTR	Neurons	Significant delay in reduction of endogenous OCT4 and NANOG expression during differentiation of iPSC-derived neurons	(Pedrosa et al., 2011)
	22q11.2 microdeletion	3 SCZ patients (with 2 COS, 1 VCFS); 3 SAD patients (with VCFS); 6 controls	Plasmids	Neurons	Multiple miRNAs differentially expressed between patient and control neurons, with targets of the differentially expressed miRNAs linked to SCZ	(Zhao et al., 2015)
	22q11.2 microdeletion	5 SCZ patients (with 4 COS, 1 VCFS); 3 SAD patients (with VCFS); 7 controls	Plasmids	Early differentiating neurons	Differentially expressed genes (DEGs) involved in MAPK signalling, cell cycle and apoptosis; 22q11.2 genes *CDC45* and *PRODH* linked to expression networks for many of the DEGs	(Lin et al., 2016)
	2.6-Mb hemizygous deletion at 22q11.2	2SCZ patients carrying 22q11.2 deletion; 2 controls	Retroviral	Neurons	Increased L1 retrotransposon copy number in SCZ patient neurons; increased insertion of L1 in genes related to synapses and schizophrenia	(Bundo et al., 2014)
	2.6-Mb hemizygous deletion at 22q11.2	2 SCZ patients carrying 22q11.2 deletion; 4 controls	Retroviral	Neurons and neurospheres	Reduced neurosphere size, neurite outgrowth and neural differentiation efficiency in patient-derived cells; reduced expression of miRNAs in miR-17/92 cluster (involved in cell proliferation and survival) and miR-106a/b (represses p38α, a regulator of gliogenic differentiation)	(Toyoshima et al., 2016)
DISC1	4 base-pair frameshift mutation	1 SCZ patient and 1 MD patient, both carrying DISC1 mutation; 2 related controls and 1 unrelated control; 1 TALEN-corrected *DISC1* line and 2 TALEN-introduced *DISC1* mutation line	Episomal vectors	Forebrain neurons	Dysregulation of genes associated with synapses, DISC1-interacting proteins and psychiatric-disorder-associated proteins; synaptic vesicle release deficits and reduction of synaptic boutons in patient neurons; isogenic lines show *DISC1* mutation is necessary and sufficient for the observed synaptic defects	(Wen et al., 2014)
	Frameshift mutation in *DISC1* exon 2 or exon 8	2 TALEN-induced *DISC1* frameshift mutation in exon 8 and 2 Cas9-induced *DISC1* frameshift mutation in exon 2; 1 healthy control	Lentiviral	NPC and dorsal neurons	Frameshift results in expression of a truncated DISC1 protein and loss of full-length protein; increased WNT signalling and altered transcriptional profile in NPC and neurons; decreased expression of NPC fate markers including *Foxg1* and *Tbr2*	(Srikanth et al., 2015)
	4 base pair frameshift mutation	2 SCZ patients carrying DISC1 mutation; 2 isogenic iPSC lines with DISC mutation; 3 healthy controls	Episomal vectors	NSCs	Increased expression of *miR-219* (promotor of oligodendrocyte differentiation) and decreased expression of *TLX* (regulator of NSC proliferation and self-processing) in DISC1-mutant cells	(Murai et al., 2016)
NRXN1	Heterozygous *NRXN1* deletion	2 H1 hESCs lines with mutations to *NRXN1* gene (1 with conditional KO of *NRXN1*, 1 with conditional truncation of *NRXN1*)	N/A	*Ngn2*-induced neurons	Decrease in presynaptic neurotransmitter release Decreased frequency of spontaneous mEPSCs and reduced amplitude of EPSCs in *NRXN1*-mutant neurons, but normal synapse number	(Pak et al., 2015)
CNTNAP2	293.3 kb heterozygous deletion in CNTNAP2 gene (exons 14–15)	1 SCZ patient carrying CNTNAP2 deletion; healthy father carrying CNTNAP2 deletion; 1 related control and 1 non-related control	Sendai viral	NPC, glutamatergic neurons and forebrain neurons	Differential expression of genes involved in synaptic transmission; altered neuronal activity	(Flaherty et al., 2017; I.S. Lee et al., 2015)

SCZ have been reported to affect the glycogen synthase kinase 3 (GSK3) signalling pathway (Lovestone et al., 2007), a pathway that the commonly used drug lithium is acting on either directly (Klein and Melton, 1996) or indirectly (De Sarno et al., 2002) by blocking the GSK3. Thus, iPSC derived models of SCZ could be used both to unbiasly screen for novel drugs and to screen candidate molecules acting on identified signalling pathways.

Gene-editing approaches have been applied using CRISPR-Cas9 to generate isogenic iPSC lines to investigate the causal role of a specific gene in SCZ development. This approach can also be utilized in a correction strategy to edit an affected gene back to normal creating an isogenic control iPSC line. For example, a heterozygous deletion of the NRXN1 gene with the CRISPR-Cas9 method resulted in a cellular model of neurons defective in neurotransmitter release but with no changes in synapse number or neuronal differentiation capacity, results which resemble the mice model (Pak et al., 2015). More recently a study investigated non-coding genomic regions and performed CRISPR-Cas9 correction of a SCZ risk SNP, which altered the expression of MIR137 expression in addition to altered dendrite arborisation and synapse maturation in iPSC-derived excitatory neurons showing the importance of non-coding regions related to SCZ for neurogenesis (Forrest et al., 2017).

Table 3
CNVs associated with schizophrenia. SCZ frequency data taken from (Kushima et al., 2017). WBS, Williams-Beuren syndrome; PWS/AS, Prader-Willi Syndrome/Angelman Syndrome; SCZ, schizophrenia.

Chromosomal location	Type	Size (kb)	Number of genes affected	SCZ risk genes in region	Frequency in SCZ (%)	Reference
1q21.1	Deletion	1380	11	*BCL9*, *GJA8*, *GJA5*	0.17	(Li et al., 2011; Ni et al., 2007)
1q21.1	Duplication	820	11	*BCL9*, *GJA5*, *GJA8*, *PDZK1*, and *PRKAB2*	0.13	(Dolcetti et al., 2013)
2q13	Duplication	1700	14	*ANAPC1*, *BCL2L11*, *MERTK*	0.48	(Gregory et al., 2014)
NRXN1	Deletion	Various; 18–420	1	*NRXN1*	0.18	(Kirov et al., 2009; Rujescu et al., 2009)
3q29	Deletion	837–1663	21	*DLG1*, *PAK2*, *FBXO45*	0.082	(Mulle et al., 2010)
7q11.23 (WBS locus)	Duplication	1400	25	*STX1A*	0.08	(Kirov et al., 2011; Mulle et al., 2014)
7q34–7q36.1	Deletion	Various; 200–10,700	1	*CNTNAP2*	Not-determined	
15q11.2	Deletion	382	4	*CYFIP1*	0.59	(Das et al., 2015)
15q11.2-q13.1 (PWS/AS)	Duplication	~4000	~15	*UBE3A*	0.083	(Ingason et al., 2011)
15q13.3	Deletion	1500	7	*CHRNA7*	0.14	(Sinkus et al., 2015)
16p11.2	Duplication	600	28	*MAPK3*	0.35	(Blizinsky et al., 2016; McCarthy et al., 2009)
16p13.11	Duplication	790	8	*NTAN1* and *NDE1*	0.31	(Ingason et al., 2009)
17q12	Deletion	1400	15	*LHX1*, *HNF1B*	0.036	(Moreno-De-Luca et al., 2010)
22q11.2	Deletion	3000	45	*COMT*, *DGCR8*, *PRODH*, *TBX1*	0.29	(Bassett and Chow, 2008; Horowitz et al., 2005; Van et al., 2017)

Cellular phenotypes and disease mechanisms caused by environmental risk factors could be feasibly modelled in iPSC-derived neurons simply by introducing the known factor to culture media. For instance, altered HSF1-HSP-signalling in iPSC-derived NPC has been observed by exposure to ethanol and methylmercury (Hashimoto-Torii et al., 2014). The challenge is to accurately and timely model the physiological concentrations of the factors that the developing brain has been exposed to for causing SCZ.

Further steps in understanding the development of SCZ would be to connect cellular phenotypes detected in genetically and/or environmentally affected iPSC to gene and protein expression differences and how they cause aberrant molecular mechanisms. Transcriptome and proteome analysis have provided a robust setup, both at bulk and single cell levels to reveal expression differences and differences in subcellular compositions. Transcriptome studies of SCZ patient-derived cells have identified hundreds of gene expression differences including gene expression alteration of synaptic genes (Wen et al., 2014) as well as glutamate receptors and adhesion molecules such as CNTN4 (Hoffman et al., 2017), many components of the cyclic AMP and WNT signalling pathways such as WNT7A, TCF4, ADCY8, and PRKCA (Brennand et al., 2011) and increased levels of oxidative stress and cytoskeletal remodelling proteins (Brennand et al., 2015). Post-mortem studies also suggested enrichment of glial markers in SCZ patients (Gandal et al., 2018) which subsequently has been traced based on differences in NPC populations (Yoon et al., 2014) or glial progenitors (Windrem et al., 2017) by iPSC modelling. Our unpublished data (under revision) confirms an increased radial glial population and a decreased neuroepithelial stem cells population of NRXN1-alpha deleted patient iPSC derived NPC as well as their differentiation tendency toward astroglia cells and immature neurons. Further, Windrem et al. reported that beside an abnormal tendency of SCZ iPSC to differentiate into astroglia, the astrocytes also showed an atypical morphology in addition iPSC-derived oligodendrocyte progenitors produced less myelin (Windrem et al., 2017). Also, other cell types have been implicated in SCZ and GWAS studies indicate variation in the major histocompatibility complex (MHC) locus including complement component 4 (C4) genes in SCZ patients, which receptors are mainly expressed by microglia cells (Sekar et al., 2016). Today's technologies create possibilities to investigate microglial-neuron interactions using iPSC-derived organoids or co-culture systems to study for example how hyperactive microglial cells eliminate synapses during the neurogenesis of SCZ patient iPSC. Indeed, new data support this idea by showing that increased C4A RNA expression (which is associated with increased SCZ risk) predicts poorer cortical performance as one of SCZ symptoms (Donohoe et al., 2018).

The more complex a disorder, the more important is to increase the amount of individuals included in the study to not oversight phenotypes that otherwise might be masked by background and to certify that we are investigating authentic disease phenotypes and not technical variations. Thus, cohort size needs to be increased, moreover, additional models like animals or post-mortem analyses are another way to increase the confidence of our data.

6. Is early neurogenesis connected to the development of schizophrenia?

The phenomenon of neurogenesis is categorized into four stages: proliferation, migration, cell survival, and neuronal differentiation (Gage and Temple, 2013). Although SCZ is known as disease of synapses (Osimo et al., 2018), which occurs at the latest part of neurogenesis, the novel mechanisms found in iPSC models indicate that SCZ could be influenced throughout the whole of neurogenesis. There are strong evidences that proliferation of neural progenitors, which is the starting point of neurogenesis might be affected in SCZ via WNT-signalling alterations (Srikanth et al., 2015). Additionally, a WNT gradient determines anterior-posterior and dorso-ventral axes during early neurodevelopment. Cells in different regions of the neural tube are exposed to and display differences in baseline WNT-signalling and in responsiveness to WNT activators and inhibitors. One current way to investigate the role of WNT-signalling in SCZ is studies of iPSC-derived models from SCZ individuals with a DISC1 mutation. Mutations in DISC1 not only increased the proliferation rate in neuroepithelial progenitors but also changed their fate by decreasing the expression levels of FOXG1 and TBR2 due to boosted levels of baseline WNT-signalling (Srikanth et al., 2015). Additional evidence for skewed WNT-signalling during early derivation of neural progenitors in SCZ patients has been presented however the genetic background of these patients was not fully investigated (Brennand et al., 2015).

Beside proliferation, cell-cell adhesion and polarity has a strong impact on regional cortical architecture during neurogenesis (Kriegstein and Alvarez-Buylla, 2009). Adherent complexes such as NRXN1-NLGN1 are determined to be involved in synapse formation in late neurogenesis and their CNVs variations have been reported in SCZ (Kirov et al., 2009). However, observations indicate that the expression

of NRXN1 and NLGN1 also peak at early human neural induction both in vitro [Cortecon data set (van de Leemput et al., 2014)] and in the early human embryo (Petropoulos et al., 2016). These observations indicate a functional role of NRXN1 and NLGN1 perhaps as adhesion molecules already in the establishment of neural stem cell and in the early neurogenesis. Dysfunctional cell-cell adhesion during early neurogenesis of SCZ patient iPSC-derived NPC carrying CYFIP1 haploinsufficiency with the 15q11.2 microdeletion resulted in a lack of adherent junctions and apical polarity (Yoon et al., 2014).

During development neurogenesis precedes gliogenesis and the neurogenic-to-glia switch is not completely mapped. Thus, the neural progenitors need to choose fate during early development and very recent postmortem transcriptional studies of major psychiatric disorders indicate altered cell identity in terms of down-regulation of neuronal genes and up-regulation of astroglial genes in postmortem brain tissue of SCZ with distinct CNVs (Gandal et al., 2018). There is growing evidence of several developmentally early de-regulated mechanisms in iPSC-derived models of SCZ with less proliferation of neural progenitors and increased levels of astrocytes, phenotypes that might be caused by altered WNT signalling and/or insufficient adhesion. However, novel single cell techniques has opened up for functional genomics and a recent study show that the expression of common genomic SCZ-variants consistently mapped to neurons and interneurons in the mouse, but much less consistently to progenitors or glial cells (Skene et al., 2018).

7. Conclusion and future perspectives

Several recent studies have established in vitro models of SCZ to reveal hidden developmental aspects of the disease using patient-derived cells. New technologies such as single cell RNA-seq analyses pinpoint alteration of cell identities compared to normal early neurodevelopment irrespective of mechanism involved in disease progress. The polygenic nature of genetic risk factors in patient population might affect the presence or absence of cellular phenotype in SCZ models and its severity. Different genetic variants could have small or large effects on the development of SCZ and the effect might be accumulated by multiple genetic and environmental risk factors. Identifying unique mechanisms underlying a specific genetic variation require large patient populations which might be a hurdle due to the rare prevalence of most of the copy number variants related to SCZ. This will necessitate advance standardization and quality control to reduce technical variation in reprogramming and differentiation and genome engineering tools to create isogenic iPSC lines.

We would like to emphasize that, although iPSC models offer a useful methodology to uncover molecular mechanisms underlying the genetic and environmental nature of SCZ, they do not provide higher information about brain activities. Interestingly, iPSC-derived brain organoids provide us with 3D cortical-like structures with the potential to authentically mimic the human brain and the neuronal complexity. However, in the fast moving field of reprogramming, iPSC and neuronal differentiation the technique development progress is amazingly speedy so we wouldn't be surprised if it is only a couple of years until we have in vitro models of the human brain in 3D containing all types of brain cells interacting properly with each other to receive and send signals, substances and messages in an authentic way. These models could then be used to understand how genes and environmental factors that have been identified as risks for developing SCZ cause molecular, cellular and tissue alterations and why those alterations eventually cause human disorder.

Conflict of interest

The authors declare no conflict of interest.

Role of the funding source

The sources of funding for this study are both Swedish governmental agencies and private foundations without any conflicting interests.

CRediT authorship contribution statement

Mohsen Moslem: Conceptualization, Writing - original draft, Writing - review & editing. **Jessica Olive:** Writing - original draft. **Anna Falk:** Conceptualization, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Acknowledgement

This study was supported by Stiftelsen för strategisk forskning, SSF (IB13-0074), the Swedish Research Council, Vetenskapsrådet (2017-03407), Fredrik and Ingrid Thurings Foundation and Jeansson Foundations. The authors thank the iPS Core (ipscore.se) at Karolinska Institutet.

References

Albuquerque, E.X., Pereira, E.F., Alkondon, M., Rogers, S.W., 2009. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol. Rev. 89, 73–120.
Amin, N., Vincan, E., 2012. The Wnt signaling pathways and cell adhesion. Front. Biosci. (Landmark Ed.) 17, 784–804.
Ballon, J.S., Dean, K.A., Cadenhead, K.S., 2008. Obstetrical complications in people at risk for developing schizophrenia. Schizophr. Res. 98, 307–311.
Bassett, A.S., Chow, E.W., 2008. Schizophrenia and 22q11.2 deletion syndrome. Curr. Psychiatry Rep. 10, 148–157.
Bitanihirwe, B.K., Woo, T.U., 2011. Oxidative stress in schizophrenia: an integrated approach. Neurosci. Biobehav. Rev. 35, 878–893.
Blizinsky, K.D., Diaz-Castro, B., Forrest, M.P., Schurmann, B., Bach, A.P., Martin-de-Saavedra, M.D., Wang, L., Csernansky, J.G., Duan, J., Penzes, P., 2016. Reversal of dendritic phenotypes in 16p11.2 microduplication mouse model neurons by pharmacological targeting of a network hub. Proc. Natl. Acad. Sci. U. S. A. 113, 8520–8525.
Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D., et al., 2011. Modelling schizophrenia using human induced pluripotent stem cells. Nature 473, 221–225.
Brennand, K., Savas, J.N., Kim, Y., Tran, N., Simone, A., Hashimoto-Torii, K., Beaumont, K.G., Kim, H.J., Topol, A., Ladran, I., et al., 2015. Phenotypic differences in hiPSC NPCs derived from patients with schizophrenia. Mol. Psychiatry 20, 361–368.
Brown, A.S., 2011. The environment and susceptibility to schizophrenia. Prog. Neurobiol. 93, 23–58.
Bundo, M., Toyoshima, M., Okada, Y., Akamatsu, W., Ueda, J., Nemoto-Miyauchi, T., Sunaga, F., Toritsuka, M., Ikawa, D., Kakita, A., et al., 2014. Increased L1 retrotransposition in the neuronal genome in schizophr[1]enia. Neuron 81, 306–313.
Casas, B.S., Vitoria, G., do Costa, M.N., Madeiro da Costa, R., Trindade, P., Maciel, R., Navarrete, N., Rehen, S.K., Palma, V., 2018. hiPSC-derived neural stem cells from patients with schizophrenia induce an impaired angiogenesis. Transl. Psychiatry 8, 48.
Chang, H., Li, L., Peng, T., Li, M., Gao, L., Xiao, X., 2016. Replication analyses of four chromosomal deletions with schizophrenia via independent large-scale meta-analyses. Am. J. Med. Genet. B Neuropsychiatr. Genet. 171, 1161–1169.
Clapcote, S.J., Lipina, T.V., Millar, J.K., Mackie, S., Christie, S., Ogawa, F., Lerch, J.P., Trimble, K., Uchiyama, M., Sakuraba, Y., et al., 2007. Behavioral phenotypes of Disc1 missense mutations in mice. Neuron 54, 387–402.
Corvin, A.P., 2010. Neuronal cell adhesion genes: key players in risk for schizophrenia, bipolar disorder and other neurodevelopmental brain disorders? Cell Adhes. Migr. 4, 511–514.
Dachtler, J., Ivorra, J.L., Rowland, T.E., Lever, C., Rodgers, R.J., Clapcote, S.J., 2015. Heterozygous deletion of α-neurexin I or α-neurexin II results in behaviors relevant to autism and schizophrenia. Behav. Neurosci. 129, 765–776.
Das, D.K., Tapias, V., D'Aiuto, L., Chowdari, K.V., Francis, L., Zhi, Y., Ghosh, B.A., Surti, U., Tischfield, J., Sheldon, M., et al., 2015. Genetic and morphological features of human iPSC-derived neurons with chromosome 15q11.2 (BP1–BP2) deletions. Mol. Neuropsychiatry 1, 116–123.
De Sarno, P., Li, X., Jope, R.S., 2002. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. Neuropharmacology 43, 1158–1164.
Do, K.Q., Cabungcal, J.H., Frank, A., Steullet, P., Cuenod, M., 2009. Redox dysregulation, neurodevelopment, and schizophrenia. Curr. Opin. Neurobiol. 19, 220–230.
Dolcetti, A., Silversides, C.K., Marshall, C.R., Lionel, A.C., Stavropoulos, D.J., Scherer, S.W., Bassett, A.S., 2013. 1q21.1 microduplication expression in adults. Genet. Med. 15, 282–289.
Donohoe, G., Holland, J., Mothersill, D., McCarthy-Jones, S., Cosgrove, D., Harold, D., Richards, A., Mantripragada, K., Owen, M.J., O'Donovan, M.C., et al., 2018. Genetically predicted complement component 4A expression: effects on memory function and middle temporal lobe activation. Psychol. Med. 48, 1608–1615.
Elkouby, Y.M., Elias, S., Casey, E.S., Blythe, S.A., Tsabar, N., Klein, P.S., Root, H., Liu, K.J., Frank, D., 2010. Mesodermal Wnt signaling organizes the neural plate via Meis3. Development 137, 1531–1541.
Falk, A., Heine, V.M., Harwood, A.J., Sullivan, P.F., Peitz, M., Brustle, O., Shen, S., Sun, Y.M., Glover, J.C., Posthuma, D., et al., 2016. Modeling psychiatric disorders: from genomic findings to cellular phenotypes. Mol. Psychiatry 21, 1167–1179.
Flaherty, E., Deranieh, R.M., Artimovich, E., Lee, I.S., Siegel, A.J., Levy, D.L., Nestor, M.W., Brennand, K.J., 2017. Patient-derived hiPSC neurons with heterozygous CNTNAP2 deletions display altered neuronal gene expression and network activity. NPJ Schizophr. 3, 35.
Forrest, M.P., Zhang, H., Moy, W., McGowan, H., Leites, C., Dionisio, L.E., Xu, Z., Shi, J., Sanders, A.R., Greenleaf, W.J., et al., 2017. Open chromatin profiling in hiPSC-

derived neurons prioritizes functional noncoding psychiatric risk variants and highlights neurodevelopmental loci. Cell Stem Cell 21, 305–318.e8.
Friedman, J.I., Vrijenhoek, T., Markx, S., Janssen, I.M., van der Vliet, W.A., Faas, B.H.W., Knoers, N.V., Cahn, W., Kahn, R.S., Edelmann, L., et al., 2007. CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. Mol. Psychiatry 13, 261.
Gage, F.H., Temple, S., 2013. Neural stem cells: generating and regenerating the brain. Neuron 80, 588–601.
Gandal, M.J., Haney, J.R., Parikshak, N.N., Leppa, V., Ramaswami, G., Hartl, C., Schork, A.J., Appadurai, V., Buil, A., Werge, T.M., et al., 2018. Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. Science 359, 693–697.
Gao, W.-J., Snyder, M., 2013. NMDA hypofunction as a convergence point for progression and symptoms of schizophrenia. Front. Cell. Neurosci. 7.
Germain, N.D., Chen, P.F., Plocik, A.M., Glatt-Deeley, H., Brown, J., Fink, J.J., Bolduc, K.A., Robinson, T.M., Levine, E.S., Reiter, L.T., et al., 2014. Gene expression analysis of human induced pluripotent stem cell-derived neurons carrying copy number variants of chromosome 15q11–q13.1. Mol. Autism 5, 44.
Gillentine, M.A., Schaaf, C.P., 2015. The human clinical phenotypes of altered CHRNA7 copy number. Biochem. Pharmacol. 97, 352–362.
Gillentine, M.A., Yin, J., Bajic, A., Zhang, P., Cummock, S., Kim, J.J., Schaaf, C.P., 2017. Functional consequences of CHRNA7 copy-number alterations in induced pluripotent stem cells and neural progenitor cells. Am. J. Hum. Genet. 101, 874–887.
Goff, D.C., Coyle, J.T., 2001. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am. J. Psychiatr. 158, 1367–1377.
Gregory, C., L.A., C., Fiona, F., S.D., J., G.M., J., M.C., R., S.S., W., B.A., S., 2014. Adult neuropsychiatric expression and familial segregation of 2q13 duplications. Am. J. Med. Genet. B Neuropsychiatr. Genet. 165, 337–344.
Guha, S., Rees, E., Darvasi, A., Ivanov, D., Ikeda, M., Bergen, S.E., Magnusson, P.K., Cormican, P., Morris, D., Gill, M., et al., 2013. A rare deletion at distal 16p11.2 is implicated in schizophrenia. JAMA Psychiatry (Chicago, Ill) 70, 253–260.
Harrison, P.J., 2015. Recent genetic findings in schizophrenia and their therapeutic relevance. J. Psychopharmacol. (Oxford, England) 29, 85–96.
Harrison-Uy, S.J., Pleasure, S.J., 2012. Wnt signaling and forebrain development. Cold Spring Harb. Perspect. Biol. 4.
Hasan, A., Falkai, P., Wobrock, T., Lieberman, J., Glenthoj, B., Gattaz, W.F., Thibaut, F., Moller, H.J., World Federation of Societies of Biological Psychiatry Task Force on Treatment Guidelines for Schizophrenia, 2012. World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for Biological Treatment of Schizophrenia, part 1: update 2012 on the acute treatment of schizophrenia and the management of treatment resistance. World J. Biol. Psychiatry 13, 318–378.
Hashimoto-Torii, K., Torii, M., Fujimoto, M., Nakai, A., El Fatimy, R., Mezger, V., Ju, M.J., Ishii, S., Chao, S.H., Brennand, K.J., et al., 2014. Roles of heat shock factor 1 in neuronal response to fetal environmental risks and its relevance to brain disorders. Neuron 82, 560–572.
Hoffman, G.E., Hartley, B.J., Flaherty, E., Ladran, I., Gochman, P., Ruderfer, D.M., Stahl, E.A., Rapoport, J., Sklar, P., Brennand, K.J., 2017. Transcriptional signatures of schizophrenia in hiPSC-derived NPCs and neurons are concordant with post-mortem adult brains. Nat. Commun. 8, 2225.
Hoffman, G.E., Schrode, N., Flaherty, E., Brennand, K.J., 2018. New considerations for hiPSC-based models of neuropsychiatric disorders. Mol. Psychiatry https://doi.org/10.1038/s41380-018-0029-1.
Holstein, T.W., 2012. The evolution of the Wnt pathway. Cold Spring Harb. Perspect. Biol. 4.
Hook, V., Brennand, K.J., Kim, Y., Toneff, T., Funkelstein, L., Lee, K.C., Ziegler, M., Gage, F.H., 2014. Human iPSC neurons display activity-dependent neurotransmitter secretion: aberrant catecholamine levels in schizophrenia neurons. Stem Cell Rep. 3, 531–538.
Horowitz, A., Shifman, S., Rivlin, N., Pisanté, A., Darvasi, A., 2005. A survey of the 22q11 microdeletion in a large cohort of schizophrenia patients. Schizophr. Res. 73, 263–267.
Inestrosa, N.C., Varela-Nallar, L., 2015. Wnt signaling in neuronal differentiation and development. Cell Tissue Res. 359, 215–223.
Ingason, A., Rujescu, D., Cichon, S., Sigurdsson, E., Sigmundsson, T., Pietiläinen, O.P.H., Buizer-Voskamp, J.E., Strengman, E., Francks, C., Muglia, P., et al., 2009. Copy number variations of chromosome 16p13.1 region associated with schizophrenia. Mol. Psychiatry 16, 17.
Ingason, Andrés, Kirov, George, Giegling, Ina, Hansen, Thomas, Isles, Anthony R., Jakobsen, Klaus D., Kristinsson, Kari T., le Roux, Louise, Gustafsson, Omar, Craddock, Nick, et al., 2011. Maternally derived microduplications at 15q11–q13: implication of imprinted genes in psychotic illness. Am. J. Psychiatr. 168, 408–417.
Jablensky, A., McNeil, T.F., Morgan, V.A., 2017. Barbara fish and a short history of the neurodevelopmental hypothesis of schizophrenia. Schizophr. Bull. 43, 1158–1163.
Kirov, G., Rujescu, D., Ingason, A., Collier, D.A., O'Donovan, M.C., Owen, M.J., 2009. Neurexin 1 (NRXN1) deletions in schizophrenia. Schizophr. Bull. 35, 851–854.
Kirov, G., Pocklington, A.J., Holmans, P., Ivanov, D., Ikeda, M., Ruderfer, D., Moran, J., Chambert, K., Toncheva, D., Georgieva, L., et al., 2011. De novo CNV analysis implicates specific abnormalities of postsynaptic signaling complexes in the pathogenesis of schizophrenia. Mol. Psychiatry 17, 142.
Klein, P.S., Melton, D.A., 1996. A molecular mechanism for the effect of lithium on development. Proc. Natl. Acad. Sci. U. S. A. 93, 8455–8459.
Kriegstein, A., Alvarez-Buylla, A., 2009. The glial nature of embryonic and adult neural stem cells. Annu. Rev. Neurosci. 32, 149–184.
Kushima, I., Aleksic, B., Nakatochi, M., Shimamura, T., Okada, T., Uno, Y., Morikawa, M., Ishizuka, K., Shiino, T., Kimura, H., et al., 2018. Comparative analyses of copy-number variation in autism spectrum disorder and schizophrenia reveal etiological overlap and biological insights. Cell Rep. 24, 2838–2856.
Kushima, I., Aleksic, B.A.-O., Nakatochi, M.A.-O., Shimamura, T., Shiino, T., Yoshimi, A., Kimura, H., Takasaki, Y., Wang, C., Xing, J., et al., 2017. High-resolution copy number variation analysis of schizophrenia in Japan. 22 pp. 430–440.
Lancaster, M.A., Knoblich, J.A., 2014. Generation of cerebral organoids from human pluripotent stem cells. Nat. Protoc. 9, 2329–2340.
Lancaster, M.A., Corsini, N.S., Wolfinger, S., Gustafson, E.H., Phillips, A.W., Burkard, T.R., Otani, T., Livesey, F.J., Knoblich, J.A., 2017. Guided self-organization and cortical plate formation in human brain organoids. Nat. Biotechnol. 35, 659–666.
Lang, U.E., Puls, I., Müller, D.J., Strutz-Seebohm, N., Gallinat, J., 2007. Molecular mechanisms of schizophrenia. Cell. Physiol. Biochem. 20, 687–702.
Lau, C.G., Zukin, R.S., 2007. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. Nat. Rev. Neurosci. 8, 413–426.
Lee, H., Kang, E., GoodSmith, D., Yoon, D.Y., Song, H., Knierim, J.J., Ming, G.L., Christian, K.M., 2015a. DISC1-mediated dysregulation of adult hippocampal neurogenesis in rats. Front. Syst. Neurosci. 9, 93.
Lee, I.S., Carvalho, C.M., Douvaras, P., Ho, S.M., Hartley, B.J., Zuccherato, L.W., Ladran, I.G., Siegel, A.J., McCarthy, S., Malhotra, D., et al., 2015b. Characterization of molecular and cellular phenotypes associated with a heterozygous CNTNAP2 deletion using patient-derived hiPSC neural cells. NPJ Schizophr. 1.
Li, J., Zhou, G., Ji, W., et al., 2011. Common variants in the bcl9 gene conferring risk of schizophrenia. Arch. Gen. Psychiatry 68, 232–240.
Li, Z., Chen, J., Yu, H., He, L., Xu, Y., Zhang, D., Yi, Q., Li, C., Li, X., Shen, J., et al., 2017. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. Nat. Genet. 49, 1576–1583.
Lin, M., Pedrosa, E., Hrabovsky, A., Chen, J., Puliafito, B.R., Gilbert, S.R., Zheng, D., Lachman, H.M., 2016. Integrative transcriptome network analysis of iPSC-derived neurons from schizophrenia and schizoaffective disorder patients with 22q11.2 deletion. BMC Syst. Biol. 10, 105.
Lovestone, S., Killick, R., Di Forti, M., Murray, R., 2007. Schizophrenia as a GSK-3 dysregulation disorder. Trends Neurosci. 30, 142–149.
Lowther, C., Costain, G., Stavropoulos, D.J., Melvin, R., Silversides, C.K., Andrade, D.M., So, J., Faghfoury, H., Lionel, A.C., Marshall, C.R., et al., 2015. Delineating the 15q13.3 microdeletion phenotype: a case series and comprehensive review of the literature. Genet. Med. 17, 149–157.
Malhotra, D., Sebat, J., 2012. CNVs: harbingers of a rare variant revolution in psychiatric genetics. Cell 148, 1223–1241.
Mao, Y., Ge, X., Frank, C.L., Madison, J.M., Koehler, A.N., Doud, M.K., Tassa, C., Berry, E.M., Soda, T., Singh, K.K., et al., 2009. DISC1 regulates neural progenitor proliferation via modulation of GSK3β/β-catenin signaling. Cell 136, 1017–1031.
Marshall, C.R., Howrigan, D.P., Merico, D., Thiruvahindrapuram, B., Wu, W., Greer, D.S., Antaki, D., Shetty, A., Holmans, P.A., Pinto, D., et al., 2016. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. Nat. Genet. 49, 27.
McCarthy, S.E., Makarov, V., Kirov, G., Addington, A.M., McClellan, J., Yoon, S., Perkins, D.O., Dickel, D.E., Kusenda, M., Krastoshevsky, O., et al., 2009. Microduplications of 16p11.2 are associated with schizophrenia. Nat. Genet. 41, 1223.
Millan, M.J., Andrieux, A., Bartzokis, G., Cadenhead, K., Dazzan, P., Fusar-Poli, P., Gallinat, J., Giedd, J., Grayson, D.R., Heinrichs, M., et al., 2016. Altering the course of schizophrenia: progress and perspectives. Nat. Rev. Drug Discov. 15, 485–515.
Moreno-De-Luca, D., SGENE Consortium, Mulle, J.G., Simons Simplex Collection Genetics Consortium, Kaminsky, E.B., Sanders, S.J., GeneStar, Myers, S.M., Adam, M.P., Pakula, A.T., et al., 2010. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. Am. J. Hum. Genet. 87, 618–630.
Mulle, J.G., Dodd, A.F., McGrath, J.A., Wolyniec, P.S., Mitchell, A.A., Shetty, A.C., Sobreira, N.L., Valle, D., Rudd, M.K., Satten, G., et al., 2010. Microdeletions of 3q29 confer high risk for schizophrenia. Am. J. Hum. Genet. 87, 229–236.
Mulle, J.G., Pulver, A.E., McGrath, J.A., Wolyniec, P.S., Dodd, A.F., Cutler, D.J., Sebat, J., Malhotra, D., Nestadt, G., Conrad, D.F., et al., 2014. Reciprocal duplication of the Williams-Beuren syndrome deletion on chromosome 7q11.23 is associated with schizophrenia. Biol. Psychiatry 75, 371–377.
Murai, K., Sun, G., Ye, P., Tian, E., Yang, S., Cui, Q., Sun, G., Trinh, D., Sun, O., Hong, T., et al., 2016. The TLX-miR-219 cascade regulates neural stem cell proliferation in neurodevelopment and schizophrenia iPSC model. Nat. Commun. 7, 10965.
Nebel, R.A., Zhao, D., Pedrosa, E., Kirschen, J., Lachman, H.M., Zheng, D., Abrahams, B.S., 2016. Reduced CYFIP1 in human neural progenitors results in dysregulation of schizophrenia and epilepsy gene networks. PLoS One 11, e0148039.
Ni, X., Valente, J., Azevedo, M.H., Pato, M.T., Pato, C.N., Kennedy, J.L., 2007. Connexin 50 gene on human chromosome 1q21 is associated with schizophrenia in matched case–control and family-based studies. J. Med. Genet. 44, 532–536.
Olney, J.W., Newcomer, J.W., Farber, N.B., 1999. NMDA receptor hypofunction model of schizophrenia. J. Psychiatr. Res. 33, 523–533.
Osimo, E.F., Beck, K., Reis Marques, T., Howes, O.D., 2018. Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. Mol. Psychiatry https://doi.org/10.1038/s41380-018-0041-5.
Pak, C., Danko, T., Zhang, Y., Aoto, J., Anderson, G., Maxeiner, S., Yi, F., Wernig, M., Sudhof, T.C., 2015. Human neuropsychiatric disease modeling using conditional deletion reveals synaptic transmission defects caused by heterozygous mutations in NRXN1. Cell Stem Cell 17, 316–328.
Pasca, A.M., Sloan, S.A., Clarke, L.E., Tian, Y., Makinson, C.D., Huber, N., Kim, C.H., Park, J.Y., O'Rourke, N.A., Nguyen, K.D., et al., 2015. Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. Nat. Methods 12, 671–678.
Paulsen Bda, S., de Moraes Maciel, R., Galina, A., Souza da Silveira, M., dos Santos Souza, C., Drummond, H., Nascimento Pozzatto, E., Silva Jr., H., Chicaybam, L., Massuda, R., et al., 2012. Altered oxygen metabolism associated to neurogenesis of induced pluripotent stem cells derived from a schizophrenic patient. Cell Transplant. 21, 1547–1559.
Paulsen Bda, S., Cardoso, S.C., Stelling, M.P., Cadilhe, D.V., Rehen, S.K., 2014. Valproate reverts zinc and potassium imbalance in schizophrenia-derived reprogrammed cells. Schizophr. Res. 154, 30–35.

Pedrosa, E., Sandler, V., Shah, A., Carroll, R., Chang, C., Rockowitz, S., Guo, X., Zheng, D., Lachman, H.M., 2011. Development of patient-specific neurons in schizophrenia using induced pluripotent stem cells. J. Neurogenet. 25, 88–103.
Pei, Y., Brun, S.N., Markant, S.L., Lento, W., Gibson, P., Taketo, M.M., Giovannini, M., Gilbertson, R.J., Wechsler-Reya, R.J., 2012. WNT signaling increases proliferation and impairs differentiation of stem cells in the developing cerebellum. Development 139, 1724–1733.
Petropoulos, S., Edsgard, D., Reinius, B., Deng, Q., Panula, S.P., Codeluppi, S., Plaza Reyes, A., Linnarsson, S., Sandberg, R., Lanner, F., 2016. Single-cell RNA-Seq reveals lineage and X chromosome dynamics in human preimplantation embryos. Cell 165, 1012–1026.
Purcell, S.M., Moran, J.L., Fromer, M., Ruderfer, D., Solovieff, N., Roussos, P., O'Dushlaine, C., Chambert, K., Bergen, S.E., Kähler, A., et al., 2014. A polygenic burden of rare disruptive mutations in schizophrenia. Nature 506, 185.
Qian, X., Jacob, F., Song, M.M., Nguyen, H.N., Song, H., Ming, G.L., 2018. Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat. Protoc. 13, 565–580.
Raciti, M., Ong, J., Weis, L., Edoff, K., Battagli, C., Falk, A., Ceccatelli, S., 2016. Glucocorticoids alter neuronal differentiation of human neuroepithelial-like cells by inducing long-lasting changes in the reactive oxygen species balance. Neuropharmacology 107, 422–431.
Readhead, B., Hartley, B.J., Eastwood, B.J., Collier, D.A., Evans, D., Farias, R., He, C., Hoffman, G., Sklar, P., Dudley, J.T., et al., 2018. Expression-based drug screening of neural progenitor cells from individuals with schizophrenia. Nat. Commun. 9, 4412.
Rees, E., Walters, J.T., Georgieva, L., Isles, A.R., Chambert, K.D., Richards, A.L., Mahoney-Davies, G., Legge, S.E., Moran, J.L., McCarroll, S.A., et al., 2014. Analysis of copy number variations at 15 schizophrenia-associated loci. Br. J. Psychiatry 204, 108–114.
Robicsek, O., Karry, R., Petit, I., Salman-Kesner, N., Muller, F.J., Klein, E., Aberdam, D., Ben-Shachar, D., 2013. Abnormal neuronal differentiation and mitochondrial dysfunction in hair follicle-derived induced pluripotent stem cells of schizophrenia patients. Mol. Psychiatry 18, 1067–1076.
Rosenfeld, J.A., Coe, B.P., Eichler, E.E., Cuckle, H., Shaffer, L.G., 2012. Estimates of penetrance for recurrent pathogenic copy-number variations. Genet. Med. 15, 478.
Roussos, P., Guennewig, B., Kaczorowski, D.C., Barry, G., Brennand, K.J., 2016. Activity-dependent changes in gene expression in schizophrenia human-induced pluripotent stem cell neurons. JAMA Psychiatry 73, 1180–1188.
Rujescu, D., Ingason, A., Cichon, S., Pietiläinen, O.P.H., Barnes, M.R., Toulopoulou, T., Picchioni, M., Vassos, E., Ettinger, U., Bramon, E., et al., 2009. Disruption of the neurexin 1 gene is associated with schizophrenia. Hum. Mol. Genet. 18, 988–996.
Sarkar, A., Mei, A., Paquola, A.C.M., Stern, S., Bardy, C., Klug, J.R., Kim, S., Neshat, N., Kim, H.J., Ku, M., et al., 2018. Efficient generation of CA3 neurons from human pluripotent stem cells enables modeling of hippocampal connectivity in vitro. Cell Stem Cell 22, 684–697.e9.
Schaaf, C.P., 2014. Nicotinic acetylcholine receptors in human genetic disease. Genet. Med. 16, 649–656.
Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421–427.
Schmidt, M.J., Mirnics, K., 2015. Neurodevelopment, GABA system dysfunction, and schizophrenia. Neuropsychopharmacology 40, 190–206.
Schmidt-Kastner, R., van Os, J., Esquivel, G., Steinbusch, H.W., Rutten, B.P., 2012. An environmental analysis of genes associated with schizophrenia: hypoxia and vascular factors as interacting elements in the neurodevelopmental model. Mol. Psychiatry 17, 1194–1205.
Sebat, J., Levy, D.L., McCarthy, S.E., 2009. Rare structural variants in schizophrenia: one disorder, multiple mutations; one mutation, multiple disorders. Trends Genet. 25, 528–535.
Sekar, A., Bialas, A.R., de Rivera, H., Davis, A., Hammond, T.R., Kamitaki, N., Tooley, K., Presumey, J., Baum, M., Van Doren, V., et al., 2016. Schizophrenia risk from complex variation of complement component 4. Nature 530, 177–183.
Shohat, S., Ben-David, E., Shifman, S., 2017. Varying intolerance of gene pathways to mutational classes explain genetic convergence across neuropsychiatric disorders. Cell Rep. 18, 2217–2227.
Simon, J., Wakimoto, H., Fujita, N., Lalande, M., Barnard, E.A., 2004. Analysis of the set of GABA(A) receptor genes in the human genome. J. Biol. Chem. 279, 41422–41435.
Sinkus, M.L., Graw, S., Freedman, R., Ross, R.G., Lester, H.A., Leonard, S., 2015. The human CHRNA7 and CHRFAM7A genes: a review of the genetics, regulation, and function. Neuropharmacology 96, 274–288.
Skene, N.G., Bryois, J., Bakken, T.E., Breen, G., Crowley, J.J., Gaspar, H.A., Giusti-Rodriguez, P., Hodge, R.D., Miller, J.A., Munoz-Manchado, A.B., et al., 2018. Genetic identification of brain cell types underlying schizophrenia. Nat. Genet. 50, 825–833.
Srikanth, P., Han, K., Callahan, D.G., Makovkina, E., Muratore, C.R., Lalli, M.A., Zhou, H., Boyd, J.D., Kosik, K.S., Selkoe, D.J., et al., 2015. Genomic DISC1 disruption in hiPSCs alters Wnt signaling and neural cell fate. Cell Rep. 12, 1414–1429.
Stachowiak, E.K., Benson, C.A., Narla, S.T., Dimitri, A., Chuye, L.E.B., Dhiman, S., Harikrishnan, K., Elahi, S., Freedman, D., Brennand, K.J., et al., 2017. Cerebral organoids reveal early cortical maldevelopment in schizophrenia-computational anatomy and genomics, role of FGFR1. Transl. Psychiatry 7, 6.
Sudhof, T.C., 2017. Synaptic neurexin complexes: a molecular code for the logic of neural circuits. Cell 171, 745–769.
Sullivan, P.F., Kendler, K.S., Neale, M.C., 2003. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch. Gen. Psychiatry 60, 1187–1192.
Szafranski, P., Schaaf, C.P., Person, R.E., Gibson, I.B., Xia, Z., Mahadevan, S., Wiszniewska, J., Bacino, C.A., Lalani, S., Potocki, L., et al., 2010. Structures and molecular mechanisms for common 15q13.3 microduplications involving CHRNA7: benign or pathological? Hum. Mutat. 31, 840–850.
T.S. Commission, 2012. The Abandoned Illness: A Report From the Schizophrenia Commission (London).
Takahashi, K., Yamanaka, S., 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676.
Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.
Tansey, K.E., Rees, E., Linden, D.E., Ripke, S., Chambert, K.D., Moran, J.L., McCarroll, S.A., Holmans, P., Kirov, G., Walters, J., et al., 2015. Common alleles contribute to schizophrenia in CNV carriers. Mol. Psychiatry 21, 1085.
The International Schizophrenia Consortium, 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 460, 748.
Topol, A., Zhu, S., Tran, N., Simone, A., Fang, G., Brennand, K.J., 2015. Altered WNT signaling in human induced pluripotent stem cell neural progenitor cells derived from four schizophrenia patients. Biol. Psychiatry 78, e29–e34.
Toyoshima, M., Akamatsu, W., Okada, Y., Ohnishi, T., Balan, S., Hisano, Y., Iwayama, Y., Toyota, T., Matsumoto, T., Itasaka, N., et al., 2016. Analysis of induced pluripotent stem cells carrying 22q11.2 deletion. Transl. Psychiatry 6, e934.
van de Leemput, J., Boles, N.C., Kiehl, T.R., Corneo, B., Lederman, P., Menon, V., Lee, C., Martinez, R.A., Levi, B.P., Thompson, C.L., et al., 2014. CORTECON: a temporal transcriptome analysis of in vitro human cerebral cortex development from human embryonic stem cells. Neuron 83, 51–68.
van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468, 203–212.
Van, L., Boot, E., Bassett, A.S., 2017. Update on the 22q11.2 deletion syndrome and its relevance to schizophrenia. Curr. Opin. Psychiatry 30, 191–196.
Weinberger, D.R., 1987. Implications of normal brain development for the pathogenesis of schizophrenia. Arch. Gen. Psychiatry 44, 660–669.
Wen, Z., Nguyen, H.N., Guo, Z., Lalli, M.A., Wang, X., Su, Y., Kim, N.-S., Yoon, K.-J., Shin, J., Zhang, C., et al., 2014. Synaptic dysregulation in a human iPS cell model of mental disorders. Nature 515, 414.
Windrem, M.S., Osipovitch, M., Liu, Z., Bates, J., Chandler-Militello, D., Zou, L., Munir, J., Schanz, S., McCoy, K., Miller, R.H., et al., 2017. Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. Cell Stem Cell 21, 195–208.e6.
Wright, Ian C., Rabe-Hesketh, Sophia, Woodruff, Peter W.R., David, Anthony S., Murray, Robin M., Bullmore, Edward T., 2000. Meta-analysis of regional brain volumes in schizophrenia. Am. J. Psychiatr. 157, 16–25.
Ye, F., Kang, E., Yu, C., Qian, X., Jacob, F., Yu, C., Mao, M., Poon, R.Y.C., Kim, J., Song, H., et al., 2017. DISC1 regulates neurogenesis via modulating kinetochore attachment of Ndel1/Nde1 during mitosis. Neuron 96, 1041–1054.e5.
Yoon, K.J., Nguyen, H.N., Ursini, G., Zhang, F., Kim, N.S., Wen, Z., Makri, G., Nauen, D., Shin, J.H., Park, Y., et al., 2014. Modeling a genetic risk for schizophrenia in iPSCs and mice reveals neural stem cell deficits associated with adherens junctions and polarity. Cell Stem Cell 15, 79–91.
Zeng, L., Zhang, P., Shi, L., Yamamoto, V., Lu, W., Wang, K., 2013. Functional impacts of NRXN1 knockdown on neurodevelopment in stem cell models. PLoS One 8, e59685.
Zhao, D., Lin, M., Chen, J., Pedrosa, E., Hrabovsky, A., Fourcade, H.M., Zheng, D., Lachman, H.M., 2015. MicroRNA profiling of neurons generated using induced pluripotent stem cells derived from patients with schizophrenia and schizoaffective disorder, and 22q11.2 Del. PLoS One 10, e0132387.